The study of experimental cancer : a review.

Contributors

Woglom, William Henry, 1879-1953.
Augustus Long Health Sciences Library

Publication/Creation

New York : Columbia University Press, 1913.

Persistent URL

https://wellcomecollection.org/works/kfz75m8u

License and attribution

This material has been provided by This material has been provided by the Augustus C. Long Health Sciences Library at Columbia University and Columbia University Libraries/Information Services, through the Medical Heritage Library. The original may be consulted at the the Augustus C. Long Health Sciences Library at Columbia University and Columbia University. where the originals may be consulted.
This work has been identified as being free of known restrictions under copyright law, including all related and neighbouring rights and is being made available under the Creative Commons, Public Domain Mark.

You can copy, modify, distribute and perform the work, even for commercial purposes, without asking permission.

wellcome collection

Wellcome Collection
183 Euston Road
London NW1 2BE UK
T +44 (0)20 7611 8722
E library@wellcomecollection.org
https://wellcomecollection.org

COLUMBIA LIBRARIES OFFSITE
HEALTH SCIENCES STANDARD
HX64120511
RC261 .W82 1913 The study of experim
RECAP

THE LIBRARIES

COLUMBIA UNIVERSITY

HEALTH SCIENCES
LIBRARY

Digitized by the Internet Archive
in 2010 with funding from
Open Knowledge Commons

http://www.archive.org/details/studyofexperimen00wogl

THE STUDY
OF
EXPERIMENTAL CANCER
A REVIEW

STUDIES IN CANCER AND ALLIED SUBJECTS

Conducted under the George Crocker Special Research Fund
at Columbia University

VOL. I. THE STUDY OF EXPERIMENTAL CANCER. A Review. By WILLIAM H. WOGLOM, M.D. Illustrated with many plates. *In two bindings, Quarto, boards, or 8vo, cloth. pp. xi + 288. Price, $5.00 net.*

VOL. II. PATHOLOGY. Illustrated with many plates and charts. *Quarto, boards, pp. vi + 267. Price, $5.00 net.*

VOL. III. FROM THE DEPARTMENTS OF ZOOLOGY, SURGERY, CLINICAL PATHOLOGY, AND BIOLOGICAL CHEMISTRY. Illustrated with many plates. *Quarto, boards, pp. ix + 308. Price, $5.00 net.*

VOL. IV. CONTRIBUTIONS TO THE ANATOMY AND DEVELOPMENT OF THE SALIVARY GLANDS IN THE MAMMALIA. Illustrated with many plates. *Quarto, boards, pp. v + 364. Price, $5.00 net.*

COLUMBIA UNIVERSITY PRESS

Sales Agents

LONDON:	NEW YORK:	TORONTO:
HENRY FROWDE	LEMCKE & BUECHNER	HENRY FROWDE
AMEN CORNER, E.C.	30–32 WEST 27TH ST.	25 RICHMOND S[illegible] W.

STUDIES IN CANCER

AND ALLIED SUBJECTS

THE STUDY

OF

EXPERIMENTAL CANCER

A REVIEW

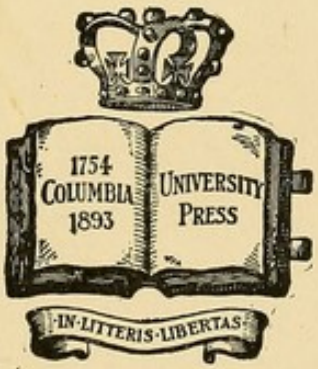
BY

WILLIAM H. WOGLOM, M.D.

ASSISTANT PROFESSOR, COLUMBIA UNIVERSITY, ASSIGNED TO CANCER RESEARCH
SOMETIME ASSISTANT TO THE DIRECTOR OF THE LABORATORIES OF
THE IMPERIAL CANCER RESEARCH FUND, LONDON

CONDUCTED UNDER THE GEORGE CROCKER SPECIAL RESEARCH FUND AT COLUMBIA UNIVERSITY

VOLUME I



New York
COLUMBIA UNIVERSITY PRESS
1913

All rights reserved

REPLACEMENT
RC261
W82
1913
c.1

COPYRIGHT, 1913,
BY COLUMBIA UNIVERSITY PRESS.

Set up and electrotyped. Published August, 1913. Reprinted September, 1913.

Norwood Press
J. S. Cushing Co. — Berwick & Smith Co.
Norwood, Mass., U.S.A.

TO THE MEMORY OF MY FATHER

WILLIAM H. WOGLOM

PREFACE

As no extensive review of the more recent experimental investigation of cancer was available, the Advisory Board of the George Crocker Special Research Fund thought it wise to prepare a summary. It was not considered advisable, however, to attempt a critical *précis* at this time, since much of the work is so new, and our ideas regarding the disease are so chaotic, that fair criticism and evaluation are well-nigh impossible. Nevertheless, the final chapter epitomizes in rough outline those results which, in all likelihood, will prove to be permanent.

Chemical studies have been omitted because a number of excellent reviews are already in existence, and because investigation in this field has thus far yielded but little of practical importance, while the bacteriological side of the question has been neglected for the reason that its discussion would amount to little more than a list of micro-organisms.

I desire to express my obligation to Dr. E. F. Bashford, Director of the Laboratories of the Imperial Cancer Research Fund, and to his colleagues, Dr. J. A. Murray and Dr. B. R. G. Russell; these gentlemen have read the book in manuscript and have made many valuable suggestions. The assistance of Dr. R. M. Rowe, of London, has been of the greatest value, while to Professor F. C. Wood, as well as to Dean Samuel W. Lambert, I am much indebted for continual advice and encouragement.

All the illustrations, with the exception of Fig. 6, have been taken from the Scientific Reports of the Imperial Cancer Research Fund, with the courteous permission of the Executive Committee of that Fund and of the Director, Dr. E. F. Bashford. For Fig. 6 I am indebted to Dr. Simon Flexner, editor of the *Journal of Experimental Medicine*.

WILLIAM H. WOGLOM.

NEW YORK, July 1, 1913.

CONTENTS

CHAPTER PAGE

I. Brief Historical Review 1

II. Attempts to Produce Tumors 20

III. Earlier Observations on the Transmissibility of Cancer . 39

Transfer from one person to another.
{ Accidental.
{ Experimental.
Transplantation of tumors into their bearers.
{ Accidental.
{ Experimental.
Attempts to transfer human tumors to animals.
Tumor transplantation within the same species.

IV. The Transplanted Tumor 58

The stroma reaction.
Increase of virulence or adaptation ?
Stimulation of growth power.
Technic of inoculation.
Relative importance of soil and graft.
Importance of uniform dosage.
Importance of uniform soil.
Inoculation site.
Interval after which growth becomes apparent.
Inoculation of stationary or receding tumors.
Transplantation of metastases.
Inoculation of tumor mixtures.
Resistance offered by the cancer cell to various agents.
Comparative growth rate of the malignant cell.
Fluctuations in growth energy.
Spontaneous absorption.
Histology of receding tumors.
Clinical course of the transplanted tumor.
Histological variations occurring during transplantation.
In the parenchyma.
In the stroma.
Cultivation of cells *in vitro*.

CHAPTER PAGE

V. RESISTANCE 128

Natural resistance.
Influence of age.
Influence of race.
Influence of health.
Influence of sex.
Influence of pregnancy.
Acquired resistance, active and passive.
Active resistance evolved by tumor.
Pan-immunity.
Active resistance probably evolved only by intact tumor cells of same species.
Active resistance evolved by normal tissue.
Active resistance probably evolved only by intact normal cells of same species.
No active resistance with autologous tissue.
Can tumor-bearing animals be made actively resistant ?
Premetastatic stage of active resistance.
Distribution of active resistance.
First appearance and duration of active resistance.
Passive resistance.
Are natural and acquired resistance transmissible by heredity ?
Nature of the resistant state.

VI. HYPERSUSCEPTIBILITY 178

Anaphylaxis

VII. THE SPONTANEOUS TUMOR 183

Frequency of tumors among the lower animals.
Structure of origin in the mouse.
The question of malignancy.
Occurrence of metastases.
Infiltrative growth.
Altmann's granules.
Ætiology.
Influence of infectivity.
Influence of age and sex.
Influence of lactation.
Influence of heredity.
Influence of inflammation.
Ætiology theoretically considered.
Histology.
Clinical course.
Relation between tumor and host.

CHAPTER PAGE

VIII. TUMORS OF A NATURE STILL UNDECIDED 227

Lympho-sarcoma of the dog.
Thyroid adeno-carcinoma of the trout.
Transmissible sarcoma of the fowl.

IX. THERAPEUTICS 256

X. GENERAL SUMMARY 270

INDEX OF AUTHORS 275

INDEX OF SUBJECTS 283

CHAPTER I

BRIEF HISTORICAL REVIEW

FROM the earliest days in the history of medicine, when cancer had already been identified to some extent as an incurable and fatal disease, conjecture regarding its nature and cure has been incessant, and theories almost without number have been advanced to explain its origin.

Speculation has been traced back to the Egyptians, who were acquainted with cancer as early as 1500 B.C., although, according to Joachim,[1] they included the most various swellings as part and parcel of the disease. But the lesion which we now recognize as cancer was probably not unknown to them, for they described an ulcerating disease and treated it with an arsenic salve.[2] It was certainly familiar, at any rate, to the Egyptians of a somewhat later period, for manuscripts prepared about 800 B.C. contain descriptions of cancer of the breast,[3] which leave no doubt that the disease was recognized at that time; even before the dates just quoted, however, cancer was being extirpated in India, and a salve applied to the wound in the hope of preventing recurrence of the disorder.[4]

With cancer of the breast Hippocrates was fairly well acquainted and he recognized the occurrence of malignant disease in certain of the internal organs as well. He believed it to be due, in common with all other pathological conditions, to a deficiency or an excess of bile, blood, or mucus.

Before the day of the Roman physician Celsus, the term carcinoma included the most bizarre collection of swellings. Celsus,[5] however, distinguished cancer from carcinoma, including under the former heading many lesions which are now recognized as simply inflammatory.

[1] *Die Lehre von der Krebskrankheit*, etc., Wolff, Jena, 1907, Teil I, 3.
[2] Arndt, cited by Wolff, 3.
[3] Oefele, cited by Wolff, 3.
[4] Wölfler, cited by Wolff, 3.
[5] Wolff, 7.

He not only separated several of the benign neoplasms (among them ganglion and lipoma) from the malignant growths, but described the enlargement of the axillary lymph nodes accompanying cancer of the breast, and seemed to be familiar with carcinoma of the liver and spleen. Nor was he unaware of the serious nature of cancer, for he expressed the opinion that it could be cured only in the first, or indurative stage, counseled ablation of the less malignant growths, devised operative measures for removal of carcinoma of the lip, and extirpated cancer of the breast.

Galen,[1] who in the second century elaborated the theories of Hippocrates, recognized with his predecessor four cardinal fluids upon whose proportion to one another depended the state of the body. These fluids were blood, mucus, yellow bile (derived from the liver), and black bile (secreted by the spleen); to the collection in undue amount of the material last mentioned he ascribed the origin of malignant growths. If the bile were sharp and irritating, there arose an ulcerating cancer; if mild, one of the non-ulcerating type. The spread of the disease was well known to Galen, but, ignorant of the existence of the lymphatics, he believed that it took place by way of the veins. His therapeutic endeavors were directed particularly toward attaining a diet which should not contain any of the substances productive of black bile, while purging played no unimportant part in the treatment. Considering both procedures of the highest utility, he nevertheless did not entirely neglect surgical intervention, which he conceived, however, to be of secondary value only, reasoning that it was illogical to treat a constitutional disease by measures so purely local as surgery.

Leonides[2] was the first investigator with courage requisite to discard the advice of Hippocrates, never to operate on an ulcerating cancer. He practised with the knife and cautery the most thorough and energetic treatment of carcinoma of the breast, and was the first to appreciate the importance of retraction of the nipple as a diagnostic sign.

From the time of Leonides, about 180 B.C., until the Renaissance, the art of the physician progressed only to a small degree, if, indeed, it advanced at all; but with the awakened interest in art, literature, and science which made this such an eventful period, there was aroused a

[1] Wolff, 10. [2] Wolff, 14.

renewed attention to medicine. Vesalius[1] exposed several of the errors of Galen and his predecessors, while his pupil Fallopius[2] made important contributions toward the diagnosis of cancer; and although he preferred to treat the disease with arsenic, his contemporary, Ambroise Paré,[3] was fully alive to the necessity of total ablation whenever its performance was possible.

Throughout more than a thousand years Galen's hypothesis of the four humors had ruled supreme in the domain of medicine, without an attempt having been made to depose it. With Paracelsus,[4] however, there appeared signs of an unwillingness to accept it in its entirety, and in the next century the final overthrow was accomplished through the simultaneous operation of several factors, the most important of which were the demonstration of the circulation of the blood, and the revelation of the red blood cells and lymph channels.

With these discoveries, and that of the cellular structure of cork by Hooke, hypothesis had begun to give place to observation, and following the improvement of the microscope came the description of the nucleus by Brown.[5] However, the cells of Hooke and his immediate successors were but chambers in the plant tissue, while it is doubtful from Brown's description whether he recognized the importance of his observation. It remained for Schleiden[6] to appreciate the significance of the cell as a unit in the organism of the plant, and for Schwann[7] to apply this conception to the animal tissues. This new work enhanced greatly the significance of Müller's[8] earlier announcement of the cellular structure of certain growths, and encouraged him to reëxamine tumors in which no cells had at first been found, as a result of which he[9] was enabled, with lenses of higher power, to perceive a cellular structure also in these.

But even after the cell had been accepted as the tissue unit, specula-

[1] Wolff, 32.
[2] Wolff, 34.
[3] Wolff, 42.
[4] Wolff, 51.
[5] *Observations on the Organs and Mode of Fecundation in Orchideæ*, etc., London, 1831.
[6] *Arch. f. Anat., Physiol.*, etc., (Müller), 1838, 137.
[7] *Mikroskop. Untersuchungen über die Uebereinstimmung in der Struktur u. dem Wachstum der Thiere u. Pflanzen*, Berlin, 1839.
[8] *Arch. f. Anat., Physiol.*, etc., (Müller), 1836, p. ccxviii.
[9] *Ueber den feineren Bau und die Formen der krankhaften Geschwülste*, Berlin, 1838.

tion regarding its actual source continued rife, and the relation of the intercellular substance to cell genesis became the subject of much debate. Believed by Schwann to be destined for transformation into new cells, its insignificance in this process was first appreciated by Remak.[1] A series of publications begun in 1841 culminated in a description[2] of cell division in which it was denied that the intercellular substance was able to produce new cells, and declared to be very probable that all animal cells were created through the progressive division of preëxisting cells.

According to Virchow's conception,[3] it was just as impossible for a non-cellular material to elaborate a cell as it was for the decomposition products of animal or vegetable matter to give origin to an infusorian. *Omnis cellula e cellula,* as animals were derived only from animals, or plants from plants.

The cells of a tissue[4] which was to become the seat of a tumor began to swell and divide, possibly as a result of irritation. If division went on rapidly, and if the members of successive generations became progressively smaller, the tissue arrived finally at the granulation stage in which, like granulation tissue, it was indifferent in appearance, the condition being analogous to that obtaining in the embryo in the early days following fertilization. Upon this state there supervened one of differentiation, leading to the final stage in which the tumor assumed its finished form.

The growth of a malignant tumor took place, not by increase of its elements, but by the fusion of accessory foci evolved in its neighborhood, a method of dissemination which proved that the primary nodule exercised a certain stimulation upon the surrounding parts through the agency of a secreted fluid. The process was exactly the same in metastatic tumors, but whether only a fluid excretion were concerned in this case was a question difficult of decision. It was not at all improbable that in some instances cells were actually transported, to act as the infective material; and although

[1] *Untersuchungen über die Entwicklung der Wirbelthiere,* Berlin, 1855.
[2] *Arch. f. Anat., Physiol.,* etc., (Müller), 1852, 47.
[3] *Arch. f. path. Anat.,* etc., (Virchow), 1855, viii, 3.
Die Cellularpathologie, 4te Aufl., Berlin, 1871, 24.
[4] *Die krankhaften Geschwülste,* Berlin, 1863, 89.

this was not a common occurrence, still, as the cells within the tumor produced the injurious fluid it was not unreasonable to imagine them able to carry it to other localities. It was certain, however, that metastases were not a result of proliferation of the transported cells, but rather the product of healthy elements in the neighborhood, incited to malignant growth by the excretions of cancer cells deposited there.

The validity of Virchow's hypothesis was seriously affected by the later work of Thiersch,[1] who advanced proof that the cells of epitheliomata developed only from epithelium — never from connective tissue. And even though the examination of serial sections failed to demonstrate in every case an actual connection between the tumor and the epithelium, it was not unreasonable to postulate a previous association with this tissue, since it might have been present in the form of cell islands which had become estranged from their surroundings during the development of the embryo. In Thiersch's opinion, metastases in the lymph nodes were to be ascribed with much more reason to the actual proliferation of transported tumor cells than to growth of the normal elements of the node under stress of stimulation by the hypothetical growth-exciting fluid. Furthermore, Waldeyer,[2] amplifying the investigations of Thiersch, demonstrated that all carcinomata were of epithelial origin and denied that a transformation of connective tissue elements into cancer cells ever occurred. He agreed with Thiersch that metastases were the product of proliferation on the part of transported cells and attributed a large share in the production of secondary deposits to the ameboid motion which, in conjunction with Carmalt,[3] he had demonstrated in the cancer cell. To this point Lambert and Hanes[4] have again called attention within the past few years.

Thus was overthrown the first half of Virchow's hypothesis. The remainder, which sought partially to explain by irritation the launching of cells upon a career of lawless growth, is still intact, and accepted

[1] *Der Epithelialkrebs, namentlich der Haut*, Leipzig, 1865, 58.
[2] *Arch. f. path. Anat.*, etc., (Virchow), 1867, xli, 470.
[3] *Arch. f. path. Anat.*, etc., (Virchow), 1872, lv, 481.
[4] *Jour. American Med. Assoc.*, 1911, lvi, 791.
Arch. f. path. Anat., etc., (Virchow), 1912, ccix, 12.

by many investigators of the present day as an explanation, in part at least, of the inception of malignant proliferation. The occurrence of cancer of the mouth in smokers, of carcinoma of the stomach upon the ground of a gastric ulcer, of cancer of the gall-bladder in connection with cholelithiasis, and many other instances of an apparent relation between chronic irritation and malignant growth have been cited time and again in favor of this conception. To mention specifically but a few observers, Neve [1] has directed attention to the natives of Kashmir who wear underneath the clothing a basket of glowing charcoal, and in whom epithelioma is very common upon the anterior abdominal wall at the site where the basket is carried, while carcinoma of the mouth is, according to the experience of Chalmers and Perry,[2] very frequent among the women of India who chew the betel-nut and retain it in the mouth during sleep. Nor are instances wanting among the lower animals. Bashford [3] has cited the observations of Captain Brodie-Mills upon the great frequency of squamous cell carcinoma at the root of the right horn among the cattle used in India for draught purposes — the right horn being used by the natives for the attachment of agricultural implements or wagons. Plicque [4] noticed that carcinoma in horses was commonly found where the bit irritated the corners of the mouth, and subcutaneous fibromata almost invariably at the point of pressure by collars or girths, while in dogs the most posterior mammæ, those most often engorged, were the ones more frequently affected by cancer. In cats, the upper lip, which was the one more likely to be wounded, was the site of election for malignant disease.

Thiersch [5] ascribed the development of epithelioma to a disturbance in the equilibrium between epithelium and connective tissue brought about by senile atrophy of the latter, while the proliferative or bioplastic energy of the epithelium remained at the same time unimpaired. Malignant growth thus occurred not by reason of an increase in the offensive power of epithelium, but through a decrease

[1] *British Med. Jour.*, 1910, ii, 589.

[2] Cited by Bashford, *Third Sci. Report, Imperial Cancer Research Fund*, London, 1908, 19.

[3] *Third Sci. Report, Imperial Cancer Research Fund*, London, 1908, 22.

[4] *Revue de Chir.*, 1889, ix, 521.

[5] *Der Epithelialkrebs, namentlich der Haut*, Leipzig, 1865, 78.

in the defenses of the connective tissue. Chronic irritation, inflammation, or trauma might under certain conditions act as the final stimulus and thus be an indirect cause of malignant growth.

Waldeyer,[1] on the contrary, urged an opposite conception: That with increasing age epithelial structures lost their vigor and became surrounded by a replacement fibrosis, the result of an increased activity on the side of the connective tissue. The isolation of epithelial elements might be attended by any one of three possible results; most frequently such cells underwent necrosis, at other times there supervened cyst formation, while in the third place they might assume malignant growth. An important prelude to this type of proliferation was the appearance of changes in the connective tissue extraordinarily like those accompanying inflammation.

Hauser,[2] although admitting that epithelium was the most vigorous of all the tissues, maintained that Thiersch's hypothesis did not explain the enormous increase in growth energy so frequently encountered in cancers of the cylindrical cell type. This phenomenon was but insufficiently explained by the assumption of a decrease in the physiological defenses, and there was required the addition of a positive quantity to the opposite side of the equation, which might well be an augmentation in the assimilative power of the epithelium coupled, perhaps, with an increased avidity for food-stuffs. Furthermore, if the hypothesis were correct, one would anticipate the more frequent occurrence of multiple cancers, whereas the disease was almost without exception local in origin and limited to a single organ, if not to one part of that organ. Finally, connective tissue was often not so passive as the hypothesis assumed, for in many scirrhous carcinomata growth of the stroma proceeded with the greatest vigor and occasionally outstripped even the epithelial proliferation.

v. Hansemann [3] criticized Thiersch's hypothesis on the ground that it explained neither the relatively uncommon occurrence of carcinoma, nor its presence in the young, and extended the latter objection to include the fact that carcinoma in early life was exceptionally malignant. Were the conception correct, the case should be reversed.

[1] *Arch. f. path. Anat.*, etc., (Virchow), 1867, xli, 470; 1872, lv, 67.

[2] *Das Cylinderepithel-carcinom des Magens und des Dickdarms*, Jena, 1890, 135.

[3] *Die mikroskopische Diagnose der bösartigen Geschwülste*, Berlin, 1902, 215.

Although Cohnheim [1] was not the first to suggest a connection between malignant growths and embryonic rests, it is with his name that the hypothesis is generally associated. Having described a congenital myo-sarcoma of the kidney, he suggested that the tumor might have originated from germinal muscle cells which had been snared off at a time when the foundations of the urinary organs were being laid down. He assumed that in early embryonal life more cells than requisite were produced and that the unutilized elements, with all their inherent power of proliferation, were cut off at a very early period, probably corresponding to the interval between differentiation of the germinal layers and completion of the foundations for the various organs.

Cohnheim sought support for his suggestions in the undifferentiated embryonal appearance of tumor cells as well as in the congenital or early postnatal occurrence of neoplasms, although admitting that it would go hard with his hypothesis were it forced to depend upon the latter alone for substantiation. Still, the conception did not in the least require that the tumor itself should be congenital, but merely the foundation for it. What were the circumstances able to initiate growth in embryonic rests he made no definite effort to establish, although offering the suggestion that one of them might be repeated arterial congestion or even inflammatory hyperemia, so that the idea of traumatic etiology might be true within limitations. It was impossible, however, to apprehend the influence which excited or released the power of indefinite proliferation, for it had not yet been learned in what manner growth was normally inhibited. It might be that normal tissues were able to exert a certain control over the germinal displacements and thus keep them from proliferating, while trauma might so weaken this physiological restraint in the neighborhood of a cell rest as to permit the inception of growth. Cohnheim believed that his explanation accounted for the variety of tissues so often found in tumors, as well as for the occurrence of certain neoplasms in specific localities. It was a clinical observation of many years' standing that epithelial growths were common at the various orifices of the body, and, indeed, this fact had been made use of by Virchow to sustain the hypothesis of mechanical insult. But if injury were the factor determin-

[1] *Arch. f. path. Anat.*, etc., (Virchow), 1875, lxv, 64.
Vorlesungen über allg. Path., Bd. i, Berlin, 1877, 634.

ing continuous proliferation it was hard to understand why tumors should be so rarely met with on the hands and feet, which of all parts of the body were most eminently exposed to trauma. An elucidation of the frequent occurrence of epithelial growths at certain sites must, therefore, be sought elsewhere, and most reasonably in the fact that at the apertures of the body there was a complication in the embryonal structure at one or another stage of development.

R. Hertwig [1] thought that a hypothesis which would refer the origin of neoplasms to embryonal rests was irreconcilable with the fact that tumors, and particularly malignant tumors, were more frequent in the higher age periods. It must be assumed that the body was everywhere provided with nutritive materials which were only awaiting utilization. How was it, then, that for decades the colonies of embryonal cells did not make use of this material? Either they were unable to do so (for lack of the very power which Cohnheim's conception emphasized and upon which it was built), or they were by some means excluded from sharing the universal food-stuff — a supposition which was inconceivable. Furthermore, Hertwig did not believe that a comparison could be instituted between embryonal and tumor cells on a common ground of absence of differentiation. Those of the embryo were undifferentiated, it was true, but they possessed an increasing tendency to differentiate, or, in other words, to exchange *cytotypic* for *organotypic* growth, while the slight disposition originally present in the elements of a tumor became progressively weaker and weaker.

The problem of malignant growth, according to Hertwig, was a double one, since it must first be explained how, in an organism which had reached its limit of size, the cells normally lost their autonomous growth and subjected themselves to their surroundings, and secondly it must be ascertained what changes had to occur in cells in order that they might escape from the control exercised over them by the organism.

Cohnheim's hypothesis has been criticized by Bashford [2] in a discussion of cancer in the cheek and abdominal wall which, almost

[1] *Festschrift Ernst Haeckel*, Jena, 1904, 347.

[2] *Third Sci. Report, Imperial Cancer Research Fund*, London, 1908, 24, and Introduction, p. ix.

unknown in Europeans, occurs with great frequency among the natives of India following chronic irritation produced by the use of the betel-nut or Kangri basket. ". . . it becomes necessary to postulate further a uniform and abundant distribution of 'embryonic rests' over the body, or to assume a different distribution of 'embryonic rests' in Europeans and native races, coinciding with the points they respectively select for the indulgence of various practices involving the application of peculiar irritations." And further: "Suffice it to say that if all forms of cancer are to be explained by such speculations and the intervention of congenital 'germs' is to hold good for all cases, then it must be assumed that such germs are as uniformly distributed, *e.g.* over the surface of the body of all vertebrates as the skin is itself, and thus the explanation becomes no explanation at all."

While, in the opinion of Ribbert,[1] many carcinomata almost certainly took their origin from embryonal cell rests, it was evident that such structures were not invariably able to assume the malignant type of growth, since many of them lay indefinitely quiescent. Although they possessed, in common with other epithelial cell groups, the normal tendency to penetrate underlying connective tissue, this latter must, under ordinary conditions, exert some sort of inhibitory influence preventing invasion on the part of the epithelium, and not until this opposition had been removed could the potential energies of the epithelium be unfolded. The release of the normal invasive faculty was brought about through the presence in the connective tissue of simple hyperemia, or of inflammatory lesions such as round cell infiltration, or proliferation of the fixed elements with a concomitant production of blood vessels, an analogous situation occurring in the development of glandular structures in the embryo, where downgrowth of epithelium was always preceded by alterations in the subjacent tissue. Nevertheless, in the fully developed organism at least, cellular changes in the connective tissue did not invariably elicit epithelial invasion, for inflammation, even though it continued over an extended period of time, was not always followed by the development of carcinoma. Hence, the inflammatory lesions which

[1] *Arch. f. path. Anat.*, etc., (Virchow), 1894, cxxxv, 433.
Geschwulstlehre, Bonn, 1904, 552.
Das Karzinom des Menschen, Bonn, 1911, 482.

had been found to precede carcinoma must be distinguished by some special characteristic, and the whole process could best be explained on the assumption that they were the product of certain agents elaborated by the epithelium itself — that the epithelial cells prepared the soil into which they were later to penetrate. Such a chain of events, however, might result merely in the occurrence of heterotopia, and to explain actual malignancy it was necessary to postulate in addition that the epithelial cells suffered a loss in differentiation,[1] through which they became enabled to proliferate more luxuriantly. In the case of embryonal rests, malignant growth was the more readily initiated because the cells had not yet undergone complete differentiation, and were therefore more ready to start upon a career of indefinite multiplication.

Ribbert's work was the anatomical expression of a biological law formulated by Weigert,[2] who had suggested that as tumors, either benign or malignant, developed without the interference of external stimuli or after the action of those which, under other circumstances, did not give rise to tumors, stimulus could be considered merely as an accessory cause. Such an etiological factor could only arouse a latent disposition toward the growth of a tumor or accelerate the proliferation of one already present. To explain this disposition, it might be imagined that there was a misrelation between the proliferative energies of some region of the organism and its neighboring tissues, which had occurred either through increased growth activity on the part of certain cells or through decreased resistance of those tissues determining the bounds of physiological growth. The former might take place in the way that Cohnheim had postulated, or else single cell groups might retain their growth energy after the embryonal period had passed. A decreased resistance, on the other hand, might be congenital for one or another locality or develop in later life by the more rapid aging of certain tissues — a conception which had been advanced by Thiersch and with which Weigert expressed his agreement. For neither non-malignant nor malignant proliferation, however, was it necessary to assume the application

[1] Ribbert distinguished very sharply between such a condition, and one of specific cancerous degeneration.

[2] *Ueber Entzündung*, Eulenburg's *Real-Encyclopädie der gesammten Heilkunde*, erste Aufl., 1880, iv, 644; zweite, 1886, vi, 325.

from without of a direct stimulus to cell growth, for in both cases there were concerned only those proclivities toward continuous multiplication which had been inherent in the cells since the embryonic period.

Hauser,[1] in a series of articles in which, to support his contention, reference was made to his own previous observations,[2] denied strenuously the correctness of the view held by Ribbert regarding the development of carcinoma. This disorder, in his opinion, could be produced only through a fundamental change in the biological properties of the implicated cells, and with the assumption of such a change certain morphological data were in entire accord. The loss of physiological function, the increase in the size of the cell and its nucleus, the higher chromatin content of the latter, the presence of abnormal mitotic figures, and finally, the enormous capacity for multiplication — all indicated an entirely new type of cell. The process was, in short, a specific cancerous degeneration, and a weakening of the defenses set up by the connective tissue against epithelium could play but a subordinate rôle in the process. While Ribbert had always contended that a new growth increased simply by the multiplication of its own cells — *aus sich heraus* — Hauser, on the contrary, maintained that it spread by a wave of cancerous degeneration which successively attacked normal structures at its margin.

In this connection the views of Adami[3] are of interest. He described an early multiple tumor of the adrenal cortex " . . . in which occasional cells in the immediate neighborhood of the small mass of new growth, while still retaining their relationship to the columns of the zona radiata, by their enlarged and deep-staining nuclei stood out as of the cancerous type." He quoted, furthermore, certain observations of Horst Oertel upon the apparently direct cancerous transformation of liver cells in a case of multiple hepatic carcinoma, and concluded with the statement: "For my part I regard it as wholly demonstrated that it does occur; and it must be taken into account in the development of any adequate theory of neoplasia."

[1] *Arch. f. path. Anat.*, etc., (Virchow), 1894, cxxxviii, 482.
Beitr. zur path. Anat., etc., (Ziegler), 1897, xxii, 587.
[2] *Das chronische Magengeschwür*, etc., Leipzig, 1883.
Das Cylinderepithel-carcinom, etc., Jena, 1890.
[3] *The Principles of Pathology*, Philadelphia and New York, 1910, Vol. i, 836.

This view has been adopted in part also by Versé,[1] a pupil of Marchand, in so far as concerned carcinomata of the gastro-intestinal tract.

An account of the argument between Ribbert and Hauser may be found in an article by the latter author,[2] whose paper is of additional value because it contains a review of all the cancer literature from 1891 to 1897.

An enthusiastic disciple of Ribbert was Borrmann,[3] who refuted Hauser's interpretation of the spread of carcinoma and quoted Kokubo,[4] Petersen,[5] Pförringer,[6] and himself [7] in support of Ribbert's belief that cancer spread only through the proliferation of its own cells. He ascribed the origin of nearly all tumors to the growth of embryonal rests, and fully supported Ribbert's view that an inflammatory reaction always preceded the abnormal growth of epithelium.

While Borst [8] did not accord such unqualified approval to Ribbert's hypothesis, he nevertheless admitted that its propounder had done no inconsiderable service in calling attention to the many mistakes which had been made in explaining the transformation of normal into abnormal epithelium and by arousing pathologists to the too exclusive attention which had been devoted to the behavior of the epithelium. His own observations led him to believe that an inflammatory infiltration of the corium occurred almost without exception — a true hyperplasia of its connective tissue, however, not so frequently. While both processes were certainly in part secondary to the carcinoma, it could not be denied that inflammation in the subepithelial tissue might frequently afford the special sort of stimulation necessary for the development of such a growth. The initiatory invasion, however, was usually on the part of the epithelium; and although he was at variance with Ribbert on one point, he nevertheless agreed entirely

[1] *Über die Entstehung, den Bau u. das Wachstum der Polypen, Adenome, u. Karzinome des Magen-Darmkanals* (*Arb. a. d. Path. Inst. zu Leipzig*, 1908, Bd. i, Heft 5).

[2] *Centralbl. f. allg. Path.*, etc., 1898, ix, 221.

[3] *Zeitschrift f. Krebsforsch.*, 1904, ii, 1.

[4] *Festschrift für Orth*, 1903.

[5] *Beitr. zur klin. Chir.*, (v. Bruns), 1902, xxxii, 543.

[6] *Beitr. zur. klin. Chir.*, (v. Bruns), 1904, xli, 687.

[7] *Das Wachstum und die Verbreitungswege des Magencarcinoms*, Jena, 1901. (Erster Supplementband, *Grenzgebiete der Medizin und Chirurgie.*)

[8] *Die Lehre von den Geschwülsten*, Wiesbaden, 1902, ii, 686.

with Ribbert's conception of the spread of carcinoma, concluding that the continuous cancerous transformation of normal glands in the vicinity of a fully developed carcinoma of a mucous membrane did not occur.

Janeway[1] expressed the conviction that the great majority of cutaneous cancers were a direct outgrowth from normal epithelial cells and that their development was associated with insignificant changes, or no changes at all, in the connective tissue.

v. Hansemann,[2] extending the observations of Klebs,[3] investigated very thoroughly the types of mitoses found in tumor cells and described three varieties, distinguished by the amount of chromatin contained — hypochromatic, normal, and hyperchromatic. He explained the increased growth energy of the cancer cell by postulating a gain in the power of independent proliferation with a concomitant loss of differentiation; this condition he called *anaplasia*. Extensively keratinized cells in a carcinoma of the skin would be less anaplastic, because departing less from the normal type, than would those of a similar tumor that were entirely free from keratinization, while, similarly, an intestinal carcinoma that still preserved some semblance of glandular arrangement or that contained cylindrical or goblet cells, would be less anaplastic than one composed of polymorphous elements. The anaplasia increased directly as the degree to which the cells were biologically removed from the tissue in which they had originated. It should not be lost sight of, however, that anaplasia was a relative, not an absolute, term — that cells were not of themselves anaplastic, but only in comparison with their mother tissue. Nor should anaplasia be likened to embryonalism from which, on the contrary, it was to be sharply distinguished, for embryonal elements were still undifferentiated, while those in a state of anaplasia had been differentiated at one time but had lost that property. Cells in becoming anaplastic did not necessarily retrace their way over the road by which they had departed from embryonalism, but rather, charac-

[1] *Zeitschrift f. Krebsforsch.*, 1909–1910, viii, 403.

[2] *Arch. f. path. Anat.*, etc., (Virchow), 1890, cxix, 299.
Studien über die Spezificität, den Altruismus und die Anaplasie der Zellen, Berlin, 1893.
Berl. klin. Woch., 1909, xliv, 1850.

[3] *Die allg. Pathologie*, Jena, 1889, Theil 2, 524.
Deut. med. Woch., 1890, xvi, 518.

teristics previously held in abeyance now became able to assert themselves.

One of the criteria of anaplasia was an unequal division of the chromosomes during mitosis; and if this were followed by an asymmetrical division of the cell it must be assumed, for the following reason, that elements of different biological character would result. In the developing ovum each unequal segmentation was followed by a series of equal divisions whose purpose it was to enlarge and perpetuate characteristic cell groups. The stages in development at which these unequal divisions occurred v. Hansemann called *generation stages*. Every generation stage was accompanied or shortly followed by a change in the direction of growth and by a degree of differentiation previously absent as, for example, in the invagination of the vegetative cells of the blastula to form the gastrula, which was the first instance of the process under discussion. To these generation stages each asymmetrical division of a cell was comparable, and it was accordingly accompanied by a change in the energy and the direction of growth.

v. Hansemann did not wish to be understood as considering anaplasia the prime cause of malignant proliferation, since according to his conception a growth stimulus was required in addition. When such a stimulus acted upon a normal cell the result was hyperplasia — upon an anaplastic cell, malignant growth.

Those mitoses were asymmetrical in which the allotment of chromosomes to the daughter stars was unequal, but as the actual number of chromosomes going to each diaster could not be counted except in rare instances, it was necessary to be content with an approximate estimation. Irregular mitoses v. Hansemann believed characteristic of the carcinomata, as he had never succeeded in finding them in sarcomata, or in hyperplastic, regenerating, or normal tissue.

Asymmetrical mitoses had, however, been discovered previously by Podwyssozki [1] in the cells of regenerating liver tissue, and soon after the appearance of v. Hansemann's first article further instances of their presence in cells other than those of carcinomata were reported.

Ribbert,[2] although able to substantiate the occurrence of asym-

[1] *Beitr. zur path., Anat.*, etc., (Ziegler), 1886, i, 301.

[2] *Deut. med. Woch.*, 1891, xvii, 1183.

metrical mitoses in carcinoma, could not agree entirely with the conclusions of v. Hansemann regarding their significance, for he had been unable to distinguish between this type of karyokinesis and other pathological division figures. In later articles Ribbert [1] denied the existence of anaplasia in the sense of v. Hansemann and strongly emphasized his belief that the characteristics of cells were so firmly fixed by heredity that single elements or their ancestors within the same organism could never suffer such a radical change as unequal and undifferentiating segmentation.

Stroebe [2] examined about a dozen carcinomata and found asymmetrical mitoses present in greater or smaller number without exception, but was successful in demonstrating them also in sarcomata, in benign tumors, in healing wounds of healthy tissue, and in the germinal centers of lymph nodes. He suggested, therefore, that they were not peculiar to any special tissue, either pathological or normal, but were to be found in any location where there was a large number of mitotic figures.

Beneke [3] believed that the mitotic anomalies were secondary to a disturbance of specific functional cell power. In the cells of malignant new growths a loss of function was accompanied by a gain in growth power and this condition, *kataplasia*, constituted the only difference between the cancer cell and the normal element. In the history of developing cells there could be no reversion and thus anaplasia, or a return from later to earlier stages through the agency of pathological mitoses, could not possibly exist.

v. Hansemann's critics had attacked him by disputing both the diagnostic value of irregular mitoses and the conception that anaplasia could give rise to malignant growth. His answer was that they had not read, or at least had not understood, the explanations of his hypothesis. To the first criticism he [4] replied that the impossibility of diagnosing carcinomata only by the presence of pathological mitoses had been recognized and emphasized in his papers; but because such

[1] *Deut. med. Woch.*, 1896, xxii, 471.
Bibliotheca Medica, 1897, Abt. C, Heft 9, 32.
[2] *Beitr. zur path. Anat.*, etc., (Ziegler), 1892, xi, 1; 1893, xiv, 154.
[3] *Arch. f. path. Anat.*, etc., (Virchow), 1900, clxi, 100; 1901, clxiii, 174.
[4] *Die mikroskopische Diagnose der bösartigen Geschwülste*, Berlin, 1902, 97, 191.

structures were not specific for carcinoma it did not necessarily follow that the entire hypothesis should be discarded. To take such a course would be "to pour out the child with the bath." In answer to the second objection he repeated that the hypothesis of anaplasia did not concern itself with ætiology.

By far the most perplexing attribute of the malignant cell has been its capacity for unlimited multiplication. As the study of the cell progressed and cytologists became familiar with the events running their course in the fertilized egg, the fact seemed significant that segmentation did not begin until another cell had entered and fused with the ovum after which, however, this element became endowed with the most remarkable proliferative power. An analogy between fertilization and the growth of the cancer cell seemed reasonable and did not escape the observant mind of Virchow,[1] who suggested that because tumor juices appeared to act on certain elements as a fertilizing agent the behavior of these tissues was, to this extent, entirely comparable to that of the ovum.

Leucocytes had often been described between or even in cancer cells and Klebs [2] suggested that they might, by conjugating with the elements of epithelium, excite malignant growth.

Farmer, Moore, and Walker [3] described the occurrence in malignant growths of heterotypical mitoses similar to the ones found in gametogenic cells and differing from those of somatic cells in possessing but one-half the number of chromosomes which, moreover, assumed the forms of rings, loops, etc. They felt justified, therefore, in correlating the appearance of these *gametoid* neoplasms with the result of a stimulus which had altered the normal somatic course of cell development into that characteristic of reproductive (not embryonic) tissue.

v. Hansemann [4] denied the presence of heterotypical mitoses in malignant growths and referred the reduction of chromosomes delineated by these authors to asymmetrical mitoses and the degeneration of occasional chromosomes.

[1] *Die krankhaften Geschwülste*, Berlin, 1863, i, 87.
[2] *Die allg. Pathologie*, Jena, 1889, Theil 2, 524.
[3] *Proc. Roy. Soc.*, 1903, lxxii, 499.
Proc. Roy. Soc., Series B, 1906, lxxvii, 336.
[4] *Biolog. Centralbl.*, 1904, xxiv, 189; 1905, xxv, 151.

While Bashford and Murray [1] at first confirmed the findings of Farmer, Moore, and Walker, dissociating themselves, however, from the conclusions which had been drawn, more extended observation convinced them [2] that the mitoses of cancer were somatic rather than heterotypical and that the heterotype appearance had been conferred by variations in the development of the achromatic figure, the peculiar forms of the chromosomes, and their mode of attachment to the spindle.

Walker and Whittingham [3] have recently reaffirmed the observation of Farmer, Moore, and Walker, adding the statement that the characteristic shapes of the chromosomes belonging to the first meiotic (heterotypical) division had been encountered in very considerable numbers among the cells of malignant growths.

Critzmann,[4] having noticed a relation between twin births and cancer in certain families, suggested that both anomalies resulted from the development of two ova, twin births following the simultaneous fertilization of two ova, cancer the inclusion of one of these within the other. A cancer was thus an abortive fetus retained within a developed one — the brother of him who bore it.

De Morgan [5] concluded that in certain persons there was a local tendency toward tumor formation, impressed upon the tissues perhaps during embryonic life, but lying dormant until finally awakened by irritation. The *slumber cell* hypothesis became more widely known, however, through its adoption by Grawitz,[6] according to whose conception some of the embryonal connective tissue elements lost their cellular nature and were no longer demonstrable with any of the known stains. They remained slumbering in this state, although still taking part in metabolism, until, awakened by stimulus or other cause, they were finally enabled to proliferate.

Beard [7] modified Cohnheim's hypothesis in conformity with his own

[1] *Proc. Roy. Soc.*, 1904, lxxiii, 67.
[2] *Proc. Roy. Soc.*, Series B, 1906, lxxvii, 226.
[3] *Jour. Path. and Bact.*, 1911, xvi, 185.
[4] *Le Cancer.* Thèse de Paris, 1894. Cited by Wolff, 443.
[5] *Lancet*, 1871, ii, 155.
[6] *Arch. f. path. Anat.*, etc., (Virchow), 1891, cxxvii, 96.
Berl. klin. Woch., 1892, xxix, 109.
[7] *The Enzyme Treatment of Cancer*, London, 1911.

views on the course of normal development as an alternation of asexual and sexual generations. He regarded cancer as an attempt of displaced or aberrant germ cells to reproduce the asexual phase of development (the chorion in mammals and its homologue elsewhere), while normally the degeneration of the asexual generation was ascribed to the activity of ferments, especially trypsin, produced by the growing embryo. On this series of assumptions he based his advocacy of the now discarded trypsin treatment of cancer.

CHAPTER II

ATTEMPTS TO PRODUCE TUMORS

THE possibility of inciting cancer in tissues previously normal has attracted and maintained the interest of a large number of experimenters. The recorded attempts are numerous and if, as is probable, there have been still others which, because they miscarried, have not been thought worthy of publication, the number of times that the experiment has been made must be very great. The ingenuity of man has been sorely taxed to find ways of conferring the power of malignant growth upon normal cells and the keenest intellects of two continents have grappled with the question year after year, eternally hoping, yet always baffled. The greater number of trials have been molded to fit one or the other of the two most widely known hypotheses of cancer genesis — the hypothesis of cell irritation and that of cell displacement. Following these two hypothetical clues, investigators of the question of malignant growth have irritated cells or misplaced them in every conceivable way, and still the riddle remains unanswered to us as it did to our forefathers. What relations, if any, obtain between irritation and cell growth can hardly be reviewed here, but those interested in them may find a full discussion of the question in the writings of Virchow, Weigert, Ribbert, and others. Nor can one consider at this point the second hypothesis which, fathered by the great Cohnheim, has become in later years the protégé of Ribbert and his school.

A number of pathologists have sought to initiate malignant growth by introducing the cells of one animal into another. None of them, however, has been able to produce a true neoplasm, and the utter hopelessness of the attempt has been emphasized in recent years by the fact that no malignant new growth has ever been reported among the thousands of mice and rats inoculated with normal tissues for the purpose of conferring immunity toward the transplantation of cancer.

Among the first to attack in this way the problem of the origin of cancer was Zahn,[1] who transplanted the tissues of adult rabbits into other rabbits without a single successful result. When, however, he substituted embryonal cartilage, the grafts proliferated, the amount of growth being proportional to the vascularization of the tissues surrounding them. The transplantation of fetal bone was equally successful.

Leopold,[2] who engaged in similar experiments, also found that while the tissues of young or adult animals were quickly absorbed after transplantation, fetal cartilage would remain alive and grow, occasionally reaching a size which exceeded by two or three hundred times that of the original graft. In this way there was established a true enchondroma which persisted for a considerable period. The younger the embryos, the more vigorous was the growth of their cartilage.

Kaufmann[3] sewed in portions of epithelium of the combs and wattles of fowl, and created in this way cysts inclosed by a giant cell tissue which grew very vigorously for weeks and exhibited no signs of regression even after months had passed.

Martin[4] injected into the jugular vein of a rabbit oil of sweet almonds which had been rendered irritating by the addition of 1 to 2 per cent of croton oil. He discovered some time later the existence of epithelial growths in the lung recalling exactly in their structure the type of epithelioma that Malassez had named *epithelioma muquoïde*.

Hanau,[5] in the course of protracted attempts to produce atypical epithelial growth, painted various preparations of tar upon the scrotum of white rats and upon the vulva, nipple, and mamma (both secreting and non-secreting) of bitches. All of the experiments, however, were entirely barren of result even when irritation was kept up for months.

Considerable interest was aroused for a time through the description of an experiment by Lack,[6] in which he left the scrapings from a

[1] *Congr. internat. des Sci. méd. de Genève*, 1877, 5^{me} Session; *Compt. rend. et Mémoires, Genève*, 1878, 658.

Arch. f. path. Anat., etc., (Virchow), 1884, xcv, 369.

[2] *Arch. f. path. Anat.*, etc., (Virchow), 1881, lxxxv, 283.

[3] *Arch. f. path. Anat.*, etc., (Virchow), 1884, xcvii, 236.

[4] Cited by Ledoux-Lebard, *Arch. gén. de Méd.*, 1885, clv, 423.

[5] *Sitzungsber. d. Gesellsch. d. Aerzte in Zürich vom* 9 *März*, 1889.

Fortschritte der Med., 1889, vii, 338.

[6] *Journal Path. and Bact.*, 1900, vi, 154.

rabbit's ovary in the peritoneal cavity until the death of the animal fourteen months later. At the autopsy, masses of new growth exhibiting the features typical of ovarian cancer were found in the abdomen and thorax. Shattock,[1] however, pointed out that as there was in this animal a uterine tumor which resembled exactly, in the gross, a columnar cell carcinoma which he himself had observed in the uterus of a rabbit, it was at least possible that the growths ascribed by Lack to his experimental measures might have been metastases from a primary carcinoma of the uterus. That such tumors in the rabbit are not uncommon may be inferred from the fact that Boycott[2] has recently reported four cases of epithelial neoplasm in the uterus of this animal, and Leitch,[3] as well as Marie and Aubertin,[4] spontaneous carcinomata in the same organ.

Fraenkel[5] carefully repeated Lack's experiment fourteen times, but always with an unsuccessful issue. He further described attempts to produce a chorion epithelioma by engrafting rabbit placenta into the rabbit from which it had been removed, into other gravid rabbits, into puerperal rabbits, and into one male. But not one of these trials was followed by the outcome that he was trying to procure, nor could he detect any evidence of growth in embryonal ova after their inoculation.

Birch-Hirschfeld and Garten[6] injected very young emulsified embryos into the livers of goats, rabbits, fowl, salamanders, and frogs, without being able to induce the formation of permanent tumors, although nodules of cartilage did grow for a time in the liver and lungs before they were finally absorbed. The embryonal cells underwent a certain amount of differentiation in spite of their unusual environment, and growth in the liver seemed to be favored when that organ was periodically stimulated by gentle heat.

Brosch[7] believed that he had succeeded in obtaining atypical epithelial growth as the outcome of the following experiment: An area of

[1] *Trans. Path. Soc. London*, 1900, li, 56.
[2] *Proc. Roy. Soc. Med.*, 1911, iv, Path. Section, 225.
[3] *Proc. Roy. Soc. Med.*, 1911, v, Path. Section, 1.
[4] *Bull. de l'Assoc. Franç. pour l'Étude du Cancer*, 1911, iv, 253.
[5] *Centralbl. f. allg. Path.*, etc., 1903, xiv, 666.
[6] *Beitr. zur path. Anat.*, etc., (Ziegler), 1899, xxvi, 132.
[7] *Arch. f. path. Anat.*, etc., (Virchow), 1900, clxii, 72.

skin on an animal's back was crushed between the blades of a forceps and a few days later a solution of paraffin in xylol was rubbed into the ulcer which remained after the removal of the scab. Further applications were not undertaken until after the infiltration of the wound had receded. The atypical proliferation was observed from six to ten weeks after the beginning of the experiment.

Stahr[1] noticed that rats fed for a long time on oats would develop papillary epitheliomata of the tongue, and he traced these tumors to the irritation set up by the lodgment of small vegetable fibers. The same author[2] produced areas of chronic irritation in rabbits, rats, and mice by repeatedly scraping a mucous membrane and painting the eroded part with xylol-paraffin, soot, or tar; still, in spite of the chronic lesion thus induced, there had been no evidences of atypical epithelial proliferation.

Kelling[3] denied that the cells of malignant growths originated in the body of the individual in whom the tumor developed, and believed rather that these elements were cells of organisms lower in the phylogenetic scale which had obtained entrance and begun to proliferate. When he submitted this hypothesis to experimental proof he was unable to demonstrate its validity to his satisfaction until the health of the animals had been deliberately undermined before inoculation, or until the injections were made in the neighborhood of wounds. By implanting emulsions of flies, gnats, snails, etc., he had been successful in producing a fibro-sarcoma, an adeno-carcinoma, and a mixed cell sarcoma, and, furthermore, in recognizing in some cases the cells of the organism introduced. In the following year he[4] described the evolution of other malignant tumors in five out of seven dogs, through the inoculation of fowl embryos emulsified in saline solution. None of Kelling's observations have received any widespread acceptance.

Ribbert[5] obtained small but typical papillomatous growths on the inner surface of the rabbit's lip by repeatedly scraping certain points, again denuding them as often as the epithelium was regenerated, and

[1] *Centralbl. f. allg. Path.*, etc., 1903, xiv, 4.
[2] *Münch. med. Woch.*, 1907, liv, 1178.
[3] *Wien. med. Woch.*, 1903, liii, 1431.
Münch. med. Woch., 1903, l, 923.
[4] *Münch. med. Woch.*, 1904, li, 1047, 1909.
[5] *Geschwulstlehre*, Bonn, 1904, 352.

finally allowing them to heal. It appeared that a state of chronic inflammation played an important rôle in the causation of this and other types of fibro-epithelial tumors, for, as Ribbert remarked, when irritation and inflammation had ceased tumors of this class not infrequently retrogressed.

Loeb[1] injected or transplanted portions of embryos into adult animals with the general result that most of the tissues not only grew, but even underwent a certain amount of differentiation. Thus epithelial cells arranged themselves in glandular form and could be seen to contain granules denoting cell activity. As in the experiments of Zahn and Leopold, the most pronounced growth was observed in the case of cartilage. The implantation of adult tissues afforded analogous results except that growth on the part of cartilage was not so marked. With tissues other than cartilage embryonal cells did not proliferate more actively than those from animals fully grown, and, indeed, in some cases the adult elements seemed to grow even more vigorously than the others. The data thus acquired did not, therefore, justify the widely accepted idea that embryonal tissues were greatly superior to adult in their power to proliferate after transplantation. In all of the experiments the displaced cells finally ceased to grow and eventually underwent absorption.[2] The same author[3] succeeded in producing, in the uterus of the guinea-pig, nodules of decidual tissue possessing the power of temporary growth. Incisions were made into the pregnant uterus, sometimes in various directions, while at other times part of one horn was split longitudinally and the mucous membrane everted. When the uterus was thus incised on the fifth or sixth day of pregnancy there developed in the wounds a number of nodules composed of typical decidual tissue. Immediate contact with the ovum was evidently not necessary, for serial sections of a number of these "deciduomata" were searched in vain for embryos. In experiments prosecuted during earlier or later stages of pregnancy decidual nodules were not evolved, although small ones could still be produced as late as the tenth day, and in one case followed an operation undertaken during the first two

[1] *Jour. American Med. Assoc.*, 1903, xl, 974.

[2] It is not quite clear whether these experiments were done by Loeb himself, or whether the author is recapitulating the work of others.

[3] *Centralbl. f. allg. Path.*, etc., 1907, xviii, 563.

days. From three to four weeks after operation the nodules were partly or entirely necrotic.

Commenting on the relation between these nodules of decidua and the malignant tumors, Loeb[1] said that during the early period of its existence the corpus luteum secreted a chemical body which united with the mucosa of the uterus and sensitized it. When incisions were made into the uterus the freeing of its inner surface from tension acted as an external stimulus which caused the sensitized tissue to react with the production of tumor-like formations, where under ordinary conditions the uterus would have shown only the usual processes of wound healing. For the initiation of malignant growth, therefore, one factor alone did not always suffice. There were necessary a chemical sensitizing substance and an external exciting cause — facts which must be kept in mind in attempting to interpret the origin of cancer.

The experiments of Wilms[2] concerned the implantation of five-to-seven-day-old embryo chicks into young fowl, a procedure which was followed by considerable growth and differentiation. In trying to overcome the reaction against the newly introduced cells, by making several successive inoculations at intervals of eight days, Wilms gained the impression that more energetic development took place. There seemed to be a certain disposition favorable to growth in one of the birds, for all of the grafts had developed into palpable nodules at a time when those in five other birds were still indistinguishable. As in the experience of other investigators, so there occurred here a certain temporary growth and differentiation of the embryonal tissue in spite of its new surroundings, succeeded by final regression and entire absorption.

Careful and extensive experiments of the same type were undertaken by Nichols[3] to determine whether normal tissue could acquire after transplantation the power of unlimited growth and give rise to metastases. Rabbits and guinea-pigs, sixty-two in all, were inoculated with the cells of various organs but always with an unsuccessful issue, at least in so far as unlimited proliferation and the production of metastases were concerned, although occasionally the implanted structures were possessed of enough proliferative energy to give origin to growths re-

[1] *New York Med. Jour.*, 1909, xc, 145.
[2] *Verhandl. d. deutschen path. Gesellsch.*, 1904, 8te Tagung, 79.
[3] *Jour. Med. Research*, 1904–1905, N.S., viii, 221.

sembling dermoid cysts or teratomata. The materials used included testis, ovary, kidney cortex, liver epithelium (adult and fetal), adrenal gland, adult or fetal epidermis, adult uterine epithelium from pregnant and non-pregnant animals, fetus (entire), fetal cartilage, and placenta. Among all these tissues only adult and fetal epidermis, uterine epithelium, entire fetus, cartilage, and placenta were capable of proliferation; these produced tumors which varied in size from a small nodule to one exceeding by ten or twenty times the amount introduced, but not one of which was at all comparable to a true malignant growth.

In an endeavor to create tumors from normal cells already possessing, or supposed to possess, high proliferative power, Levin[1] implanted or injected rabbits with cells from the ovary, with embryonic cells or those from healing wounds in the adult liver, with epithelium, and with pigmented cells from the iris. In no case, however, could he convince himself that any of the elements so transplanted had taken on malignant growth.

Reinke[2] recorded an example of atypical epithelial growth which he had procured by the injection of 4% ether into the eye of the adult salamander. The proliferating epithelium of the crystalline lens thus obtained was transplanted intraperitoneally into other salamanders, where it continued its growth, lost its similarity to lens epithelium, and finally came to resemble carcinoma. Reinke had found, also, that the mitosis educed by treatment with ether could be inhibited. He rubbed down salamander lens with ether, which he poured off and allowed to evaporate, afterward making a saline emulsion of the residue so gained. This he inoculated into the eyes of salamanders that had undergone an injection of 4% ether eight days previously and found subsequently no mitoses, or only a few at the most. The conclusion was accordingly drawn that there was present in the tissues a material which inhibited mitotic division and which could be extracted with ether. The continued action of ether upon normal cells dissolved this substance or altered it in such a way as to permit the occurrence of mitotic division or atypical growth; but if the material were supplied

[1] *Jour. Med. Research*, 1901, N.S., i, 145.

[2] *Deutsche medizinal Zeitung*, 1907, xxviii, 579.
Münch. med. Woch., 1907, liv, 2381.

in sufficient amount, it could be taken up anew by the cells and again exert its inhibitory action upon division.

Askanazy,[1] stimulated by these experiments, mixed emulsions of rat embryo with 4% ether before inoculation, and obtained a series of large rapidly growing teratoid tumors such as he had never seen following the inoculation of untreated embryos, even in pregnant animals. The fat stain, Scharlach R, which Fischer believed capable of exciting epithelial growth exerted, on the contrary, an inhibitory effect upon the proliferation of embryonic tissue.

Freund[2] succeeded in producing teratoid tumors in 74% of rats that had been inoculated intraperitoneally with an emulsion of rat embryo. The age of the injected rats was immaterial, but the results were more uniformly successful in females. Previous treatment of the embryo tissues with ether water or with solutions of indol with or without ether had no effect upon their subsequent proliferation, nor was growth any more vigorous after autoplastic than after homoplastic transplantation. Finally, tumors followed the intraperitoneal inoculation of animals in which subcutaneous injection had been unsuccessful, a fact proving that resistance had not been conferred by the single antecedent unsuccessful treatment.

v. Hippel[3] observed the evolution of a teratoma from the head of a twelve-day-old rabbit embryo which had been injected as a saline emulsion into the eye of an adult rabbit. Six weeks after the beginning of the experiment, the tumor had reached a length of 1 centimeter and a thickness of 0.8 centimeter and was composed of elements of the outer and middle germinal layers, but contained none of the tissues of the hypoblast.

Embryos were transplanted into adult animals by v. Tiesenhausen[4] also. In his earlier experiments he made use of mammalian material, but in later investigations he employed the chick, because of the readiness with which embryos of known age could be procured from artificially incubated eggs. About one hundred and fifty inocu-

[1] *Wien. med. Woch.*, 1909, lix, 2518, 2578.
Centralbl. f. allg. Path., etc., 1909, xx, 1039.
[2] *Beitr. zur path. Anat.*, etc., (Ziegler), 1911, li, 490.
[3] *Verhandl. d. deutschen path. Gesellsch.*, 1906, 10te Tagung, 56.
[4] *Arch. f. path. Anat.*, etc., (Virchow), 1909, cxcv, 154.

lations were made into young and old fowls at different sites with chicks varying in age from one to eight days. Five-day embryos gave the best results, whereas inoculation with those less than two days old was attended by a uniformly negative outcome. The sites chosen for implantation included the anterior and posterior chambers of the eye, the brain, the pectoral muscles, the anterior abdominal wall, the great omentum, the peritoneal cavity, the comb, and the wattles. In no case was progressive growth obtained, even in the birds from which the chick embryos had been derived, nor were any metastatic deposits observed. Growth went on actively for about eight weeks, but gradually ceased after from six to twelve months.

Fichera[1] inoculated rats subcutaneously with a bouillon suspension of rat embryos, causing nodules which grew progressively until, at the expiration of two or three months, they were at least twenty times the original size. But by the end of six months their volume had greatly diminished, and they could not be considered in any sense as malignant new growths. When several successive inoculations had been made grafts became necrotic as early as the tenth day.

The question whether pregnant animals might not perhaps offer a more favorable soil for the growth of embryonic tissue has not escaped investigation. Askanazy[2] and his pupil Jentzer[3] thought that pregnancy in the host exerted a distinctly adjuvant effect upon the growth of experimental teratomata in white rats, and Féré[4] noticed increased growth in brooding hens; but Shattock, Seligmann, and Dudgeon,[5] on the contrary, did not find that the proliferation of ingrafted fetal cartilage was stimulated in rabbits that repeatedly bore young.

Rous[6] cited certain investigations of Fichera[7] which seemed to establish the fact that embryonic grafts grew better in the rats from which they had been removed than they did either in non-pregnant or in other pregnant rats. The observations of Rous, which were

[1] *Arch. de Méd. exp. et d'Anat. path.*, 1909, xxi, 617.
[2] *Verhandl. d. deutschen path. Gesellsch.*, 1907, 11te Tagung, 82.
[3] *Rev. méd. de la suisse Romande*, 1908, xxviii, 329.
[4] *Compt. rend. Soc. Biol.*, 1899, li, 824; 1900, lii, 737.
[5] *Proc. Roy. Soc. Med.*, 1909–1910, iii, Path. Section, 132.
[6] *Proc. Soc. Exp. Biol. and Med.*, 1909–1910, vii, 71.
Jour. Exp. Med., 1911, xiii, 248.
[7] *Policlinico, Sez. pratica*, 1909, xvi, 692.
Etiologia del cancro, Rome, 1909.

undertaken independently of Fichera's, demonstrated that embryonic mouse tissue obtained at operation and implanted in the mother would grow well if she no longer carried young; and although growth was no more rapid than in favorable non-pregnant aliens, yet it persisted for a longer period and resulted in a greater variety of tissues. On the other hand, when a mouse still bearing embryos was implanted with embryonic tissues from her own uterus the grafts failed either to grow or to differentiate, although they did become vascularized. Still, they did not die, and sometimes proliferation was inaugurated after the conclusion of pregnancy.

Freund[1] concluded that pregnant rats offered a somewhat more suitable soil for the growth of embryo emulsions than did non-pregnant females, although the difference amounted to but 2 %.

Rous[2] investigated in mice the behavior of embryonal cells mixed with tumor, hoping that as some transplantable carcinomata were able to originate sarcomatous transformation in their stroma, so close association with tumor cells might cause embryonic tissue to assume malignant properties. He found that growth of both components took place and that there was often an intimate histological association of the two; but for the occurrence of these conditions a balance of avidity was necessary, otherwise the embryonic cells were necessarily soon overgrown by those of the more rapidly proliferating tumor. It seemed, therefore, that the enormous proliferative capacity exhibited by embryonic tissue *in utero* depended at least as much upon the excellent nutritive arrangement as upon inherent cell energy. In a mixed graft that had only partially succeeded, tumor and embryo tended to grow or to fail together, while in a number of quantitative experiments it was found that tumor and embryo proliferated better alone than when mixed.

Gougerot and Laroche[3] obtained a keloid in a tubercular guinea-pig at the point where an inoculation of tuberculin had been made. The ridge of growth was twenty-five millimeters long and five millimeters wide, appeared to involve the needle track, and when excised

[1] *Beitr. zur path. Anat.*, etc., (Ziegler), 1911, li, 490.
[2] *Proc. Soc. Exp. Biol. and Med.*, 1909–1910, vii, 73.
Jour. Exp. Med., 1911, xiii, 239.
[3] *Compt. rend. Soc. Biol.*, 1908, lxv, 342.

and examined, was found to consist of collagenous fibers inclosing a few cells. This case was the sole success in a long series of experiments upon fourteen guinea-pigs.

The development of sarcoma in a rat that had been exposed repeatedly to X-rays was reported by Clunet.[1] Fragments of the growth were transplanted into eight rats, in one of which there was found a nodule the size of a pea about two months after inoculation. Recession, however, set in immediately, and two months later the tumor had disappeared entirely.

Considerable discussion has ensued upon Fischer's description[2] of a series of experiments in which he had succeeded in producing lesions imitating very closely the histological structure of carcinoma. After having failed to produce a suitable irritation by means of agar impregnated with calcium salts he tried a saturated solution of the stain Scharlach R in olive oil, injecting it into the ears of rabbits just beneath the epithelium and under considerable pressure. After a few days an augmented number of mitoses, of which a few were atypical, was detected in the germinal layer of the epithelium, hair follicles, and sebaceous glands, and there was an increase in the thickness of the whole epithelial layer with an attendant over-production of keratin. In later stages the epithelial proliferation was still more marked, hair follicles and sebaceous glands had either disappeared or become strands of squamous epithelium, and these structures then began to invade the underlying parts. When the intrusions reached the particles of oil lying in the connective tissue the epithelium grew around the globules in an irregular way, producing a lesion which in appearance was comparable only to a squamous cell carcinoma in man. Such areas could not, however, be called true epitheliomata because they were not destructive and because, furthermore, growth did not continue after the oil had been absorbed. It was found that the two fat stains Sudan III and indophenol, chemically unrelated though they were, exerted a similar action. A series of experiments on the epithelium of the stomach, intestine, and mamma, had been inaugurated, but at the time of writing the subepithelial inflammation resulting from

[1] *Recherches exp. sur les Tumeurs malignes*, Paris, 1910, 297.

[2] *Verhandl. d. deutschen path. Gesellsch.*, 1906, 10te Tagung, 20, 22. *Münch. med. Woch.*, 1906, liii, 2041.

the inoculation of solutions of Scharlach R produced no trace of epithelial growth in these locations. Other solutions of the dye were investigated, among them a saturated ethereal solution which was injected into four of the mammary glands of a rabbit. In all four, numerous lobules were transformed into islets and strands of squamous epithelium of exactly the same shape as a gland lobule and connected with the duct. It seemed most probable that the parenchyma had been destroyed and then replaced through regeneration (often of duct epithelium), and that during the process there had resulted the substitution of squamous epithelium for gland lobules. No such lesions were to be found in the mammæ that had not been injected. To explain the action of Scharlach R Fischer suggested that there might be a specific substance — an *attraxin* — contained in it, able to exercise a positive chemotactic influence on epithelial cells. The growth of the epithelium about the oil globules could, at any rate, hardly be explained by the well-known disposition of epithelium to grow along a free surface, for it did not take place about collections of other oils and fats; and, moreover, if Scharlach R exerted only an ordinary stimulus upon the epithelium it was hard to see why the effects above set forth did not follow its external application, even when this was continued for months. The demonstration of a chemotactic action on the part of the dye seemed to Fischer, therefore, the most significant feature of the experiment, and pursuing his hypothesis further he interpreted the limitless growth of malignant tumors as the product of a hypothetical attraxin occurring naturally and exerting its influence upon embryonal cell rests.

McConnell,[1] in repeating these experiments with rabbits, Belgian hares, guinea-pigs, and white rats, found that Belgian hares were on the whole the most suitable animal in which to reproduce the lesions described by Fischer.

Jores[2] published a general confirmation of Fischer's results in which, however, he laid somewhat more stress upon the rôle which was played by the germinal epithelium of the hair follicles. Indeed, when moderate pressure had been employed the outcome of the injection was mainly a proliferation of this layer, and the hypertrophy of the surface epi-

[1] *Jour. American Med. Assoc.*, 1907, xlix, 1498.
[2] *Münch. med. Woch.*, 1907, liv, 879.

thelium occurred only when the oil had been injected under greater pressure. The sebaceous glands, at first unaffected, were gradually lost to view amid the encroaching hair follicles. If the epithelial growth exceeded a mere thickening of the hair follicles a lesion of cancerous appearance was the result; still, such a condition was difficult of attainment and Jores had seen it only once. He had never been able to reproduce it in scar tissue even after repeated injections, possibly because of the absence of hair follicles. The process as a whole seemed to be the sum of two components — action by the dye upon the upper part of the hair follicles, and proliferation of the epithelium which lay in direct contact with the oil globules. The latter phenomenon one might be tempted at first to refer to the tendency of epithelium to grow over any surface. But particles of Scharlach oil were more readily inclosed than other foreign bodies, a fact which, in conjunction with the characteristic action of the stain upon the hair follicles, led the author to assign to the dye an actual influence upon squamous epithelium, although it might not be chemotactic. It was very unlikely, too, that Scharlach R exerted any real stimulus to growth, and the proliferation attending its injection might be considered with more justification as a replacement process exceeding, in the manner described by Weigert, the bounds of physiological regeneration.

Helmholz[1] confirmed and extended the experiments begun by Fischer, and proved that the effects of Sudan III and Scharlach R were exercised not only upon various types of epithelium but also upon those connective tissues which produce cartilage. He did not adopt, however, the conclusions which Fischer had drawn, and expressed the belief, not that the dyes possessed a specific attraxin, but rather that they contained some substance which, by interaction with connective tissue, produced a soil appropriate for epithelial development.

In contrast to the foregoing workers Snow[2] could not substantiate Fischer's observations, although it should be noted that his injections were not made under the high pressure upon which Fischer had insisted.

[1] *Johns Hopkins Hosp. Bull.*, 1907, xviii, 365, 369.

[2] *Jour. Infect. Diseases*, 1907, iv, 385.

Stahr,[1] on the other hand, was able to corroborate Fischer, but only by following strictly the technic which that author had described; wherefore he emphasized the necessity of injecting the oil under very high pressure. He found that atypical epithelial growth would follow even a single injection of a saturated solution of Sudan III in olive oil. While Stahr's results were thus confirmatory, he disagreed with Fischer's theory of a specific chemotactic action and was inclined to refer the lesions to the co-result of a stimulus plus a complex of conditions, not the least among the latter being the anatomical structure of the tissue into which the dye had been injected.

Wyss[2] had concluded from observations on X-ray carcinomata that such tumors were consequent upon altered nutrition, and this upon a narrowing or obliteration of the subepithelial blood vessels supplying a certain group of cells. Areas deprived of nourishment in this way underwent proliferation and finally became parasitic on the neighboring tissues. Wyss believed that the proliferation described by Fischer was not due to chemotactic influences but to a withdrawal of normal nutrition through the obliteration of blood vessels, and emphasized the fact that the most striking results were seen after injection had been made at high pressure.

Levin[3] investigated in white rats the action of a saturated solution of Scharlach R in paraffin or oil when injected subcutaneously or into the mamma. Only in the connective tissue elements could any proliferation be demonstrated, an observation well in accord with a previous finding by the same author,[4] that the rat was prone to vigorous reactions on the part of its connective tissues.

Seyberth[5] confirmed the observation of Rehn, that tumors of the bladder were not infrequent among those who worked in aniline dye factories, and recorded five such cases, three non-malignant although showing excessive epithelial proliferation, and two malignant. Of the latter, one was an adeno-carcinoma and the other a carcinoma. These growths, the author believed, were all without doubt the prod-

[1] *Münch. med. Woch.*, 1907, liv, 1178.
[2] *Münch. med. Woch.*, 1907, liv, 1576.
[3] *Jour. Exp. Med.*, 1908, x, 815.
[4] *Med. Record*, 1907, lxxii, 974.
[5] *Münch. med. Woch.*, 1907, liv, 1573.

uct of one cause, namely, chronic stimulation of the bladder wall by urine containing aniline bodies, and Fischer's experiments had thrown a significant light upon the relations between aniline dyes and epithelial proliferation.

Stoeber[1] experimented with certain components of Scharlach R and Sudan III, all of which were found to elicit epithelial proliferation. Some of these, however, which were toxic, and others which caused necrosis, were of course unsuitable for further investigation. Contrary to Fischer's belief the chief source of the new epithelium was not the surface layer but, as Jores had contended, the germinal epithelium of the hair follicles. The same writer[2] recorded the next year an experiment in which oily solutions of Scharlach R, amidoazotoluol, and *a*-naphthylamine had been inoculated into the foot of an old man with ununited fracture of the leg, before amputation. When this was performed fourteen days later it was found that Scharlach R and amidoazotoluol had induced epithelial changes identical with those evolved in the rabbit's ear. They were not so extensive, however, partly because hair follicles and sebaceous glands were only sparsely distributed in the inoculated region. The overlying epithelium was thickened, and the down-growing projections appeared to have been derived from thickened hair follicles and the ducts of sweat-glands. No sebaceous glands were recognized in the sections.

In conjunction with Wacker,[3] Stoeber sought to discover what effect would follow the injection of various products found in the body under physiological or pathological conditions, and especially of such as resulted from the splitting and decomposition of albumins. It was apparent that only organic substances of a basic nature and soluble in fats, had any definite action upon epithelium. Employing Fischer's technic, but using small amounts and making two injections with two or three days intervening, the authors obtained epithelial proliferation with 2 and 5 % solutions of pyridin in olive oil, and with 5 % indol or skatol in rabbit fat. The inoculation of indol solution followed by a small amount of skatol produced a tumor which attained the size of a hazel-nut within fifteen days. The histology of the lesions provoked by pyridin were less characteristic than in the case of indol and skatol.

[1] *Münch. med. Woch.*, 1909, lvi, 129. [2] *Münch. med. Woch.*, 1910, lvii, 739.
[3] *Münch. med. Woch.*, 1910, lvii, 947.

The picture produced by the two last mentioned was similar to that of human squamous cell carcinoma, so similar, indeed, that it would have been difficult for even the most skilled diagnostician to distinguish one from the other.

Meyer[1] injected Scharlach oil into the renal arteries, under the capsule, and into the parenchyma of the kidney, in a series of rabbits and dogs, but without being able to produce any effect upon the renal epithelium. In certain other experiments, in which inoculations had been made under the skin, he found that when a vein or an artery had been ligated the action of the stain was hastened, and that under these conditions even oil alone, or oil and paraffin, would inaugurate epithelial proliferation. The growth of epithelium in the rabbit's ear was, therefore, according to this author, due to chronic inflammation associated with other circulatory disturbances.

This view was challenged by Greischer,[2] who, working with Schmincke, induced anemia in one ear of each of a series of rabbits by ligating the carotid, arterial hyperemia in others by cutting the cervical sympathetic, or finally, venous hyperemia by the ligation of veins at the base of the ear. The lesions accompanying inoculation of the operated ear were always the same as those obtaining in the normal one of the opposite side, and epithelial proliferation could never be discovered in the prepared ear when it had not occurred in the normal one. Disturbances of circulation, therefore, played a rôle only in so far as they shortened or protracted the length of time during which the injected material could act upon the epithelium.

White[3] introduced oleic acid, either pure or with the addition of carbon dioxide or methyl oxalate, into the ears of rabbits, the mammary glands of guinea-pigs, and the backs of mice. Notwithstanding the fact that his findings corresponded so exactly with those of Fischer that, as he himself said, the illustrations published by that author might have been prepared from his own specimens, White could not find the slightest evidence of any chemotactic influence. Thus, there was no intrusion by the epithelium, and hair follicles outside the inflam-

[1] *Beitr. zur path. Anat.*, etc., (Ziegler), 1909, xlvi, 437.
[2] *Inaugural Dissertation*, München, 1911.
Münch. med. Woch., 1911, lviii, 1608.
[3] *Jour. Path. and Bact.*, 1910, xiv, 450.

matory zone remained entirely unaltered; and although it was true that epithelial cells lined the abscesses, this was merely an expression of the normal tendency of these elements to grow over a free surface and could not be attributed to any specific property of the fatty acids employed. The injected materials appeared to act merely as chronic irritants.

Although White's results were negative with oleic acid in liquid paraffin and with a mixture of oleic acid and cholesterin, a positive action was claimed for these substances by Wacker and Schmincke.[1]

Benthin[2] injected the ears of animals, usually rabbits, with paraffin, olive oil, agar, agar with calcium carbonate and calcium phosphate, Sudan III, Scharlach R, indophenol, amidoazobenzol, paratoluidin, amidoazotoluol, *a*-naphthylamine, indol, skatol, paraffin with soot, gum arabic alone and with Sudan III, oleic acid, and glycerine. No growth was obtained after olive oil, agar, or gum arabic alone, and none following agar with calcium carbonate or calcium phosphate, while the injection of paraffin and soot gave rise only to epithelial cysts. Oleic acid, glycerine, paratoluidin, and amidoazobenzol were unsuitable, for, as Stoeber had already pointed out, they caused too much necrosis. The most marked growth accompanied the use of Sudan III and Scharlach R, and Fischer's statements regarding the action of these dyes were verified by Benthin, who, nevertheless, did not believe that the far-reaching conclusions drawn by that author could be justified. The growth of epithelial cells around the oil droplets seemed to occur only when there was contact between the two, and deeply lying masses of oil, even though of considerable size, did not become inclosed by epithelium. The author ascribed the epithelial proliferation to a complex of causes among which inflammation, tissue tension, perhaps circulatory disturbances, and, most important of all, the presence of certain chemicals, were jointly responsible. It was noticed that wounds following operative removal of the injected areas healed with thick keloid-like scars as though the fat stains had the property of stimulating growth.

Wacker and Schmincke,[3] reviewing the various materials used by themselves and others to educe epithelial proliferation, pointed out

[1] *Münch. med. Woch.*, 1911, lviii, 1607.
[2] *Zeitschrift f. Krebsforsch.*, 1910–1911, x, 227.
[3] *Münch. med. Woch.*, 1911, lviii, 1607.

that all which had afforded a positive result had this factor in common, that they possessed the solubilities of lipoids — produced, that is, a physical change in the lipoid membrane of the epithelial cell. Still, a few materials were unable to inaugurate proliferation, possessed of this property though they were. That the stimulating action of the various agents was not primarily a chemical process, in the sense of a splitting up of the lipoid membrane surrounding the cells, the authors inferred from the following experiment. Bergel[1] having already demonstrated the presence of a fat-splitting ferment in lymphocytes, Wacker and his associate injected the ears of rabbits with emulsions of rabbit spleen, lymph nodes, or pleural lymphocytic effusions, without, however, being able to find any evidence of the epithelial growth which should have taken place were lipoidolysis a factor in its initiation.

McConnell[2] tested the question whether Sudan III dissolved in oil would exert any influence upon the cells of an epithelial tumor. Human carcinoma was suspended in saline solution and injected into sixteen white rats, of which eight were subsequently inoculated at the same site with three minims of Sudan oil. No growth took place either in the inoculated animals or in the controls.

Werner[3] investigated the effect of Scharlach R upon growing mouse tumors and found that concentrated solutions in oil excited growth, while alcoholic solutions caused the tumor at first to shrink and finally to slough away. The excitation of growth caused by the oily solution was not the result of chemotactic influences, but of a true growth stimulus.

Powdered Scharlach R was added to tumor emulsions before inoculation by Albrecht and Hecht,[4] to see whether the vigor of growth would be augmented. In contrast to Werner's findings regarding tumors already established, the dye neither incited the cells to increased proliferation nor did it inhibit their growth.

A review of certain aspects of the more recent attempts to establish malignant growth has been published by Herxheimer and Reinke.[5]

[1] *Münch. med. Woch.*, 1909, lvi, 64.
Münch. med. Woch., 1910, lvii, 1683.
[2] *Jour. Med. Research*, 1908, N.S., xiii, 381.
[3] *Münch. med. Woch.*, 1908, lv, 2267.
[4] *Centralbl. f. allg. Path.*, etc., 1909, xx, 1039.
[5] *Ergebnisse der allg. Path.*, etc. (Lubarsch and Ostertag), Abt. ii, 1909, xiii, 416.

The effect of ether upon the cells of the salamander, discovered by Reinke, corresponded entirely with experiments in plants where it had been found that hibernation could be overcome with ether, and premature blossoming brought about. It was further parallel with hemolysis, which was due to solution of the lipoids of red blood cells, and Reinke had therefore suggested that *blastosis*, or stimulation to mitotic cell division, might perhaps be interpreted as the final outcome of a melting, saponification, or solution of lipoids in the cells and their nuclei, taking place in the same way as in hemolysis. The suggestion was strengthened by Askanazy's observation of the increased growth power of embryo rat tissue after exposure to ether. Somewhat later, and independently of Reinke, J. Loeb had reached similar conclusions, deciding that it was the fat-dissolving properties of the substances with which he was working that enabled him to achieve artificial parthenogenesis. Furthermore, Scharlach oil and the affiliated substances that had been utilized by Fischer and his successors were also solvents of lipoids, a circumstance that could be correlated with Overton's theory of a lipoid ectoplasm. Although any serious injury to the cell was followed by cytolysis, as could be demonstrated most easily through the application of heat, if the action of this agent were not so intense as to lead to destruction the protoplasm became more active, metabolism was increased, ferments were set working, and latent vital processes were awakened. Ether caused a change in the condition of those important elements, the lipoids, whose rôle in cell division could be readily harmonized with Weigert's conception of proliferation, according to which tissue injury was interpolated between interference with the cell and subsequent growth. Herxheimer and Reinke could see no reason, therefore, to abandon Weigert's view that there could be no direct stimulation of cell growth, and it seemed justifiable to accept with him the existence of an external functional stimulus, but to refer growth to a preceding injury.

CHAPTER III

EARLIER OBSERVATIONS ON THE TRANSMISSIBILITY OF CANCER

LONG before it was realized that the question of the transmissibility of cancer could be approached through experiment upon the lower animals, its solution had been attempted by means of the much less exact method of clinical observation; and although numerous instances of transfer of the disease have been recorded, most of them so seriously exceed the limits of credulity that the occurrence of cancer in the two patients may be attributed to chance.

Butlin[1] has laid down with admirable clearness certain conditions which reasonably may be demanded before it is agreed that any given case illustrates the transmission of malignant disease from one person to another. "... all cases of reported contagion of cancer in which the disease is not of the same variety must be unhesitatingly rejected; ... cases must not be accepted where there is no evidence that the affected parts of the two individuals were from time to time in contact; ... it is extremely improbable that contagion should take place from a covered cancer, such, for instance, as a cancer buried in the breast; or that the disease could be implanted in a part the covering of which has not been broken."

A few cases of the reputed transmission of cancer may now be examined to see in how far they conform to Butlin's requirements.

TRANSFER FROM ONE PERSON TO ANOTHER

Accidental

Zacutus[2] reported the following case, perhaps the first to appear in the literature. "A poor woman who had suffered for many years from an ulcerated cancer of the breast, slept at night on the same

[1] *British Med. Jour.*, 1907, ii, 255.

[2] *Praxis Medica Admiranda*, p. 31, obs. 124 (Appendix to Opera, tome ii, Lugduni, 1649).

couch with her three sons. All three became affected with a like disease. Five years after the death of the mother, two of the sons died; but the third, being more robust, recovered with difficulty after having submitted to excision of the cancer at the hands of a surgeon."

Tulpius [1] expressed surprise that there should exist any doubt as to the contagiousness of ulcerating cancer, and related a case that had come within his own experience. "Adriana Lambert, a woman advanced in years, was afflicted with a cancer of the breast that had advanced to such a putrid condition as to infect by its exhalations her body-servant who attended upon her, living in close contact with the mistress. Some evil spark lighted such a conflagration as to destroy the maid no less than her mistress, for such a foul, irregular ulcer invaded the breast and armpit of each that I am undecided which of these two was tortured with the greater savagery."

Juncker [2] believed that cancer was contagious, but not virulently so unless it were implanted in situations suitable for its reception, and especially in a place where solution of continuity of the tissues had been effected.

That there existed in Juncker's day a popular belief in the contagiousness of cancer is shown by an episode which Guelliot [3] has reported. Cancer patients having been refused admission at the Hôtel Dieu in Reims, Jean Godinot, a canon celebrated for his charity, offered the sum of twenty-five thousand livres for the construction of a hospital for such patients, who until then had been able to subsist only through what they could gain from compassionate worshippers by exposing their sores at the church door. A house was bought in the center of the town and opened as the Hôpital Saint-Louis, and here cancer patients were cared for, but on condition that they were not to leave the building. The neighbors, however, complained that, as cancer was contagious, the health of the public would be endangered if such an establishment were tolerated in a populous quarter, and the administration was finally compelled to transport its charges to a lazaret formerly used for victims of the pest. It was not until 1841

[1] *Observationes Medicae*, Amstelredami, 1672, editio nova, 292.
[2] *Conspectus Chirurgiae tam Medicae*, Halle, 1721, 327,
[3] *Gaz. des Hôpit.*, 1892, lxv, 1210.

that cancer cases were admitted to a general hospital, and even then they were kept in a separate ward.

Viel-Hautmesnil [1] reported the three following cases as apparent instances of the transmission of cancer. Mr. Smith, of St. Thomas's Hospital in London, tasted the contents of a small cyst in a mammary cancer which he had just removed. The acrid taste remained permanently in his mouth, and he became subject to attacks of vomiting which ultimately determined his death. A doctor was said to have died of cancer which had apparently been communicated to him by his wife. The third example was a case reported by Tulpius, concerning a surgeon whose wife suffered from mammary cancer. Accustomed to apply his mouth and tongue to the affected part in order to relieve the intense pain, he soon afterward died of a terrible cancer which destroyed the interior of his mouth.

The foregoing cases of transference are interesting, of course, only in so far as they show the age of the question now under discussion, for before the time when the microscope had come into general use the diagnosis of cancer was often so highly problematical that reports of transmission of the disease may be dismissed with scant curtesy. Still, even after the importance of histological diagnosis had been established, examples of contagion continued to be described, most of them, however, on a par with the earlier instances through the neglect of the observers to advance adequate microscopic justification for their claims.

Budd [2] has related the case of a strong and healthy girl of nineteen who regularly washed dressings and linen soaked with the discharge from an advanced case of uterine and vaginal cancer. Six months after the death of the patient the young woman was admitted to the hospital with a large axillary cancer, which eventually proved fatal. Budd noted, further, that five surgeons of the same institution had died of cancer within a few years, and expressed the opinion that such a mortality could hardly be conceived except on the supposition that the disease had been communicated, at least to some of them, during manipulations on patients suffering from cancer.

Whitehead [3] had under his care a man with an extensive epithe-

[1] *Consid. gén. médico-chir. sur le Cancer;* Thèse de Paris, 1807, 24.
[2] *Lancet,* 1887, ii, 1091. [3] *Lancet,* 1887, ii, 1040.

lioma of the lower lip, attributed by the patient to his habit of drinking daily out of a small vessel used by his father, who had died during the previous year of the same disease.

Adam [1] described the case of a medical man who married a woman subsequently found to be the victim of mammary and uterine cancer, from which she soon afterward died. Within a short time the widower had developed cancer of the liver and stomach, but before his death he married again, and a year or two afterward it was discovered that his widow was the subject of cancer of the breast.

Smith [2] reported a woman with sloughing carcinoma of the mamma, whose husband was attacked by cancer of the stomach, and Behla [3] collected fourteen examples of contagion which had occurred among patients under his own observation. The first in the series was a man of seventy-six who died of carcinoma of the kidney three years after his wife had died of cancer of the liver. The other thirteen instances were similar. To these cases of *cancer à deux*, Behla added one of *cancer à trois* which had been communicated to him by Elsler. Herr B, who died of carcinoma of the rectum, was nursed by his son-in-law who for six months administered daily nutrient enemata. A short time after the death of the patient the son-in-law developed a carcinoma of the lip, and during his illness a cancer of the breast was discovered in his wife.

The following examples of "cancer houses" have been drawn from Behla's monograph.[4]

In the village of Breitenbach, with a population of four hundred and fifty, four persons died of cancer in 1906, among them a man with a cancer of the lip who lived in the house where a man had died of carcinoma of the esophagus in 1905. A citizen of Rehfeld died of cancer of the stomach, and the man to whom the house was sold selected for his living room the one in which the previous owner had died, erecting his bed in the place where that of his predecessor had formerly stood. Although he had been in perfect health before moving into this house, the new tenant died after about a year from carcinoma of the stomach.

[1] *Lancet*, 1887, ii, 766.
[2] *Med. Record*, 1895, xlviii, 383.
[3] *Deut. med. Woch.*, 1901, xxvii, 427.
[4] *Die Bestätigung d. künstlichen Züchtung des Krebserregers*, Berlin, 1910, 17.

Newsholme [1] has criticized the usual published accounts of cancer houses on the score that the writers always forgot that they were dealing with small figures; they forget, for instance, that if a number of persons were set to tossing coins, it would happen eventually that one of them would toss a thousand heads without a break. One who remembered the occurrence of such coincidences as this would attach little importance to numbers of cases in particular houses.

The same idea has been expressed also by Adami,[2] who said: ". . . by the law of chance, just as one individual in a thousand may be of gigantic proportions, so one house in a thousand may show a great excess of cases of cancer — or of twin births — over the ordinary run of houses."

Experimental

Although the attempt has been made to decide, by means of experimental inoculation, the question regarding the transmission of tumors from one person to another, the number of these experiments is so small that no deduction can with any safety be drawn from them.

On October 17, 1808, at the Hôpital de St. Louis, and in the presence of several physicians and students, Alibert [3] allowed himself to be injected with ichorous material from a cancer of the breast. The experiment was performed at the same time upon M. Fayet, a medical student, and the next morning upon MM. Lenoble and Durand. Except for an inflammatory reaction the experiment was without sequelæ. A week later Alibert inoculated himself a second time, and his colleague, M. Biett. He himself escaped with a result similar to that which followed the first trial, but M. Biett developed a somewhat more severe infection, which involved the axillary and cervical lymph nodes.

Senn [4] transplanted into himself a fragment of lymph node in which carcinomatous invasion had been demonstrated by the microscope. Although a nodule the size of a pea appeared at the implantation site and remained stationary for two weeks, it vanished soon

[1] *Trans. Epidemiological Soc. London*, 1906–1907, N.S., xxvi, 73.
[2] *Principles of Pathology*, Philadelphia and New York, 1910, Vol. i, 839.
[3] *Description des Maladies de la Peau*, Paris, 1825, 118.
[4] *Jour. American Med. Assoc.*, 1901, xxxvii, 811.

afterward, and seven weeks later a red linear scar was the only indication of the experiment.

Lanz [1] inoculated his gardener on the back of the hand with finely minced common warts (*verruca vulgaris*) removed from another person, arranging the series in the shape of the letter "J." The first sign of growth did not appear until fully one and a half months had elapsed, when two or three very small nodules were recognizable. Two months after implantation there were eight warts forming a "J" and each about the size of a pinhead, while a month later the number had increased to twelve. Lanz said, further, that as a result of rubbing down the warts of a patient, he himself had acquired several of these tumors.

TRANSPLANTATION OF TUMORS INTO THEIR BEARERS

Accidental

Although it may be true that tumors can be transplanted by contact under certain conditions, not a few of the instances that have been reported to illustrate this type of implantation may have been cases either of multiple tumors or of retrograde metastasis; and the amount of care which is necessary to eliminate error is well shown in an article by Petersen.[2] In a case of inoperable carcinoma of the uterus, the skin in the neighborhood of the vulva contained a number of small nodules and a few indurated ulcers which seemed clearly enough to be the outcome of auto-inoculation. Examination of serial sections, however, proved definitely that they were the result of retrograde lymphatic metastasis.

Full as the literature is of examples of reputed transfer by contact, there are but few which satisfy Ewing's demand [3] that " . . . the transferred tumor shall exhibit a structure similar to that of the original, but different from that spontaneously arising in the invaded tissue."

A useful set of standards for the guidance of those engaged in the critical study of these cases has been formulated by Butlin [4] as follows: "The disease must be of the same variety in the primary carcinoma

[1] *Deut. med. Woch.*, 1899, xxv, 313.
[2] *Arch. f. Dermat. u. Syph.*, 1904, lxx, 313.
[3] *Arch. Internal Med.*, 1908, i, 177.
[4] *British Med. Jour.*, 1907, ii, 256.

and in the reputed contact-cancer. The identity of the disease must be proved by microscopical examination. The primary disease must have been exposed at the time at which the contact is known to have taken place; and there should be such evidence of contact of the primary carcinoma with the seat of the reputed contact-cancer as would satisfy a jury."

The necessity for microscopical control was illustrated by an anecdote. A specimen that had been exhibited as an example of contact transfer having been finally cut and examined, it was found that an ulcer on the cheek immediately opposite a carcinoma of the gum was a simple inflammatory lesion.

Klebs [1] described three instances of squamous cell epithelioma of the stomach, two of which were, in his opinion, undoubtedly due to infection of the mucous membrane by particles implanted on it, while the third had probably been so produced. The primary tumors involved respectively the esophagus, the face, mouth, and throat, and the tongue.

Kaufmann [2] recorded the case of a woman with an ulcerating epithelioma on the dorsal surface of the right hand, who was in the habit of rubbing her eye with the back of the affected member. About three years after the discovery of the tumor a small ulcerating growth developed on the inner side of the right lower lid. Both ulcers had all the microscopic characteristics of a cancroid.

Kraske [3] had seen two patients with primary cylindrical cell carcinoma situated high up in the rectum, who had developed similar growths close to the anus and separated by a considerable distance of healthy tissue from the higher tumor. The lowermost were in the region normally covered by squamous epithelium, a circumstance which seemed to exclude the possibility of their having been primary.

v. Bergmann [4] discussed a patient with an ulcerating epithelioma on the lower lip and a tumor of the same type on the upper at a precisely corresponding point. The former growth had appeared three months before the man came under observation and the latter six or seven weeks later, but not until after the lower tumor had ul-

[1] *Handbuch d. path. Anat.*, Berlin, 1868, Bd. i, erste Abt., 190.
[2] *Arch. f. path. Anat.*, etc., (Virchow), 1879, lxxv, 317.
[3] *Centralbl. f. Chir.*, 1884, xi, 801. [4] *Berl. klin. Woch.*, 1887, xxiv, 891.

cerated. In answer to an inquiry from Butlin[1] the author replied that the growths had been subjected to microscopical examination and found to be epitheliomata.

A woman under the observation of Hamburger[2] was admitted to hospital with an epithelioma of the left labium minus about the size of a hen's egg, first noticed two years before. On admission there was discovered at a corresponding point on the right labium minus a small, raised, ulcerating tumor which the history stated was of only two months' duration. Microscopical examination proved that both growths were squamous cell epitheliomata.

Thorn[3] found a carcinoma of the cervix uteri coupled with a carcinomatous ulcer in the left side of the vagina, but without involvement of the fornix. Perimetritic adhesions had drawn the uterus to the right, tilting an exceptionally long cervix to the left so that the cervical lesion had been kept in constant contact with the left vaginal wall. Both tumors presented the same histological structure. In a second case, a carcinoma involved both right labia and a shallow carcinomatous ulcer occupied a corresponding point on the left side, although the tissues intervening had remained intact. No statement was made regarding the structure of the tumors in the latter case.

Butlin[4] collected two cases in which an epithelioma had arisen on one labium at the exact place of contact with a similar tumor on the other side, and one where a labial tumor opposite to an epithelioma was not malignant. He further reported three examples of apparent transfer from one side of the larynx to the other, and a fourth instance in which an ulcer of one vocal cord immediately opposite to an epithelioma on the other was demonstrated by microscopical examination to be merely an inflammatory lesion. Among various examples regarding other parts of the body, there was described an ulcerated epithelioma of the lower lip in a patient who had the habit of pressing his forefinger against the ulcer and then rubbing it on his nose. Twenty months after the excision of the tumor the patient presented a large ulcerated patch on the nose. This lesion, first noticed three months after the operation on the lip, was proved by the microscope to be of identical structure with the first tumor.

[1] *British Med. Jour.*, 1907, ii, 259.
[2] *Hospitalstidende*, 1892, x, 81.
[3] *Centralbl. f. Gynäkol.*, 1894, xviii, 228.
[4] *British Med. Jour.*, 1907, ii, 257.

Secondary growths of the Fallopian tube arising by implantation of cells from malignant tumors in the abdominal organs have been described by many authors, one of the most recent being Wakasugi.[1] The nodules are found on the outer aspect of the tube, or, as occurs not infrequently in the case of ovarian carcinoma, in the mucous membrane.

It has often been observed that particles of tumor, eluding the vigilance of the surgeon at operation, have been spread through the wound to demonstrate later in the most unhappy way the possibility of ingrafting a tumor into the individual in whom it has originated.

Thus Quincke[2] recorded a case of peritoneal carcinosis in which, as the result of abdominal puncture for ascites, there was found in the subcutaneous tissue at autopsy a carcinoma identical in structure with the peritoneal nodules, although the intervening tissues were free from growth. The secondary tumor had been clinically recognizable as early as the tenth day after puncture.

Thorn[3] saw a patient who had submitted two years previously to vaginal hysterectomy, during which lateral incisions had been made to overcome the narrowness of the vaginal orifice. Renewed hemorrhage necessitating subsequent examination, a small recurrence was discovered in the scar, and upon microscopical examination it was shown that this nodule exactly resembled the original carcinoma of the cervix. A second and similar instance of implantation was described by the same author.

Lack[4] discussed two cases in which the rupture of a carcinomatous lymph node during operation was followed by recurrence in the scar, a third where recurrence supervened at the site of a tracheotomy wound after excision of a sarcoma of the lower jaw, and two examples of apparent implantation in tracheal wounds during operation for cancer of the larynx.

Richardson[5] reported a patient with carcinoma of the breast in whom an exploratory puncture had been performed four weeks previ-

[1] *Beitr. zur. path. Anat.*, etc. (Ziegler), 1910, xlvii, 483.
[2] *Deut. Arch. f. klin. Med.*, 1875, xvi, 134.
[3] *Centralbl. f. Gynäkol.*, 1894, xviii, 228.
[4] *Lancet*, 1896, i, 1638.
[5] *Boston Med. and Surg. Jour.*, 1898, cxxix, 414.

ously. At operation a small nodule was found in the pectoralis minor, and the entire mass was therefore thoroughly removed. "The examination of the tumor and the pectoralis major showed a clear space between the two. A nodule was found among the fibers of the pectoralis major corresponding in position and size to that in the pectoralis minor, and in the same general line with the two just described there was a stellate malignant mass in the axillary fat, not connected with the enlarged lymph nodes.

"The infection of the pectoralis major, the pectoralis minor, and the axilla, in a straight line, shows, I think, a direct contamination of previously healthy parts by the exploring punch, for the nodules were of about the same size and age, and in the muscles, at least, they were the only ones found." Discussing the transplantation of tumors into their bearers, Richardson added that he had seen the ". . . stitch-holes of a closed abdominal wound, after nephrectomy for sarcoma of the kidney, burst out with luxuriant masses of recurrent disease."

Experimental

The problem whether or not a tumor can be transplanted successfully into its bearer has even been subjected to experimental investigation, although, as Ewing[1] has said: "No experimental evidence is needed to show that a malignant tumor may often be grafted from one part of the patient's body to another, since the several recognized modes of metastasis daily demonstrate this process."

Hahn[2] seems to have been the first to approach this question from the experimental standpoint. In a case of inoperable recurrence of a mammary carcinoma, three portions of infiltrated skin were transplanted into the sound integument overlying the normal breast on the opposite side. About eleven weeks after the operation all three grafts had developed into nodules which were demonstrated by the microscope to be carcinomata. Virchow[3] objected, however, that this experiment did not demonstrate the transplantation of a tumor into its bearer, because, as the entire skin had been transferred,

[1] *Arch. Internal Med.*, 1908, i, 176.

[2] *Berl. klin. Woch.*, 1887, xxiv, 892.
Berl. klin. Woch., 1888, xxv, 413. See also Frank, *Deut. med. Woch.*, 1891, xvii, 933.

[3] *Berl. klin. Woch.*, 1887, xxiv, 892.

the tumor had grown in its new location only in so far as it was situated in the implanted integument. The case, therefore, was entirely analogous to the ingrafting of a portion of skin containing hair, which, even though successful, could not be held to have demonstrated the transplantability of hair.

Senn [1] transferred a fragment of epithelioma to the subcutaneous tissue of the patient's leg, but nothing remained of the graft after the expiration of four weeks.

Cornil [2] reported two cases of the inoculation of a tumor into its bearer, by a surgeon whose name was withheld. In the first instance a graft from a spindle cell sarcoma of the mamma, inserted into the normal breast on the other side, had reached the size of an almond at the end of two months. This nodule, when removed and subjected to microscopical examination, proved to be a spindle cell sarcoma similar to the primary tumor. The patient died somewhat later of an intercurrent disease, but no secondary tumors were demonstrable at autopsy in spite of the most painstaking search. The second case was similar except that the implanted growth was an adeno-carcinoma. The secondary nodule, while it had all the clinical characteristics of a new growth, was not removed, on account of the patient's reluctance to submit to another operation, and microscopical proof of its nature could not, therefore, be advanced.

Thorn [3] wrote that in six cases of inoperable carcinoma of the uterus he had made about twenty implantations in the respective patients, either by sewing small pieces of growth into the mucous membrane of the vagina or by rubbing fragments into small vaginal wounds. Although one of these grafts seemed to proliferate for three weeks it disappeared soon afterward, and the outcome in all the other experiments had been negative.

To the subject of implantation cancer as a whole Milner [4] has contributed an exhaustive critical review which includes two hundred references.

[1] *Surgical Bacteriology*, Philadelphia, 1889, 261.
[2] *Semaine méd.*, 1891, xi, 259.
Bull. de l'Acad. de Méd., 1891, xxv, 906.
[3] *Centralbl. f. Gynäkol.*, 1894, xviii, 228.
[4] *Arch. f. klin. Chir.*, (v. Langenbeck), 1904, lxxiv, 669, 1009.

ATTEMPTS TO TRANSFER HUMAN TUMORS TO ANIMALS

Since the day when Peyrilhe [1] made the first recorded experiment, attempts to transfer cancer from man to the lower animals have been almost continuously in progress, and although a positive result has been reported more than once, the consensus of opinion has been for many years that such claims cannot be seriously entertained.

Peyrilhe's account of his investigation appears in the English translation of the Dissertation in the following words: "I will relate here an experiment which I myself made with the cancerous virus. I procured about two drachms of it from a cancerous breast, and introduced it by means of a syringe, into a small wound made in the back of a dog. I covered the wound with a plaster and bandage, and in three days removed the dressing; the retraction of the skin afforded an ulcer, which already afforded a very disagreeable smell: it was of a dark violet color, and the parts all around it were emphysematous. I covered it again with the same plaster, and in forty-eight hours opened it again for the second time. The effects were then more violent. The whole skin from the head to the tail was completely emphysematous. A little ichorous blackish matter flowed from the wound. The eyes of the animal were vivid, and he seemed to have a great thirst: in this state the poor creature was perpetually howling. At length my maid, disgusted by the stench of the ulcer, and softened by the cries of the animal, put an end to his life, and thus prevented my observing the ultimate effects of this disease."

Dupuytren [2] fed animals with cancerous material, introduced it into the abdomen, injected cancer juice into the peritoneal cavity and the veins, and inoculated the pus of an ulcerated cancer, but without succeeding in transmitting the disease.

Langenbeck [3] injected into the femoral vein of a dog the juice from a medullary carcinoma of the humerus, mixed with the dog's own serum, and two months afterward, when the animal was autopsied,

[1] *Dissertatio Academica de Cancro*, Antverpiae, 1775.
A Dissertation on Cancerous Disease, London, 1775.

[2] Cited by Viel-Hautmesnil, *Considérations générales médico-chirurgicales sur le Cancer*, Paris, 1807, 23.

[3] *Schmidt's Jahrbücher*, 1840, xxv, 99.

several small, round, bluish nodules were found in the lungs. When submitted to the microscope, they appeared without doubt to be carcinomata. Virchow,[1] however, who saw the drawings of these tumors, said that the structure was more similar to that of spontaneous cancer in the dog than to that of carcinoma as it occurred in the human subject.

Lebert's treatise on cancer[2] contained the description of an experiment performed in conjunction with Follin. Part of a mammary cancer was emulsified in water and about sixty or eighty grams of the resulting fluid, in which the presence of cancer cells had been confirmed by the microscope, was injected into the jugular vein of a dog. The animal died fifteen days afterward, and the autopsy disclosed a number of nodules in the walls of the heart, beside some of smaller size in the liver, all of which contained cancer cells with round or elliptical nuclei possessing one or more nucleoli, while numerous free nuclei were also present in the preparations. Lebert did not believe, however, that this single experiment was of any great significance and declined to draw definite conclusions therefrom, realizing that the dog might have been the subject of spontaneous cancer when the injection was first undertaken.

Many investigators beside Dupuytren had reported their failure to transplant human tumors into animals, and with the idea that the miscarriage of their efforts might have been due to the length of time intervening between removal and implantation, Billroth[3] planned a series of experiments in which this period should be reduced to a minimum. He inoculated three dogs subcutaneously and six intravenously, but with negative results in every case despite his careful technic. For the subcutaneous inoculations two carcinomata and a giant cell sarcoma were used, while for the intravenous three carcinomata, a struma, a lymphoma, and a giant cell sarcoma were chosen.

Alberts[4] was the first to appreciate that the lesions so often de-

[1] *Die krankhaften Geschwülste*, Berlin, 1863, i, 87 (footnote).

[2] *Traité pratique des Maladies cancereuses et des Affections curables confondues avec le Cancer*, Paris, 1851, 136.

[3] *Wien. med. Woch.*, 1867, xvii, 1137, 1153.

[4] *Das Carcinom in historischer u. experimentell-pathologischer Beziehung*, Jena, 1887, 183.

scribed as transplanted carcinomata might have been septic emboli, and to conduct experiments in such a way as to exclude sepsis. Tumors that had been aseptically removed by operation were inoculated into dogs, but in no case did a successful result ensue.

Klebs[1] examined fragments of human carcinoma removed from the peritoneal cavities of white rats at varying intervals after introduction, and found that in the great majority of cases the epithelial constituents of the graft had vanished by the third day.

Duplay and Cazin[2] transplanted various types of tumors from man into different localities in animals of several species, but the outcome was always unsuccessful, even when they tried to produce by trauma a soil suitable for implantation. Nor did they meet with any more favorable results after the introduction of fragments that had been kept for a period outside the body to allow the life cycle of a hypothetical parasite to be completed, in accordance with the suggestion of Metchnikoff.[3] The authors decided that malignant tumors did not appear to be transmissible from one species of animal to another.

This conclusion may be accepted as representative of most authorities, and even where inoculations have been made into species so closely related to man as the anthropoid apes, the attempted transfer has failed, as has been shown by the work of Roux and Metchnikoff,[4] and of Jobling.[5] The reputedly successful results have all been submitted to careful scrutiny and found wanting in one respect or another, v. Hansemann,[6] for example, having pointed out that typical metastases have never been described, although their presence would be a most important proof that implantation had been accomplished. Ribbert[7] has said that transplantation could be performed only

[1] *Deut. med. Woch.*, 1890, xvi, 710.

[2] *Semaine méd.*, 1892, xii, 61.
Compt. rend. de l'Acad. des Sc., 1892, cxiv, 325.
Semaine méd., 1893, xiii, 329.

[3] *Ann. de l'Inst. Past.*, 1892, vi, 158.

[4] *Bull. de l'Acad. de Méd.*, 1903, l, 101.

[5] See Flexner, *Med. Record*, 1909, lxxv, 783.
Monographs on Medical and Allied Subjects, Rockefeller Institute, New York, 1910, No. 1, 120.

[6] *Berl. klin. Woch.*, 1905, xlii, 314.

[7] *Verhandl. d. deutschen path. Gesellsch.*, 1904, 8te Tagung, 104.

between individuals of the same or of a very closely related species, and has extended this rule to include tumors, since no satisfactory evidence of their transfer from man to the lower animals has yet been adduced.

TUMOR TRANSPLANTATION WITHIN THE SAME SPECIES

Hanau [1] described the first successful transfer of carcinoma within the same species. Involving the vulva of an old rat there was discovered an ulcerated cancer which had metastasized in the inguinal lymph nodes on both sides and in those of the axilla on the right. Microscopical examination showed that it was a keratinized squamous cell carcinoma. A fragment from one of the still unulcerated nodes was sewn under the skin of the scrotum in two old rats. One of these animals died forty-seven days after inoculation, presenting a few small nodules on the quasi-mesentery of the right vas deferens. The omentum-like structure which, in the rat, accompanies the vasa spermatica interna, was studded with tumors, while the great omentum had been converted into a nodular mass. The small omentum was also involved, and behind the stomach and between this organ and the spleen, were still other growths. Microscopical examination of two of the nodules in the omentum showed that they were carcinomata of exactly the same character as the tumor which had been inoculated. Encouraged by these findings, Hanau examined the second rat on the fifty-ninth day after implantation and discovered a round ulcer affecting the prepuce, and a firm movable tumor in the right half of the scrotum about one-half the size of the testicle. On the sixty-first day this rat was sacrificed. No trace of epithelial proliferation was found in the ulcer, but on the right gubernaculum hunteri there was a flat, white nodule about 2.5 millimeters in diameter, while between the testicle and the tail of the epididymis lay the larger growth which had been felt during life. A complete histological description of Hanau's material has been published by Jenny.[2]

Pfeiffer,[3] in an article on the pathogenic protozoa, said incident-

[1] *Fortschritte der Med.*, 1889, vii, 321.
Arch. f. klin. Chir., (v. Langenbeck), 1889, xxxix, 678.
[2] *Arch. f. klin. Chir.*, (v. Langenbeck), 1895, li, 269.
[3] *Centralbl. f. Bakt.* etc., erste Abt., Orig., 1890, viii, 802.

ally and without giving any details regarding the experiment, that he had succeeded two years previously in transferring a melanotic carcinoma of the mouse to other mice.

v. Eiselsberg [1] discovered a tumor in an adult rat involving the right shoulder and about the size of a hen's egg, hard, nodular, and movable on the underlying parts. The examination of fragments removed for diagnosis proved that the growth was a spindle cell sarcoma. Some days later portions of the growth were excised and sewn into mesenteric folds in two half-grown rats. The tumor rat having died under the anesthetic, an autopsy was immediately undertaken, during the course of which it was found that the neoplasm was connected by dense adhesions to the periosteum of the scapula, the bone itself, however, having been spared. Nothing was said of the presence of metastases. In the second month following inoculation the two rats were examined under narcosis. In one there was nothing abnormal, but in the abdomen of the second there was discovered a tumor the size of a nut. Five months after inoculation this rat died during the night, and examination twelve hours later disclosed a firm nodular tumor in the mesentery, about the size of a hen's egg. No other pathological condition was encountered. The microscopic structure of the daughter tumor was identical with that of the spontaneous growth except that a larger number of spindle cells were present. A fragment was immediately transplanted into another rat but without result — doubtless, said v. Eiselsberg, because the tumor cells were already dead.

A tumor about as large as a hazel-nut was found by Morau [2] in the axilla of a white female mouse of unknown age. The nodule was reduced to an emulsion, which was inoculated subcutaneously into other mice, and among these a number of tumors were found three months later. From one of the growths of this, the first generation, cultivation was continued for about three years, seventeen transfers being made in all. Morau drew the following conclusions from his series of experiments: Cylindrical epitheliomata can be transferred to other mice by

[1] *Wien. klin. Woch.* 1890, iii, 927.

[2] *Compt. rend. Soc. Biol.*, 1891, xliii, 289.
Compt. rend. de l'Acad. des Sc., 1893, cxvii, 62.
Arch. de Méd. exp. et d'Anat. path., 1894, vi, 677.

inoculation; heredity plays a considerable rôle in the development and evolution of these tumors; traumatism hastens and favors their generalization; pregnancy accelerates their growth; the tumors possess a variable toxicity, which may destroy the host's life; they seem to lose in virulence as they develop in new animals; when they are not ulcerated, they do not contain microbes.

Firket [1] published a preliminary note describing the transfer of a spindle cell sarcoma from rat to rat. A fragment from one of the many abdominal tumors in the animal primarily affected was transplanted into the peritoneal cavity of another rat, which died about six weeks later with generalized growths in the abdomen. Grafts from this generation were transplanted into three rats, in all of which growth took place, and with one of the resulting tumors two other rats were ingrafted, one of them living long enough to develop small nodules. The tumor retained its original sarcomatous structure throughout the experiment.

Velich [2] discovered in the femur of a white rat a subperiosteal sarcoma, a portion of which, transplanted subcutaneously into another rat, produced a nodule as large as a walnut after a week's growth. This increased so rapidly in size that in three weeks it had attained a length and breadth of five centimeters, and a thickness of three. At the end of six weeks, when the rat died, the tumor was about one-third the length of its body. Before the death of the animal pieces had been removed from the tumor and implanted in three rats, in all of which nodules were appreciable a few days after inoculation. Altogether this sarcoma was carried through eight generations, but the growth energy became progressively more feeble until finally the tumor died out. Velich recorded the fact that the rats used after the eighth generation were from another source and considered the possibility that a strange breed might not have offered favorable conditions for growth, dismissing it, however, because a decrease in proliferative energy had set in before the new strain was introduced into the experiment. The daughter tumors were all spindle cell sarcomata and similar in every respect to the primary growth.

To none of these communications was any especial attention devoted,

[1] *Bull. de l'Acad. Royale de Méd. de Belgique*, 1892, vi, 1147.
[2] *Wien. med. Blätter*, 1898, xxi, 711, 729.

for their significance was not grasped at the time of their appearance; and it was only after the earlier articles of Jensen, Loeb, and Borrel had been published that experimental pathologists, appreciating the possibilities which they had until then neglected, commenced a concerted attack upon the biological side of the cancer problem. A communication from the pen of Jensen[1] which initiated this era of activity, has became a classic in the literature of cancer research as the masterly paper of Koch had become classic in the annals of tuberculosis — for its evidences of patient, accurate, and exhaustive investigation. Jensen[2] had described previously the results attending the transplantation of tumors from mouse to mouse, but the fruits of his labor first became accessible to the general pathological public upon the appearance of an article in the German literature. The facts recorded in the earlier papers were, in short, that he had been able to transmit a mouse cancer through eight generations, that no microorganisms had been found in it, and that experiments dealing with the resistance of the cancer cell to various agents had been undertaken.

In the German paper Jensen described in great detail the tumor and the results attendant upon its transplantation. The primary growth was about the size of a hazel-nut, situated under the skin of the back, and microscopical examination disclosed the fact that it was a typical carcinoma. Daughter tumors were of entirely similar architecture, a relatively sparse stroma inclosing a large number of alveoli containing cells of various contours. The cell bodies were large and fairly homogeneous, the nuclei large and provided with nucleoli and a prominent chromatin network. As a rule the mitotic figures, which were by no means rare, were of the normal type, although atypical forms were occasionally to be found. A part of the nodule was emulsified in physiological saline solution and a small amount of this suspension was inoculated subcutaneously into five mice, three of which developed tumors that were afterward transplanted into other series of mice. The experiments had extended over a period of two and a half years, the tumor had been carried through nineteen generations, and would grow in about half of the mice inoculated. During all the time that

[1] *Centralbl. f. Bakt.*, etc., erste Abt., Orig., 1903, xxxiv, 28, 122.

[2] *Hospitalstidende*, 1902, x, 489.
Hospitalstidende, 1903, xi, 549, 581.

the neoplasm had been under cultivation metastases in internal organs had never been discovered, and the regional lymph nodes, while usually swollen, were only once found to contain anything like a secondary deposit. Unfortunately, however, the specimen had been mislaid before it was ready for examination.

The details of Jensen's experiments, in common with those of the investigations which have been prosecuted by the two succeeding authors, will be discussed in the following chapters under their appropriate headings.

The period that saw the publication of Jensen's first article witnessed also the accounts of Loeb's early work.[1] Loeb discovered a cystic sarcoma of the thyroid in a white rat and succeeded in transmitting it to other rats by inoculation, but not by causing its ingestion. During the fifteen months that the growth had been under propagation the histological structure had been preserved unaltered. Neither the primary tumor nor those belonging to subsequent generations had produced metastases, although in one instance tumor cells had been found penetrating a blood vessel.

In a succeeding paper Loeb[2] reported that the neoplasm in question had been carried through about forty generations in the course of twenty months, but had finally become so infected that further propagation had been rendered impossible. Other rat tumors had, however, been successfully transplanted.

While the experiments of Jensen and Loeb were in progress, Borrel[3] was engaged upon the same problem. Although transplantation succeeded in only about 10% of the mice inoculated, daughter tumors were obtained of such rapid growth that after the expiration of forty days they weighed more than the mice themselves. Metastases were discovered at autopsy in the blood vessels of the lungs as well as in the lymph nodes, always preserving the structure of the main growth.

A critical review of the work of Jensen's antecedents will be found in a summary by Sailer[4] of the literature relating to the inoculability of carcinoma.

[1] *Jour. Med. Research*, 1901, N.S., i, 28.
Arch. f. path. Anat., etc., (Virchow), 1902, clxvii, 175.
[2] *Jour. Med. Research.*, 1902, N.S., iii, 44. [3] *Ann. de l'Inst. Past.*, 1903, xvii, 112.
[4] *American Jour. of the Med. Sciences*, 1900, cxx, 190.

CHAPTER IV

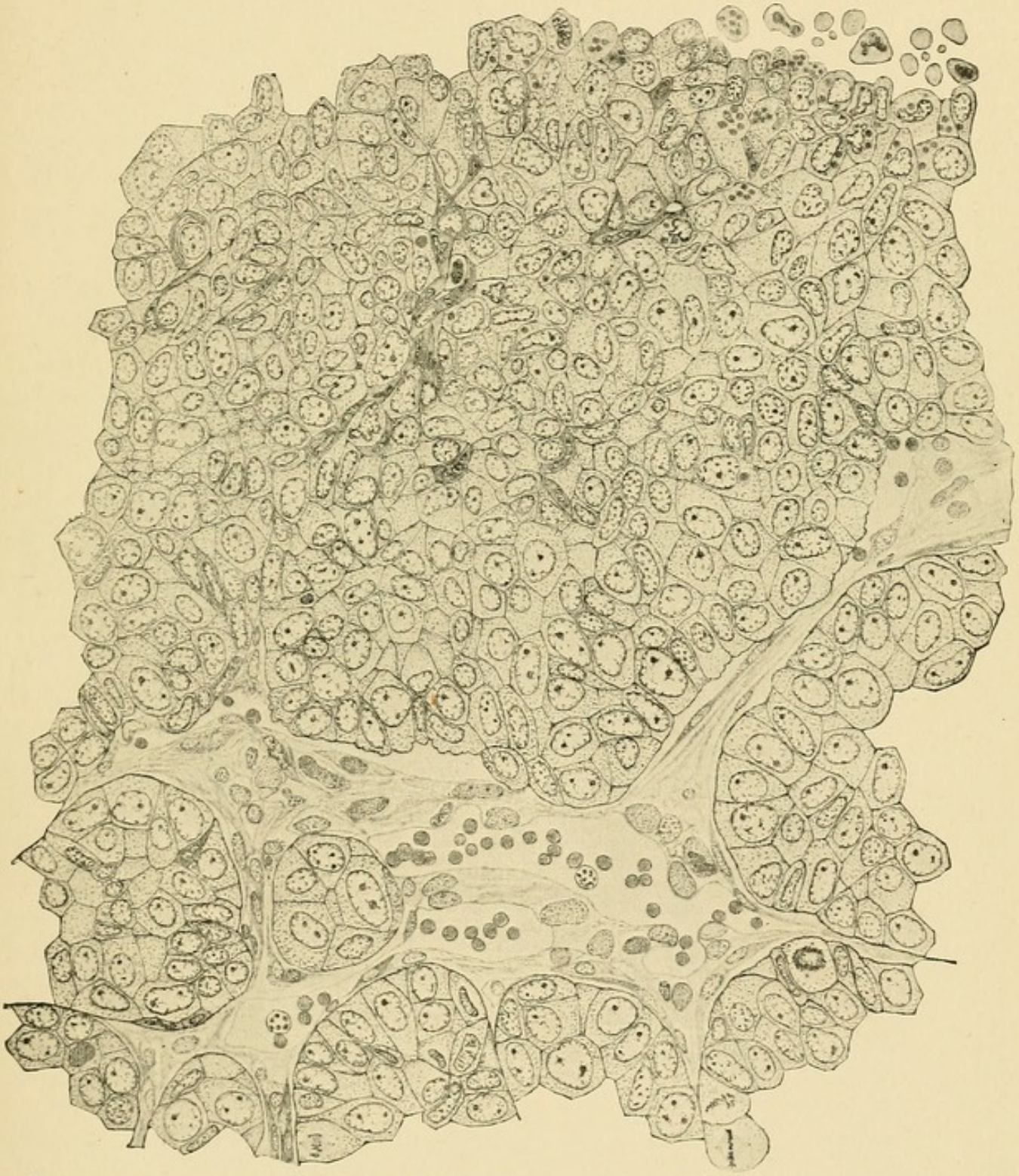
THE TRANSPLANTED TUMOR

THE STROMA REACTION

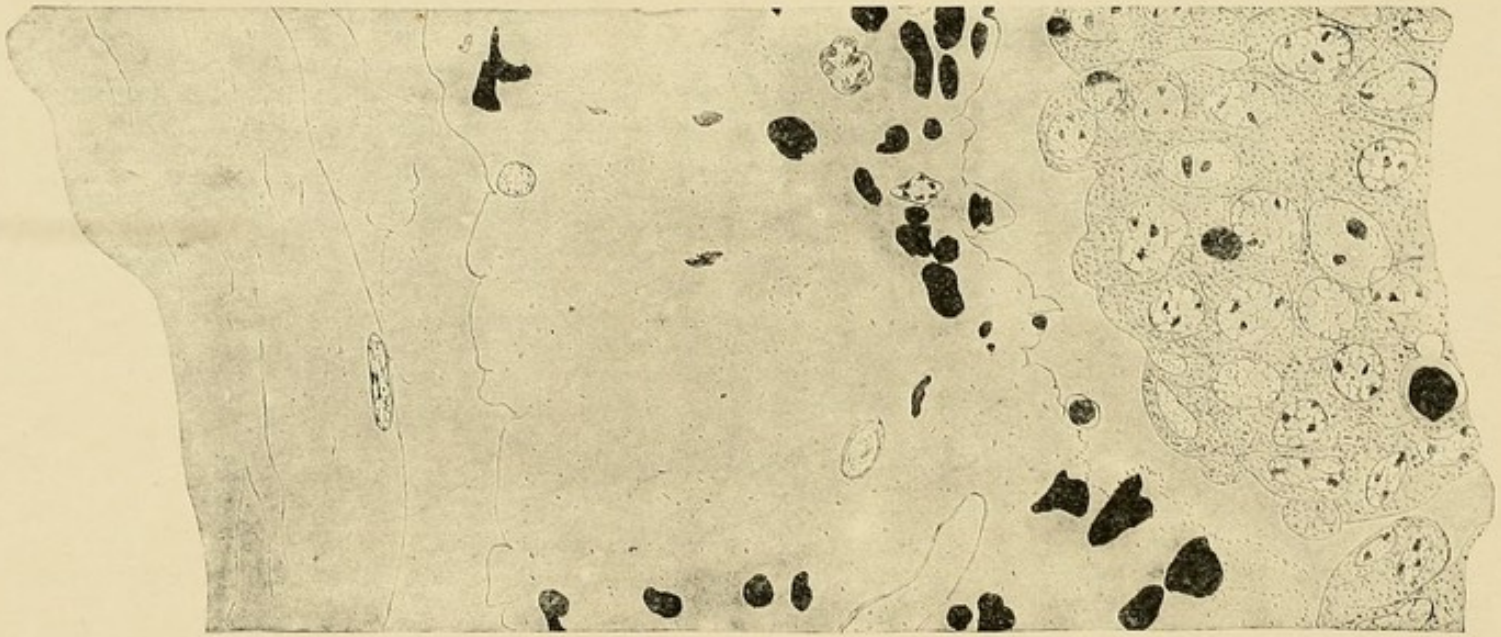
As soon as it had been demonstrated that malignant growths were transmissible, it was necessary to know whether the cells of the daughter tumors were direct descendants of those that had been introduced in the graft, or whether they arose from the tissues of the new host. This was, of course, tantamount to asking: Is the transfer of a tumor an instance of transplantation or of infection?

The vital importance of settling this question did not escape Jensen.[1] He followed carefully the fate of implanted fragments, examining them at daily intervals after transplantation, and found that, while many of the parenchymal cells perished soon after their introduction into the new animal, there was still a fairly large number that remained alive, and that in larger grafts the cells able to retain their vitality were those situated at the periphery. The stroma of the newly ingrafted tumor became hyaline and nearly all the connective tissue cells in the central parts disappeared, while at the same time many recently formed blood vessels and fibroblasts appeared about the fragment and in its margin. The final fate of the stroma was not absolutely clear to Jensen, but it seemed as though it were gradually penetrated from without by blood vessels and fibroblasts and that it eventually suffered absorption, although there remained the possibility that part of it might continue to live. The surviving cells of the parenchyma were always sharply demarcated from those surrounding them, and there was no evidence of the creation of tumor cells from any of the elements of the host. Without doubt, therefore, the process was a real transplantation.

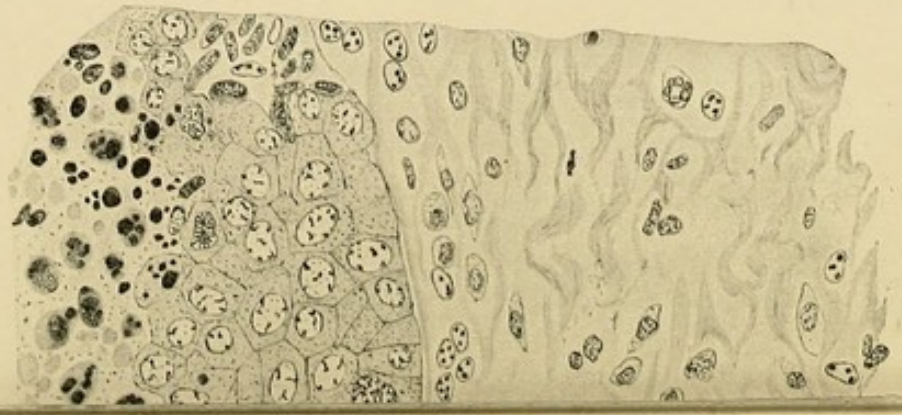
[1] *Centralbl. f. Bakt.*, etc., erste Abt., Orig., 1903, xxxiv, 127.



Jensen's mouse carcinoma: fully developed tumor, from which grafts were used in the investigation of the early stages shown in the three following plates.



Graft of Jensen's carcinoma, removed 2 hours after transplantation. Absence of reaction at this period; (*a*) unaltered connective tissue; (*b*) cleft with coagulated lymph; (*c*) necrotic tumor; (*d*) healthy tumor. $\times \frac{1000}{1}$.



Graft of Jensen's carcinoma, removed 6 hours after transplantation. Reaction beginning in tissues of host. Immigration of polymorphonuclear leucocytes. $\times \frac{400}{1}$.

Graft of Jensen's carcinoma, removed 15 hours after transplantation. Commencing proliferation of host's connective tissue. $\times \frac{1000}{1}$.

Loeb[1] was unable to decide definitely between transplantation and infection, possibly because his initial observations were conducted with a sarcoma — a tumor type in which the analysis of early growth is fraught with the greatest difficulty. In a later article he[2] expressed the view that the peripheral cells of the graft remained alive and developed, mingling with the surrounding elements of the connective tissue. It was very probable that tumor cells themselves were transplanted in addition to the tumor-producing agency.

v. Leyden,[3] while admitting that inoculated tumors resulted from the proliferating cells of the transplanted graft, could understand this fact only by assuming the presence of an intracellular parasite. This supposition, he thought, did not oppose the conceptions which had been developed by the pathologist, and was, moreover, the only one capable of explaining the phenomena associated with cancer in man.

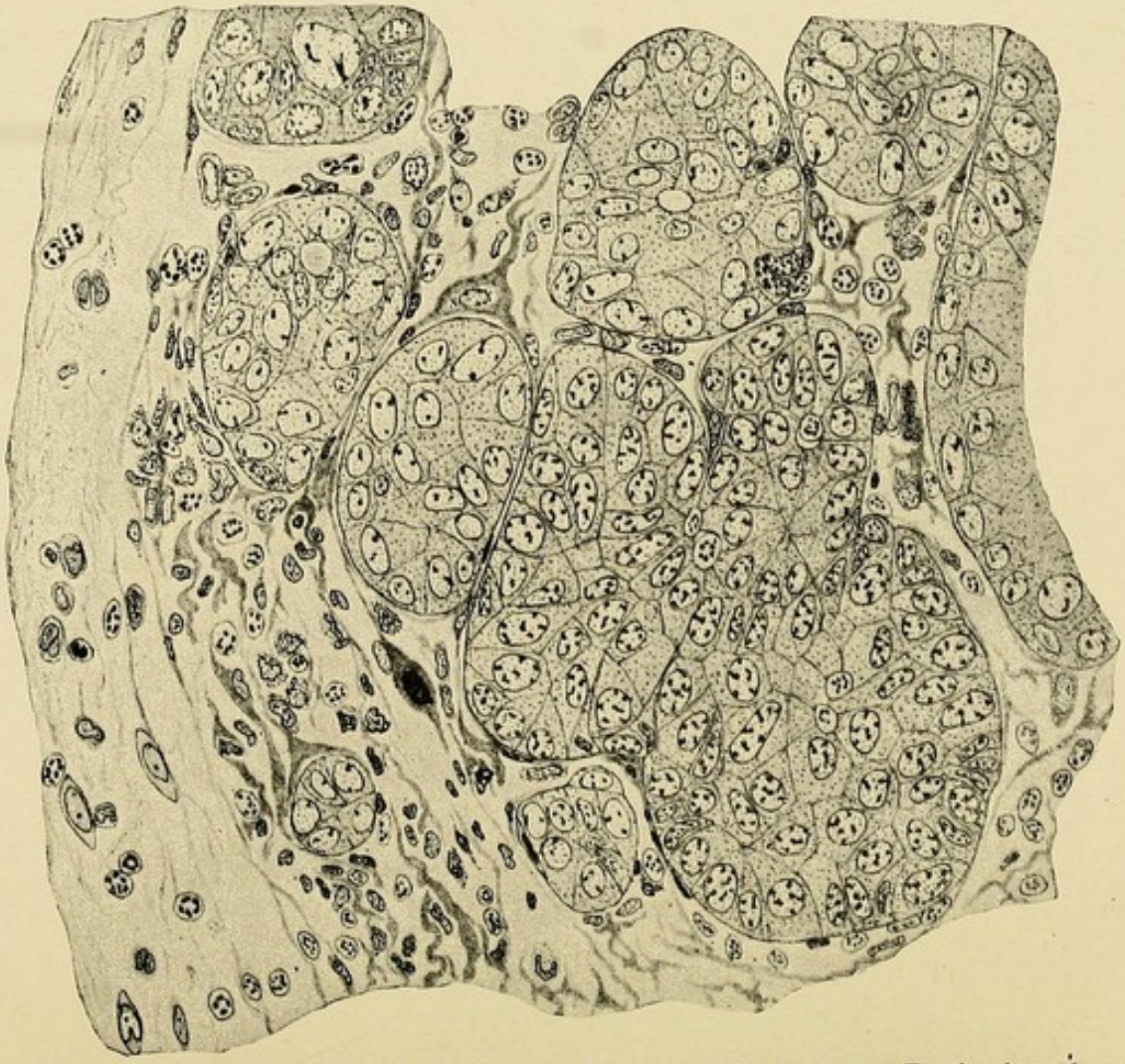
Jensen's findings were confirmed and amplified by Bashford and Murray[4] in conjunction with Cramer,[5] through the investigation of several tumors with parenchyma and stroma of different types. The results, which were similar in all the growths studied, were described most fully for Jensen's tumor, which had been submitted to a more extensive examination than the others. The authors found that the first evidence of reaction to the graft was a rapid aggregation of polymorphonuclear leucocytes in the surrounding tissues of the host, beginning about two hours after the introduction of the tumor fragment and of short duration only. The leucocytes collected about any necrotic material present and even penetrated between the tumor cells. About fifteen hours after the introduction of the graft there occurred proliferative changes in the surrounding areolar connective tissue of the host, the cells of which became shorter and thicker, while their nuclei divided by amitosis. The proliferating cells migrated into the cleft which originally separated the connective tissue of the host from the newly introduced fragment, and applied themselves to the surface of the graft. The stroma had already become hyaline after twenty-four hours, and commencing degeneration could often be detected in its cells. Such changes were even more distinct after thirty-six hours, and by this time

[1] *Jour. Med. Research*, 1901, N.S., i, 37.
[2] *Jour. Med. Research*, 1902, N.S., iii, 52.
[3] *Berl. klin. Woch.*, 1905, xlii, 348.
[4] *Proc. Roy. Soc.*, 1904, lxxiii, 70.
[5] *Sci. Reports, Imperial Cancer Research Fund*, London, 1905, No. 2, Part ii, 24.

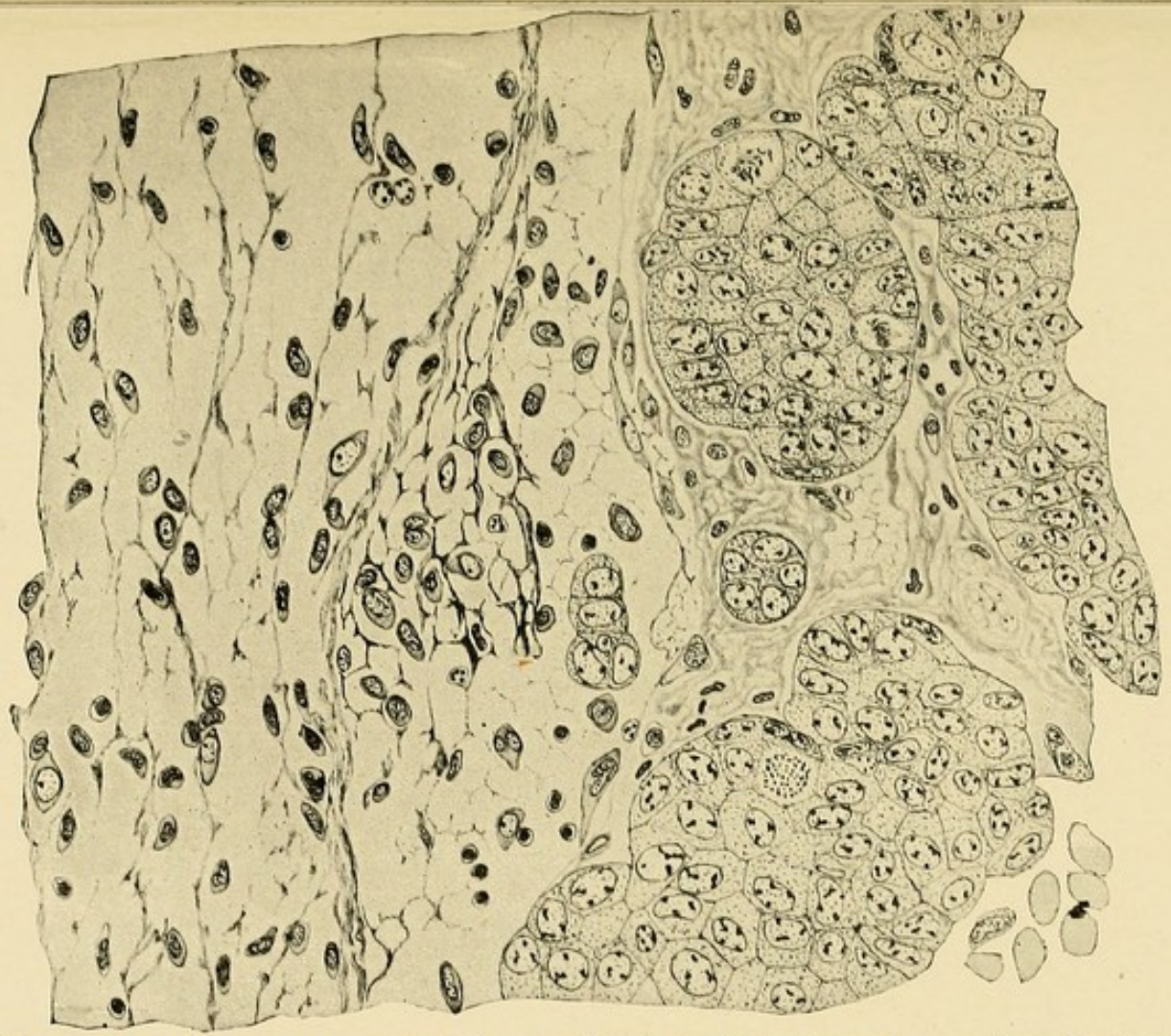
the nuclei had become small, irregular, and deeply staining, while fatty degeneration of the cytoplasm had set in. The capillaries of the graft with their blood corpuscles were still recognizable, although the endothelium showed degenerative changes. Outside of the graft fibrin filaments had appeared in the exudate and amitotic division of the connective tissue cells was still in progress, while spindle-shaped wandering cells had begun to penetrate the transplanted tumor. Three days after inoculation the cleft between the tumor and the host's tissues was almost obliterated, and wandering cells could be distinguished in the interstices of the graft, where they were dividing by mitosis. Mitotic division was in evidence, also, in the fibroblasts of the host nearest the tumor. In the transplanted stroma the collagenous fibrils had fused into homogeneous glassy bundles and the connective tissue cells exhibited, beside chromatolysis, unmistakable fatty degeneration of their protoplasm. No development of new blood vessels had yet occurred, and the whole mass of transplanted tissue remained without vascular supply. Four days after transplantation, however, an ingrowth of capillaries had taken place and vascularization was in such rapid progress that all stages of new capillary formation might be found in the same specimen. Fibroblasts from the host were streaming into the tumor from every side, some of them appearing to exercise a phagocytic function and finally to degenerate, while others survived to form the new stroma. The old connective tissue still remained, but was in the last stages of degeneration. From four days onward there was a continual recession of the stroma originally introduced, and an orderly progress of vascularization until, by the eleventh day, fibrils of new-formed collagen could be found in the new stroma which, at the eighth, had been very cellular.

Thus the cancer cells manifested the faculty of continuous growth and a power to make the tissues of the new host subservient to their needs. The proliferative power, however, attached only to the parenchymal cells, and was not acquired by any of the elements with which they had come in contact in successive hosts.

This *stroma reaction* was shown further to be a specific one. That is to say, the character taken on by the newly formed scaffolding was determined not by the reacting tissues themselves, but by some unknown influence exerted upon them by the parenchymal cells, which



Graft of Jensen's carcinoma, removed 24 hours after transplantation. Beginning degeneration of introduced stroma. $\times \frac{400}{1}$.



Graft of Jensen's carcinoma, removed 36 hours after transplantation. Progressive degeneration of introduced stroma and proliferation of surrounding connective tissue. $\times \frac{400}{1}$.

Graft of Jensen's carcinoma, removed 3 days after transplantation. Active proliferation in surrounding connective tissue. $\times \frac{400}{1}$.

were able to force the tissues of each new host to furnish a stroma typical for the tumor to which they belonged.

Ehrlich,[1] in accepting the stroma reaction, assigned to the tumor cell two specific powers, one of which acted upon fibroblasts, the other upon angioblasts. The former property was exercised by the transplantable carcinomata and sarcomata while the latter was very distinct in a transplantable chondroma, the cells of which could, however, be robbed of their chemotactic action upon the angioblasts by exposure to heat or cold or by transplantation into an animal highly resistant to the growth. In such cases the tumors proliferated without the participation of the vascular system, but grew slowly, were white in color, and not infrequently necrotic.

Gierke[2] also was of the opinion that differences could be discerned in this reaction in accordance with the varying extent to which the connective tissues and blood vessels responded to stimulation, and described a *fibroplastic* and an *angioplastic* type. If the two influences were in equilibrium well-nourished carcinomata would result, but should the fibroplastic type predominate nutrition of the cell nests would suffer, and in this way might be explained the necrosis of part or all of a neoplasm. If the angioplastic sway preponderated there would be formed a rich supply of vessels and but little connective tissue, a condition which had been described by Apolant in the case of the hemorrhagic tumors.

INCREASE OF VIRULENCE OR ADAPTATION?

Although all observers have agreed that once a tumor has been transplanted it is the rule for it to yield a gradually increasing number of successes upon protracted cultivation, it has not been decided whether this is to be looked upon as an increase in virulence on the part of the cancer cell, or as an augmentation of its power to adapt itself to a new host. The former hypothesis was advanced by Ehrlich and has been consistently upheld by him, while the latter has found defenders in Bashford and the adherents of his school.

[1] *Zeitschrift f. Krebsforsch.*, 1907, v, 70.

[2] *Beitr. zur. path. Anat.*, etc., (Ziegler), 1908, xliii, 340.
Third Sci. Report, Imperial Cancer Research Fund, London, 1908, 129.

Ehrlich[1] held it possible to achieve increased or even maximal virulence by transplanting at short intervals the rapidly growing tumors in each series. In this way he had obtained tumors with an energy of growth that had never been equalled either in the laboratory or in clinical experience, although such a result could not be attained with every strain. An analogy for the increase of tumor virulence could be found in bacteriology where it was necessary, in order that the virulence of a culture might be maintained, to subject it to continual *passage* through animals or to transfer it frequently to a favorable medium.

Apolant[2] distinguished sharply between two factors comprised in the conception of virulence — *transplantability*, measured by the number of daughter tumors, and *proliferative energy*, evaluated by the rate of growth. While in general slowly growing tumors gave a meager, and rapidly growing a generous outcome after transplantation, there were exceptions to the rule, among which Ehrlich's propagable chondroma was a striking example, for although this growth had yielded from the first a maximal number of daughter tumors, the proliferative energy of its cells had remained extremely feeble in spite of several years of cultivation. Notwithstanding the fact that it had been possible with many tumors to augment both the transplantability and the energy of proliferation, such a transmutation could not always be brought about, certain strains showing individual peculiarities as regarded the increase of their virulence. These differences were exhibited both in the degree to which transplantability could be stimulated and in the rapidity at which the optimum inoculation result was reached, but the rate of growth apparently could be hastened only to a certain point specific for each individual strain. It was at its lowest in the chondroma, variable among the carcinomata, and enormous among the sarcomata, the tumors last named often reaching within three or four weeks a size equal to that of the mouse in which they were growing. Such energy of growth was without analogy in human oncology.

Apolant did not think that Bashford's hypothesis of *adaptation* sufficed to explain either the fact that tumors could be made to grow

[1] *Arb. a. d. Königl. Inst. f. Exp. Therap.*, 1906, Heft i, 80.
Zeitschrift f. Krebsforsch., 1907, v, 62.
[2] *Zeitschrift f. allg. Physiol.*, 1909, ix, Sammelreferat, 69.

after a time in strange races of mice, or that it was possible to achieve an increase in virulence. Conceptions of the real nature of these phenomena had been hitherto very vague, and words rather than ideas had been employed in explaining them. It was accordingly very gratifying to find that the question had been simplified through the following investigations carried out upon trypanosomes by Ehrlich, in common with Röhl and Fräulein Gulbranson.

If an infected mouse were treated with one of the newer and very active arsenic preparations, but without being given an amount quite large enough to cure it entirely, the trypanosomes would disappear from the blood for a considerable time and, through the absorption of the parasites killed by the medicament, there would occur the elaboration of an abundant quantity of antibodies. But, as all the parasites had not been destroyed, recurrence would take place sooner or later, the advent of which could be explained by one of two possibilities — either that the antibodies had disappeared, or that the trypanosomes had become proof against them. Actually the latter was what happened, for if one introduced trypanosomes cultivated during a recurrent attack into mice which, having been cured of an infection, enjoyed the possession of antibodies, these injected organisms would be able to flourish as vigorously as in normal animals, although inoculation of the immune mice with their original strain would be at first unsuccessful, because this strain would have become susceptible to the action of the antibodies which had conferred immunity on these mice. There was here in play a fundamental, hereditary, biological alteration, the nature of which Ehrlich had explained as follows. The original parasites had a certain type of nutriceptor (a receptor for assimilating nutriment), A, which, after having been killed, acted as antigen in producing an antibody which could only be fastened by this group. If all the A-receptors of a parasite were occupied by antibodies and thus eliminated from the absorption of nourishment, the cell died, unless through the stimulation of hunger a new group, B, already potentially present and unrelated to the antibody A, could be produced. The trypanosome that formerly obtained food through its A-nutriceptors now nourished itself by means of its B-receptors, so that there had occurred the disappearance of one class of receptor in favor of a new one.

But in the same cell there might be still other potential rudiments and Ehrlich had, in fact, succeeded in provoking in one species of trypanosome ten different kinds of receptor. Generally, however, only one group was produced — A *or* B *or* C, etc. This was the *unio* type; when two or more groups were elicited, A *and* B *and* C, etc., the varieties were called *binio*, *ternio*, etc. If now a binio containing, for example, groups A and B, were to attract a single antibody, that is, only antibody A or antibody B, death would not occur and the binio would live on with the other receptors. It followed, therefore, that the antibody in such a case would have no deleterious effect except in so far as it blockaded one source of nourishment; and, as from one trypanosome ten different stable unios might be evolved, it must be true that this parasite was able to obtain its nutrition in ten different ways in the mouse alone. The observation demonstrated that one single occurrence could produce a persistent change in the cell protoplasm. An alteration like this would be sufficient to confer on the cell and its descendants a permanent increase in growth energy, for to a case of this sort Weigert's law of the hypercompensation of damaged functions was quite applicable. The disappearance of one type of receptor, A, represented a cell injury which was overcompensated by the abundant production of other receptors. A substance able to bring about immediately such a transformation Ehrlich had called a *growth substance* ("Wuchsstoff"), contrasting it with the *food-stuffs* ("Nährstoffe"), which, after assimilation, maintained the constant growth of the cells, and of which ten varieties had been demonstrated for the trypanosomes.

These conceptions could be transferred to the study of malignant growths without further formality. In a tumor cell there must be present a number of potential rudiments beside the actual nutriceptors suited to the food offered by the affected animal. If the tumor were transplanted to another host, three occurrences were possible: either the new soil would afford the same nourishment as the old and the tumor could accordingly grow; or the host would proffer food that none of the nutriceptors was able to grasp, in which case transplantation would be unsuccessful; or, finally, there might be tendered food not exactly like the old, but for which there were present at least the potential rudiments of nutriceptors. In this case those cells would die in which the

development of potential nutriceptors had not occurred with sufficient promptitude; but the cells which had been stimulated by hunger to produce fresh nutriceptors would be able to survive in the new host, and, furthermore, their proliferation through succeeding generations would be assured, the newly acquired properties being hereditary as they were in the trypanosomes.

Apolant considered this to be an exact scientific explanation for the sudden transformations so frequently seen after the introduction of tumors into strange races, transformations which, as with the trypanosomes, might include a loss of sensitiveness toward the original race. The conception explained also increase of virulence for, through an excess of nutriceptors produced in answer to the stimulus of hunger, there were assured both an easier establishment and a more vigorous proliferation in the new host.

The English school, headed by Bashford, preferred the term *adaptation* to *increase of virulence*. Bashford[1] thought that objective statements should be substituted for loose descriptions like "virulent" or "avirulent," and that the initial dose should be stated, since it so largely determined the size of the resulting tumor. When this dose was unknown no judgment of the growth rate was possible.

Murray[2] called attention to the reservations which should be made in comparing the growth of transplantable carcinomata to the cultivation of a pathogenic micro-organism through a succession of susceptible animals. The augmentation in the percentage of successful inoculations and in the speed of growth could be referred with a high degree of probability to the rapidity with which the parenchymal cells adapted themselves at this stage. In consequence of such adaptation, a progressively larger number of cells survived the injuries inseparable from the act of transplantation, and the size attained by the tumors in equal periods of time was, therefore, greater. Once this preliminary stage had been passed the rate of growth and percentage of success fluctuated between somewhat wide limits.

Bashford, Murray, Haaland, and Bowen[3] had found that the initial transfer of a malignant new growth from the spontaneously affected

[1] *Third Sci. Report, Imperial Cancer Research Fund*, London, 1908, Introduction, xxiii.
[2] *Third Sci. Report, Imperial Cancer Research Fund*, London, 1908, 161.
[3] *Third Sci. Report, Imperial Cancer Research Fund*, London, 1908, 269.

animal to the first series of normal ones was in most cases attended with great technical difficulties, and they believed that this was the result of a failure by the tumor cells to adapt themselves to altered conditions of existence upon their removal from the animal in which they had first acquired malignant properties, and their introduction into normal ones. With successive transplantation, however, there came an increase in the number of cells surviving and proliferating

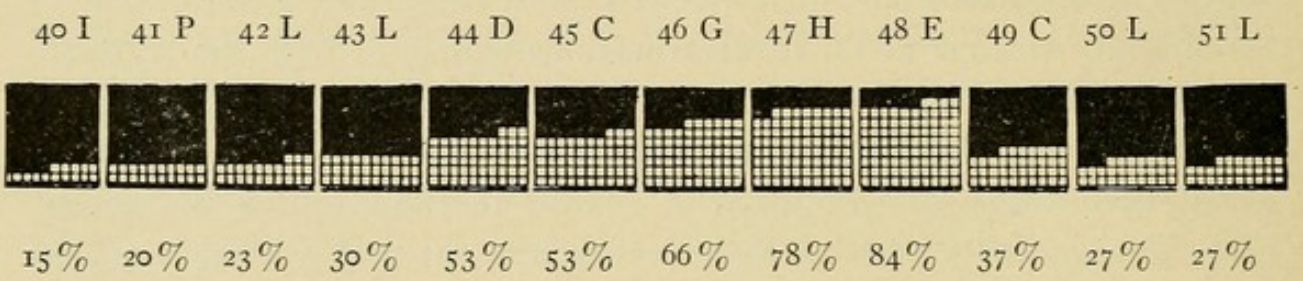


FIG. 1.—Diagram to illustrate the way in which the elimination of degenerating cells by repeated transplantation may result in a progressive increase in the percentage of success in a strain of transplantations. Each large square represents the constitution of the parent tumor of the batch of inoculations whose label is printed above it, as measured by the percentage of success printed below. One hundred inoculations are supposed to be made in every case, and the number of small squares left clear, corresponding to the percentage, shows the number of fragments which developed into tumors.

in each graft. Whether this were due to the elimination of cells less able to conform to varying conditions, or, on the other hand, to the acquisition of greater adaptability, growth power, and resistance to injury, by cells at first barely able to survive, was not of any great moment. It seemed probable that both factors were realized in propagation, and if the authors inclined to ascribe more importance to the former it was only because on that assumption a greater number and range of facts could more easily be harmonized and brought under review.

Bashford, Murray, and Cramer,[1] during the propagation of Jensen's tumor and other growths, had frequently obtained series of inoculations with a maximal percentage of success. The method of repeated subdivision of the parenchyma into the small grafts necessary for the analysis of the phenomena of proliferation showed that each maximum

[1] *Proc. Roy. Soc.*, Series B, 1907, lxxix, 164.
Third Sci. Report, Imperial Cancer Research Fund, London, 1908, 325, 336.

was followed by a rapid and great diminution in the percentage of success and that this, in turn, was succeeded by an increase to a fresh maximum. By choosing a suitable interval for the inoculation of tumors which had been selected from series with from 90 to 100% of success, and especially by increasing the initial dose, the authors had been able to evade for a considerable number of transferences the diminution usually following each maximum. This outcome, however, was artificial, and indicated neither an increased "virulence" of the tumor cells nor a uniform energy of growth.

They ascribed great importance to the nature of the inoculated material. Where small doses gave a maximum success, large doses might do so as well and produce very much bigger tumors in the same time; but on the other hand, in those cases where growth could be inhibited by the simultaneous absorption of tumor, the larger doses would be much less successful. The absorption of tumor tissue was inversely proportional to the number of cells growing in the graft, and whereas full doses of cells in the positive phase of growth led to large tumors and no immunity, a similar amount in the negative phase yielded very few tumors, permitted of much absorption, and produced a high proportion of resistant animals. Thus the "virulence" of the cells of a single tumor fluctuated between negative and positive phases of growth energy.

Spontaneous tumors varied considerably in the ease with which they could be propagated, and these differences had been regarded as degrees of virulence. Nevertheless there was a certain amount of evidence to show that such tumors fluctuated in growth energy in a manner similar to transplantable growths. Any direct conclusion regarding the "virulence" of a spontaneous tumor drawn, therefore, from one primary transplantation, might be upset when the same growth gave an opposite result, *i.e.* when, after having recurred, it had been transplanted a second time.

The term "virulence" was for this reason unfortunate, and had to be used with so much reservation that it would be better discarded altogether.

STIMULATION OF GROWTH POWER

A few observers have reported an increase of proliferative energy on the part of tumor cells exposed to various physical or chemical influences.

Thus Clowes and Baeslack [1] found that neoplasms of relatively low growth power might be so stimulated by incubation at a temperature of 39° to 41° C. or by exposure to mercuric chloride, potassium cyanide, ammonium fluoride, and mercuric iodide, in solutions of suitable concentration, as to afford a larger yield of more rapidly proliferating growths. The curious paradox was noted that in the case of highly virulent tumors incubation decreased the growth energy.

Ehrlich,[2] in an endeavor to reproduce the phenomenon described by Clowes and Baeslack, exposed three tumors to 39° C. for twenty minutes, but without being able to observe any increase in their capacity for growth.

Exposure to a temperature of 44° C., according to Michaelis,[3] favored the growth of tumor fragments. Furthermore, as Hertwig had shown that sea-urchin eggs subjected to the action of a narcotic developed irregularities in their division figures, and v. Hansemann had described irregular mitoses in the cells of malignant growths, Michaelis investigated the action of chloral hydrate upon the cells of a mouse tumor, and found that in weak solution it increased their growth power.

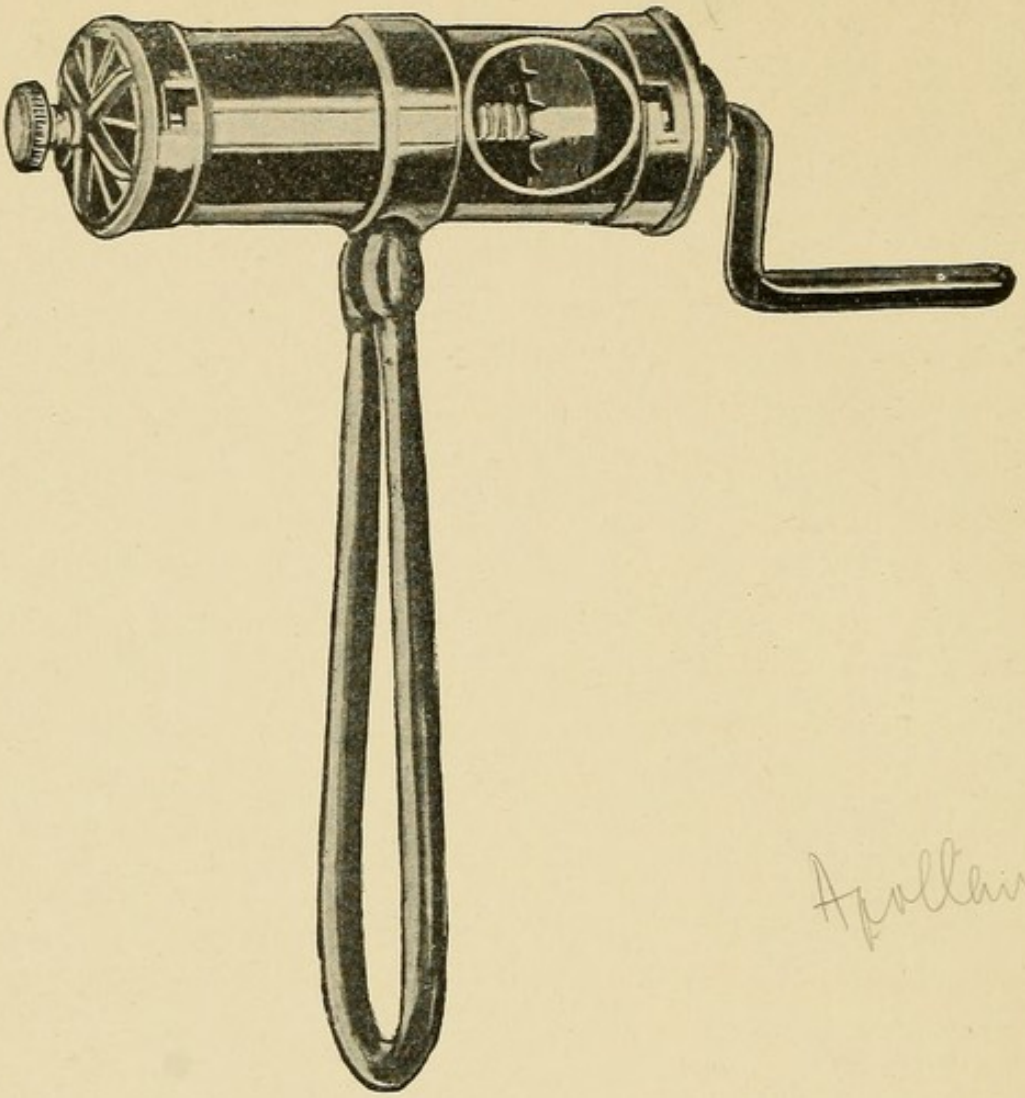
TECHNIC OF INOCULATION

The methods generally employed for inoculating a tumor have been to inject it with a syringe or a Pasteur pipette after reducing it to an emulsion, or to break it into small fragments and inoculate these with a hollow needle. Jensen [4] tried both of these plans, and thought that the results of the latter were more reliable.

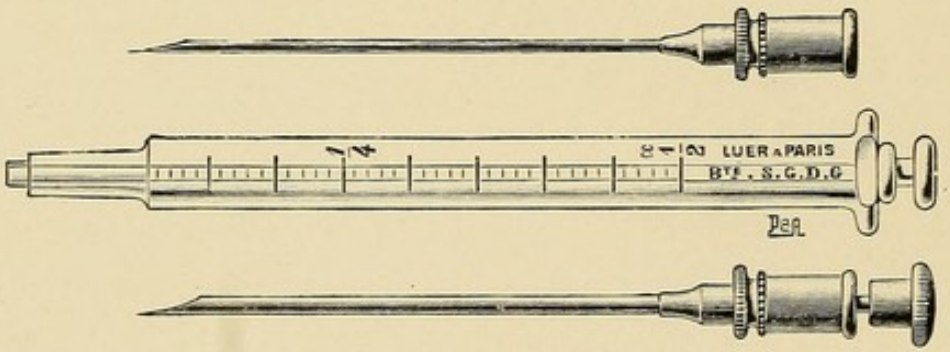
At the Pasteur Institute, where the two methods have been employed in Borrel's laboratory, both Haaland [5] and Bridré [6] found that the results following the needle method were the more satisfactory.

[1] *Jour. Exp. Med.*, 1906, viii, 486.
British Med. Jour., 1906, ii, 1548.
[2] *Zeitschrift f. Krebsforsch.*, 1907, v, 66.
[3] *Zeitschrift f. Krebsforsch.*, 1907, v, 194.
[4] *Centralbl. f. Bakt.*, etc., erste Abt., Orig., 1903, xxxiv, 124.
[5] *Ann. de l'Inst. Past.*, 1905, xix, 187.
[6] *Ann. de l'Inst. Past.*, 1907, xxi, 762.

Graft of Jensen's carcinoma, removed 4 days after transplantation. Late stage in degeneration of introduced stroma and early stage of vascularization. $\times \frac{400}{1}$.



Small mincing machine devised by Haaland for emulsifying firm tumors. Natural size.



All-glass syringe of 0.5 cubic centimeter capacity and graduated to 0.01 cubic centimeter, employed to inoculate accurate doses of tumor emulsion. Four-fifths natural size.

Hollow platinum-iridium needle for the inoculation of intact tumor fragments. Natural size.

In Ehrlich's institute,[1] on the other hand, the emulsion method has been found preferable, and the technic there employed was described as follows. The mouse bearing the tumor for inoculation is decapitated with scissors, placed in alcohol-sublimate, and, having been rinsed off in alcohol, is laid out on a board freshly cleansed with sublimate solution. The tumor is removed with sterile instruments, chiefly by blunt dissection, minced with scissors and forceps, and then broken down in a mortar into an emulsion without the addition of any extraneous fluid. Inoculation is done with capillary pipettes carrying a stopper of cotton wadding at the mouth end, the pipette being entered at a point over the lower abdomen which has been shorn and washed with alcohol, and the emulsion deposited in the axilla. Absolute asepsis must accompany all the steps of the operation.

For the more ready preparation of tumor emulsions, Haaland[2] devised a small mincing machine constructed on the principle of the ordinary meat grinder.

In Bashford's laboratory[3] both needle and syringe have been employed. Of the two methods, that in which small fragments of tumor were introduced intact by means of a hollow needle was preferred, for the reason that it caused less damage to the tumor cells, a fact which explained the superiority of the results achieved. Although accurate doses could be administered by means of this method under certain conditions, the English workers, reflecting on the dissimilar results obtained in various laboratories, felt the necessity for a method which would permit of more uniform dosage without serious damage to the cells. The following procedure was accordingly adopted. After removal the tumor was reduced to an emulsion, which in the case of firm tumors was most conveniently effected with Haaland's mincing machine, although softer growths could be emulsified by repeatedly clipping them with sharp scissors. The Pasteur pipette, which did not permit of accurate dosage, was replaced by a small all-glass syringe of 0.5 cubic centimeter capacity, calibrated to 0.01 cubic centimeter. A hypodermic needle of slightly wider bore than that used in a serum syringe was fitted to the nozzle,

[1] *Arb. a. d. Königl. Inst. f. Exp. Therap.*, 1906, Heft i, 79.

[2] *Berl. klin. Woch.*, 1907, xliv, 714.

[3] *Third Sci. Report, Imperial Cancer Research Fund*, London, 1908, 267.

and the injections were made in the axillary region or along the flank by introducing the needle into the groin, pushing it toward the axilla, and injecting the emulsion during withdrawal. Between injections the needle was wiped carefully on a pledget of sterile cotton-wool moistened with absolute alcohol. It was unnecessary, except in special cases, to epilate the site of inoculation, although it might with advantage be moistened with alcohol.

With a hollow needle,[1] the dose could be varied between 0.005 and 0.03 gram.

Bashford, Murray, Haaland, and Bowen[2] recorded that the doses used by them in many thousands of experiments varied between 0.005 and 0.03 gram where a tumor fragment was inoculated by means of a needle, and between 0.025 and 0.25 cubic centimeter where an emulsion was injected with a syringe. While practically all spontaneous mammary tumors of the mouse could be induced to grow by inoculating large numbers of young animals with small intact fragments, the number yielding a successful result was much smaller when 0.05 to 0.1 cubic centimeter of tumor emulsion had been inoculated.

The foregoing authors, therefore, as well as Murray[3] and Gierke,[4] were of the opinion that variations in success were to be explained by technical differences, pointing out that the conversion of a tumor into an emulsion involved considerable mechanical injury to the parenchyma, and, furthermore, that the inevitable absorption of part of a large dose of emulsion might readily be responsible for the evolution of sufficient acquired immunity to prevent the establishment of the tumor.

According to Bashford[5] the optimum conditions of transplantation for different strains could only be reached after trial and error more or less prolonged. Most tumors were best transferred by the frag-

[1] These platinum-iridium needles are provided with plungers which fit closely enough to allow the tumor fragment to be aspirated by negative pressure upon their withdrawal. The needles are sterilized between inoculations by being flamed off with alcohol. (W. H. W.)

[2] *Third Sci. Report, Imperial Cancer Research Fund*, London, 1908, 269.

[3] *Third Sci. Report, Imperial Cancer Research Fund*, London, 1908, 105.

[4] *Beiträge zur path. Anat.*, etc., (Ziegler), 1908, xliii, 332.
Third Sci. Report, Imperial Cancer Research Fund, London, 1908, 119.

[5] *Fourth Sci. Report, Imperial Cancer Research Fund*, London, 1911, 137.

ment method, and some could be propagated continuously only by this means. Others, again, gave consistently better results when larger doses of emulsion were inoculated. The intervals between successive transplantations were of great importance, for while some tumors could be cultivated only by rapid *passage*, in the case of others this was unsuitable. The influence of short intervals was particularly noticeable in the frequent experience that rapid *passage* might lead to a temporary exhaustion of the tumor cells, from which they could recover only after a prolonged sojourn in one animal. It was not always the most rapidly growing tumors that could be transplanted at the shortest intervals, and a permanent improvement in the rate of growth and the inoculation percentage might merely indicate that the optimum interval for grafting had been selected, either deliberately or accidentally.

Other means of implantation have been employed, although less extensively. Thus Hertwig and Poll,[1] as well as Stahr,[2] imbedded fragments in small incised wounds which were sutured afterward with silk. In Gaylord's laboratory the method preferred was, as described by Clowes,[3] emulsification of the tumor in a mortar with the addition of salt solution and removal of the connective tissue residue by means of fine rakes.

Relative Importance of Soil and Graft

It was found, however, that in spite of the most careful technic, transplantation was not always successful, and that in some animals the tumor cells, unable to proliferate, underwent final absorption.

Thus Jensen [4] had attempted the transplantation of several tumors before he succeeded in getting one to develop, and Borrel [5] found that even those growths which could be successfully transferred might yield but about 10 % of daughter tumors.

Ehrlich[6] wrote that only a small proportion of spontaneous tumors were propagable, and that of one hundred and eight such growths

[1] *Abhandl. d. Königl. Preuss. Akad. d. Wissenschaften*, 1907, 6.
[2] *Centralbl. f. allg. Path.*, etc., 1909, xx, 869.
[3] *British Med. Jour.*, 1906, ii, 1549.
[4] *Centralbl. f. Bakt.*, etc., erste Abt., Orig., 1903, xxxiv, 29.
[5] *Ann. de l'Inst. Past.*, 1903, xvii, 112.
[6] *Zeitschrift f. Krebsforsch.*, 1907, v, 61.

only about 8 % had been cultivated continuously. The hemorrhagic cyst-adenomata seemed to resist all efforts at transplantation.

Bashford, Murray, and Cramer,[1] however, succeeded in transplanting hemorrhagic tumors, and ascribed their success to the fact that they had implanted small fragments into a large number of mice instead of inoculating large doses into a few animals.

But even though the most suitable dose be chosen the outcome is still uncertain, for it is determined not alone by the power of the malignant cell to proliferate in a strange host. Another condition for continuous growth is that the new animal shall offer a proper soil.

Loeb[2] found that, as a general rule, when two tumor fragments were transplanted into a rat, either both of them grew or else neither one did, and this, he thought, indicated differences in the soil rather than in the grafts introduced.

The relative importance of these two factors was investigated also by Bashford, Murray, and Cramer,[3] who inoculated the members of one group of mice with a single graft each and those of a second series each with five fragments of tumor. If idiosyncrasy of individual mice alone determined whether a tumor would grow or not, the percentage of success per animal should be the same in both series, with multiple tumors in the successful cases; but the series with multiple inoculations gave a higher percentage of success than those with single implantations. The authors accordingly attributed a minor importance to the influence of the soil in determining success or failure, and a much greater to the introduced cells. This decision received additional support from the constancy with which different sporadic tumors gave a uniformly low percentage of success or even completely negative results, while others yielded a much higher outcome in most cases. The authors were driven to the same conclusion by the subsequent behavior of the different descendants of one and the same tumor, when one series grew quickly and another slowly, with high and low percentages of success, especially since this result might be reversed in subsequent series. Even more remarkable was

[1] *Proc. Roy. Soc.*, Series B, 1907, lxxix, 170.
Third Sci. Report, Imperial Cancer Research Fund, London, 1908, 321.

[2] *Jour. Med. Research*, 1902, N.S., iii, 58.

[3] *Sci. Reports, Imperial Cancer Research Fund*, London, 1905, No. 2, Part ii, 51.

the disparity sometimes seen between two tumors arising alongside each other and derived from separate groups of cells at a single inoculation, one growing quickly and the other slowly. These same characters were again presented by the daughter tumors which occurred when the two growths were transplanted into new mice.

Importance of Uniform Dosage

The importance of accurate dosage was first suggested by Loeb,[1] emphasized later by Clowes and Baeslack,[2] and Gaylord and Clowes,[3] and has been upheld consistently by Bashford and his colleagues.

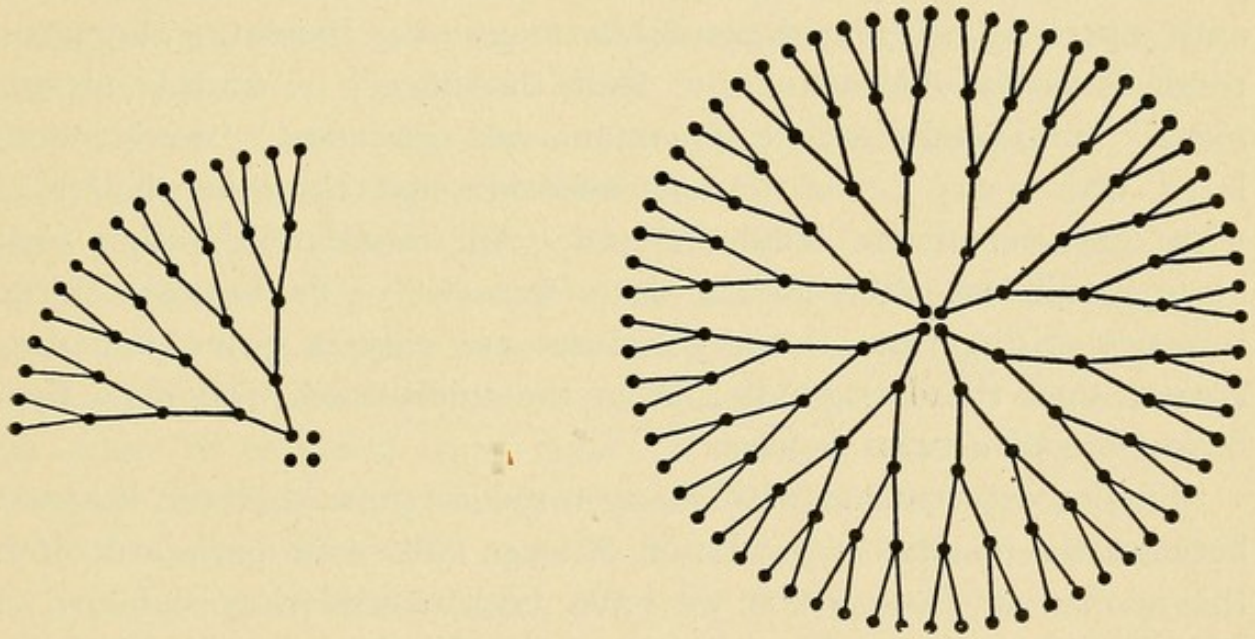


FIG. 2.— Diagram illustrating the effect on the amount of tissue produced of increase in the number of cells introduced or surviving after transplantation (effective initial dose), under conditions where the arithmetical factor alone is assumed to be of moment.

Thus:[4] "The size the tumours of any one strain attain in a given time is in part determined by the proportion of the introduced cells which adapt themselves to the new conditions: *i.e.* by the size of the *effective* initial dose. That this is the case can be demonstrated by experiments in which the initial dose of tumour material varies.

[1] *Jour. Med. Research*, 1902, N.S., iii, 59.
[2] *Med. News*, 1905, lxxxvii, 970.
[3] *Surgery, Gynecology, and Obstetrics*, 1906, ii, 634.
[4] *Third Sci. Report, Imperial Cancer Research Fund*, London, 1908, 273 *et seq.*

"The simplest phenomenon is encountered in the experiments with tumours which grow with a rapidity proportional to the initial dose introduced. A transplantable spindle cell sarcoma of the rat, for which we are indebted to Professor Jensen, illustrates the subject now under discussion in a diagrammatic manner. . . . The size attained by the tumours in the animals inoculated with the larger dose,[1] at the end of ten days, and their subsequent progress, present a striking contrast to those of the tumours arising from the small dose. They are nearly twenty times as large, and the animals succumb more quickly. It is important to note that the initial percentage of successes is the same in both series, viz. 100 per cent. . . .

"When experiments with *these doses* are made with other tumours, and especially with transplantable mouse carcinomata, the same result is rarely obtained. . . . Half the mice . . . of this experiment" (inoculation with a squamous cell carcinoma) "were inoculated with 0.025 c.c. of tumour emulsion, and the other half . . . with 0.15 c.c. of the same material. An initial proliferation took place in all, but although the doses were as 1 : 6, the sizes of the tumours arising from the larger doses are only in a few instances greater than those originating from the small doses, and even then little more than twice as large. . . .

"Similar experiments with many tumours show that the contrast between the results of inoculation of large and small doses are often the reverse of those which we have been considering so far. . . . This is the most usual result when mice are inoculated with large and small doses. Small doses grow progressively and well, whereas large doses, even when followed by a more pronounced proliferation, give tumours which remain stationary or disappear spontaneously. The natural resistance of the mice cannot be invoked to explain the anomaly. . . .

"The different behaviour of the tumours arising from larger and small doses, and the temporary diminution in size or disappearance after initial proliferation, must be referred to the effects of the absorption of more or less tumour material, inducing an adequate specific resistance of the animals in the one case and not in the other. The nature of the difference between tumours which support transplanta-

[1] The doses employed were 0.2 c.c. and 0.01 c.c. of an emulsion. (W. H. W.)

tion by large doses, and those which do not, is by no means clear. It is not merely a difference in capacity for independent life of the cells. . . . The strength of the specific resistance following absorption of the same quantities of different tumours seems to vary from one strain to another, as does also the susceptibility of the tumour cells to such altered resistance, and it is obvious that differences of this kind will manifest themselves also by varying susceptibility to dosage.

"This process, which may be described as a concomitant active immunization, is in part responsible for the low percentage of success attending the primary transplantations of sporadic tumours. The ratio of absorbed tissue in them is higher, and the initial proliferation is less, in conformity with the failure of adaptability. This effect is exaggerated when primary transplantation is carried out with large doses, and is diminished when minute grafts are introduced. Where mice are naturally resistant to the tumour inoculated, concomitant immunization greatly enhances it."

In a paper by Bashford, Murray, and Haaland,[1] there were described two experiments with the same tumor strain, in one of which progressively growing tumors were obtained from 0.05 cubic centimeter of tumor emulsion, but not from 0.1 cubic centimeter. In the second, large doses of emulsion (0.15 cubic centimeter) gave a higher percentage of more quickly growing tumors than did simultaneous inoculation of similar mice with a small dose (0.025 cubic centimeter). The explanation of this different behavior at different times was, the authors thought, to be sought in alternations of the biological qualities of the tumor cells and corresponding alternations in their vulnerability to unfavorable environment.

Equal emphasis was given in this paper to the importance of accurate dosage when tumors or normal tissues were used to produce the resistant state.

An amount of material much less than the smallest dose mentioned in the preceding paragraphs may give rise to a tumor. Uhlenhuth, Haendel, and Steffenhagen [2] described an experiment in which growth took place after the Jensen rat sarcoma had been rubbed into a scarified area in the skin, and another in which this growth was transferred

[1] *Third Sci. Report, Imperial Cancer Research Fund*, London, 1908, 364.

[2] *Arb. a. d. Kaiserl. Gesundheitsamte*, 1911, xxxvi, 477.

through the medium of a pin which had been plunged into it and afterward inserted under the skin of a healthy rat and immediately withdrawn. These results led them to try the transmission of tumors through the bites of bed bugs, stinging flies (*Stomoxys*), and leeches, but of six trials with bed bugs and flies and an equal number with leeches, not one was successful.

Importance of Uniform Soil

The desirability of using mice of the same age and race was pointed out almost coincidently by Bashford, Murray, and Bowen,[1] and by Clowes and Baeslack.[2] The former authors, having observed differences in the suitability of animals of different colors, even among the ordinary English tame mice, were convinced of the necessity of using the same race throughout any one experiment. The wild mouse, they thought, would probably offer more uniform conditions than the tame mouse, but a sufficient stock of uniform age was difficult to obtain and supervise. Besides having the mice of the same race it was important that they should be of the same age, preferably from five to seven weeks old. When the indicated precautions were observed, the individual variations in the general suitability of different mice of the same race and age were negligible, if implantation were performed at the same site, and provided sufficiently large numbers of animals were used.

Inoculation Site

In Bashford's laboratory,[3] the subcutaneous tissue of the back was at first elected, attempts to perform collateral series of intraperitoneal inoculations having been abandoned owing to the frequency with which growth within the peritoneum occurred secondarily from tissue implanted in the abdominal muscles. The axilla was finally[4] chosen, however, as the most suitable location for the following reason: "On

[1] *Proc. Roy. Soc.*, Series B, 1906, lxxviii, 196.
Third Sci. Report, Imperial Cancer Research Fund, London, 1908, 285.
[2] *Jour. Exp. Med.*, 1906, viii, 484.
[3] *Proc. Roy. Soc.*, Series B, 1906, lxxviii, 197.
Third Sci. Report, Imperial Cancer Research Fund, London, 1908, 286.
[4] *Proc. Roy. Soc.*, Series B, 1967, lxxix, 175.
Third Sci. Report, Imperial Cancer Research Fund, London, 1908, 327.

a series of 11 *passages*, it was found that the axilla was invariably a more suitable site than the dorsal subcutaneous tissue: 59 mice out of 286 developed tumours both back and front, 18 mice had tumours only on the back, and 59 only on the front. . . . The most natural explanation of the difference would seem to be that the connective tissue reaction . . . without which the grafts cannot grow, is more readily supplied by the connective tissue of the mammary region."

Ehrlich[1] also preferred the axilla, and routine inoculations were always made in that location.

Implantation has been undertaken in still other locations, as, for example, successfully in certain of the intra-abdominal organs by Goldmann,[2] and with indifferent success in the testicle by Flexner and Jobling.[3]

Interval after which Growth becomes Apparent

When implantation has been successful a tumor appears at the inoculation site after a certain period, which was set by Jensen[4] at about fourteen days, or in exceptional cases six to eight days. Borrel[5] found small nodules after from twelve to twenty days, Clowes and Baeslack[6] after two to four weeks, and Michaelis[7] stated that one could tell by about the third week whether an inoculation had been successful or not, although occasionally the nodule might not be palpable until more than two months after implantation. Still, the appearance of the tumor may occasionally be delayed for as long as five and a half months,[8] eight months as described by Bridré,[9] or even between eight and ten months as recorded by Stahr.[10]

[1] *Arb. a. d. Königl. Inst. f. Exp. Therap.*, 1906, Heft i, 79.

[2] *Arb. a. d. Königl. Inst. f. Exp. Therap.*, 1906, Heft i, 79 (footnote).

[3] *Monographs on Medical and Allied Subjects*, Rockefeller Institute, New York, 1910, No. I, 35.

[4] *Centralbl. f. Bakt.*, etc., erste Abt., Orig., 1903, xxxiv, 125.

[5] *Ann. de l'Inst. Past.*, 1903, xvii, 113.

[6] *Med. News*, 1905, lxxxvii, 968.

[7] *Med. Klin.*, 1905, i, 204.

[8] Oral communication regarding a sarcoma of the rat, by Dr. J. A. Murray of the Imperial Cancer Research Fund, London.

[9] *Ann. de l'Inst. Past.*, 1907, xxi, 762.

[10] *Centralbl. f. allg. Path.*, etc., 1909, xx, 874.

It is essential to distinguish early tumors from inflammatory swellings, as has been pointed out by Clowes and Baeslack,[1] Bashford, Murray, and Cramer,[2] and Michaelis.[3] In the experiments of Clowes and Baeslack, inflammatory swellings frequently occurred soon after inoculation, but differed from malignant tumors either by undergoing absorption or by ulcerating out in the course of two or three weeks. A true tumor, on the other hand, developed somewhat later and was generally firmer than an inflammatory mass.

Inoculation of Stationary or Receding Tumors

Loeb[4] removed half of a stationary tumor from a rat and re-implanted it in the same animal, after which both the new graft and the remaining piece began to grow at a rapid rate. In this case it was suggested that the liberation of the tumor from the tension of its capsule might have permitted the resumption of proliferation. A fragment taken from a growth actually diminishing in size grew so well after inoculation into another rat that a number of tumors were afterward transplanted from it. The original tumor, however, continued to recede until only a few small nodules were left.

White and Loeb[5] later entered more fully into this question and found that, in general, the tumors resulting from the transplantation of stationary or receding growths did not have a proliferative activity equal to those descended from vigorously growing tumors.

In the opinion of Bashford, Murray, and Bowen,[6] diminished transplantability was due to a real alteration in the parenchymal cells, an inability to establish themselves in new hosts, coinciding with the spontaneous cessation of proliferation in an animal in which growth had already become established.

[1] *Med. News*, 1905, lxxxvii, 968.

[2] *Sci. Reports, Imperial Cancer Research Fund*, London, 1905, No. 2, Part ii, 63 (footnote).

[3] *Med. Klin.*, 1905, i, 204.

[4] *Jour. Med. Research*, 1901, N.S., i, 34.
Arch. f. path. Anat., etc., (Virchow), 1902, clxvii, 175.
Jour. Med. Research, 1902, N.S., iii, 47.

[5] *Centralbl. f. Bakt.*, etc., erste Abt., Orig., 1910, lvi, 488.

[6] *Proc. Roy. Soc.*, Series B, 1906, lxxviii, 208.
Third Sci. Report, Imperial Cancer Research Fund, London, 1908, 299.

Transplantation of Metastases

The lung metastases of four spontaneous tumors were transplanted by Murray,[1] with success in two cases.

Uhlenhuth and Weidanz[2] also attempted the transplantation of pulmonary metastases, but their mice died of sepsis before the completion of the experiment. A somewhat more encouraging issue attended their endeavors to cultivate lymph node metastases, and one tumor was obtained among four inoculations. Unfortunately, however, the mice into which this growth was implanted died of mouse typhus before the experiment was finished.

Gay[3] was successful in transplanting metastases of the Flexner-Jobling rat carcinoma, and ventured the opinion that there occurred after several generations an increase in the virulence of the resulting tumors which was exemplified by a greater proliferative activity and the production of wider spread metastases of a more pronounced epithelial type.

Inoculation of Tumor Mixtures

Mixtures of tumors have been transplanted in a few instances. Apolant and Ehrlich[4] recorded a series of inoculations with a mixture of three alveolar carcinomata and one malignant adenoma, in which the first generations showed no departure from the structure of an ordinary transplantable alveolar carcinoma. Sarcoma development occurred in this strain between the twelfth and fourteenth generations.

Haaland [5] mixed Jensen's carcinoma with a sarcoma from Ehrlich's laboratory, and obtained the carcinomatous strain pure again in Danish mice and the sarcomatous in Berlin mice. The Danish mice were susceptible to carcinoma and refractory to sarcoma, while the Berlin strain was highly susceptible to sarcoma and resistant to Jensen's carcinoma.

Ehrlich[6] and Apolant[7] described the results following the trans-

[1] *Third Sci. Report, Imperial Cancer Research Fund*, London, 1908, 106.
[2] *Arb. a. d. Kaiserl. Gesundheitsamte*, 1909, xxx, 441.
[3] *Proc. Soc. Exp. Biol. and Med.*, 1908–1909, vi, 74.
[4] *Berl. klin. Woch.*, 1906, xliii, 38. [5] *Berl. klin. Woch.*, 1907, xliv, 716.
[6] *Zeitschrift f. Krebsforsch.*, 1907, v, 67. [7] *Zeitschrift f. Krebsforsch.*, 1907–1908, vi, 251.

plantation of mixtures of carcinoma and sarcoma, carcinoma and chondroma, and chondroma and sarcoma. Mixed tumors in which both components were intermingled, and which were exactly analogous to the carcinoma sarcomatodes of v. Hansemann, were readily obtained by the inoculation of a mixture of sarcoma and carcinoma. It was much more difficult to produce a mixed tumor with combinations of chondroma and carcinoma, or sarcoma, because the elements varied so much in vitality and in proliferative energy that pure chondromata, carcinomata, or sarcomata, were usually the outcome of the experiment. But even when both components grew there was never the intimate commingling which would allow one to claim the production of a distinct new variety of tumor, for both remained isolated and each one produced its own typical form of neoplasm. It had been found that the inoculation yield was much less affected by low degrees of temperature than was the proliferative energy, and yet when sarcoma that had been exposed to -10° C. for periods even as long as nineteen days was inoculated together with chondroma, it was impossible to produce a mixed tumor with an equal distribution of the two components. There occurred merely two independent tumor tissues growing side by side — never an amalgamation into a distinct new type. The importance of these experiments for general pathology lay in the demonstration that a mixed tumor could result or persist only when the biological conditions of growth for the two components were approximately equal.

Resistance Offered by the Cancer Cell to Various Agents

Jensen[1] discovered that no growth followed the inoculation of crushed cells, and that the resistance which the cancer cell was able to oppose toward various injurious agents was perhaps somewhat lower than that displayed by the cells of normal tissue. While his tumor was still capable of successful transplantation after an exposure of about eighteen days to a temperature of 1° to 3° C., and of about twelve days to room temperature, it remained alive hardly twenty-four hours if kept at body heat. When warmed for five minutes at 47° C., or cooled for a few minutes at -20° C., the tumor was killed.

[1] *Centralbl. f. Bakt.*, etc., erste Abt., Orig., 1903, xxxiv, 129, 131.

Intense light (Finsen), in so far as it was able to penetrate the tissue, was fatal, and partial drying, or a five-minute exposure to a (¼ %) solution of carbolic acid, also robbed the cells of their power to proliferate.

Loeb[1] investigated the maximum temperature to which a rat sarcoma could be exposed without losing its power to grow. He found that fragments kept at 43° to 44° C. for forty minutes remained viable, as did one heated for twenty-five minutes at 43° C. and then for fifteen minutes at 45° C. On the contrary, pieces exposed for thirty minutes to a temperature of 45° C. as well as those exposed for a similar length of time to still higher degrees (up to 50° C.), did not grow in any single instance. Fragments of tumor kept on ice for five days were successfully transplanted, and it was thus clear that the tumor-producing factor did not materially lose in power after having been kept for this length of time at a temperature of from 2° to 4° C. That intact cells were necessary for growth was demonstrated by the negative result which attended the injection of filtered emulsions.

Michaelis[2] achieved a successful outcome from the transplantation of Jensen's tumor after it had lain in an ice-box for five days, and even after it had been exposed to liquid air for half an hour. Chloroform water, however, robbed the cells of their power to grow.

Moore and Walker,[3] several years later, observed that a half-hour's exposure to liquid air at a temperature of about −195° C. was not always fatal for cancer cells, while Gaylord[4] found that they could withstand this temperature for eighty minutes.

In the experiments of Clowes,[5] all tumor cells seemed to be destroyed at 45° C. Toward organic disinfectants, however, their resistance was very high. Treatment for an hour with mercuric chloride at a concentration of 1–3500 was insufficient to prevent the development of a small proportion of slowly growing tumors, and it was not until a strength of 1–2000 was reached that complete destruction of the cells was effected. They were killed also by mercuric iodide in a solution

[1] *Jour. Med. Research*, 1902, N.S., iii, 62.
Arch. f. path. Anat., etc., (Virchow), 1903, clxxii, 345.
[2] *Med. Klin.*, 1905, i, 204.
[3] *Lancet*, 1908, i, 226.
[4] *Jour. Inf. Diseases*, 1908, v, 443.
[5] *British Med. Jour.*, 1906, ii, 1549.

of from 1–2000 to 1–2500 and ammonium fluoride in a concentration of 1–1000, but potassium cyanide in an $\frac{N}{100}$ solution was unable to effect the death of either the Jensen or the Brooklyn tumor, although bacteria are entirely destroyed by an $\frac{N}{200}$ solution.

Ehrlich [1] considered that the maxima of temperature given by Jensen and Loeb represented the maxima for his own carcinomata and sarcomata. But the few minutes' exposure to −20° mentioned by Jensen as having sufficed to destroy his tumor was, in Ehrlich's experience, not enough, for he had often seen growth power preserved after exposure for forty-eight hours to a temperature of from 25° to 30° below zero C., and in one case he had been able to transplant a carcinoma kept for two years at 8° to 10° below zero. In the case of a chondroma he had been able to discover microscopic evidence of temporary growth in material heated for an hour at 50° C., an exposure which would have destroyed the cells of a sarcoma or a carcinoma, while a successful result had attended the transplantation of a chondroma that had been kept three days at the temperature of liquid air. In these experiments, however, as the vitality of the cells had been very seriously impaired, tumors larger than a pea were never obtained, and these gradually disappeared instead of growing progressively.

Bridré [2] found that mice developed growths only exceptionally after the inoculation of finely ground tumor, and never after the injection of an emulsion in saline solution (filtered or unfiltered), or of tumor dried or heated above 50° C. In short, it was necessary to inoculate intact cells.

From the work of Haaland,[3] it appeared that the cells of a sarcoma were better able to resist heat than those of a carcinoma, for he was able to purify a mixed tumor of its carcinomatous elements by exposure to 44° C. for thirty-five minutes or longer.

Lewin [4] was able to substantiate for a carcinoma of the rat all the statements which had been applied to mouse carcinomata, and for an

[1] *Zeitschrift f. Krebsforsch.*, 1907, v, 65.
[2] *Ann. de l'Inst. Past.*, 1907, xxi, 767.
[3] *Berl. klin. Woch.*, 1906, xliii, 40.
[4] *Zeitschrift f. Krebsforsch.*, 1907–1908, vi, 304.

artificial mixed tumor the observation of Haaland just cited. He succeeded also in repressing the development of keratin by exposure to 46° C. for a quarter of an hour.

The experiments of Uhlenhuth and Weidanz[1] yielded results quite analogous to those of other observers. Tumor cells were killed by an exposure to 56° C. for twenty-five minutes, but not by being subjected for the same length of time to 45° C.; nor was a several days' sojourn in an ice and salt mixture, at a temperature far below the freezing point, fatal to them.

Loeb and White[2] kept mouse tumor at 44° C. for varying lengths of time and plotted the results of inoculation in a series of curves depicting the growth energy, the latent period, and the number of receding tumors. The curves corresponded rather closely, and showed that up to a certain temperature the result was roughly proportional to the degree of heat applied. In all the curves there was a critical point between forty-five and fifty-five minutes, and at the end of the latter period fundamental alterations in the cell set in. The three properties above mentioned probably depended, therefore, upon one and the same factor of the cell protoplasm.

The cancer cell, however, may be prevented from growing by damage more subtle than any of the comparatively gross injuries so far described, for Haaland[3] showed that the action of radium was fatal to its life, although the anatomical structure remained unaltered.

COMPARATIVE GROWTH RATE OF THE MALIGNANT CELL

While much has been written in the past about the enormous proliferative capacity of the cancer cell it may very well be that such statements have been unwarranted, and that the true key to the nature of malignant growth lies in the direction of an explanation of the continuous, rather than the rapid, growth of the cancer cell. Thus the power of this cell to multiply proves but a sorry accomplishment when compared with the growth energy of the bacteria, organisms

[1] *Arb. a. d. Kaiserl. Gesundheitsamte*, 1909, xxx, 441.
[2] *Centralbl. f. Bakt.*, etc., erste Abt., Orig., 1910, lvi, 325.
[3] *Proc. Roy. Soc.*, Series B, 1909–1910, lxxxii, 297.
Lancet, 1910, i, 789.

which Minot [1] has estimated are able to add 1000% to their original weight within a few hours, while according to the same author the embryos of rabbits and other mammals, no less than those of birds, may be said with safety to grow at least 1000% a day.

A close comparison has been drawn by Bashford,[2] who weighed mouse embryos at different periods of gestation and estimated the rate at which growth took place after the embryo had reached a

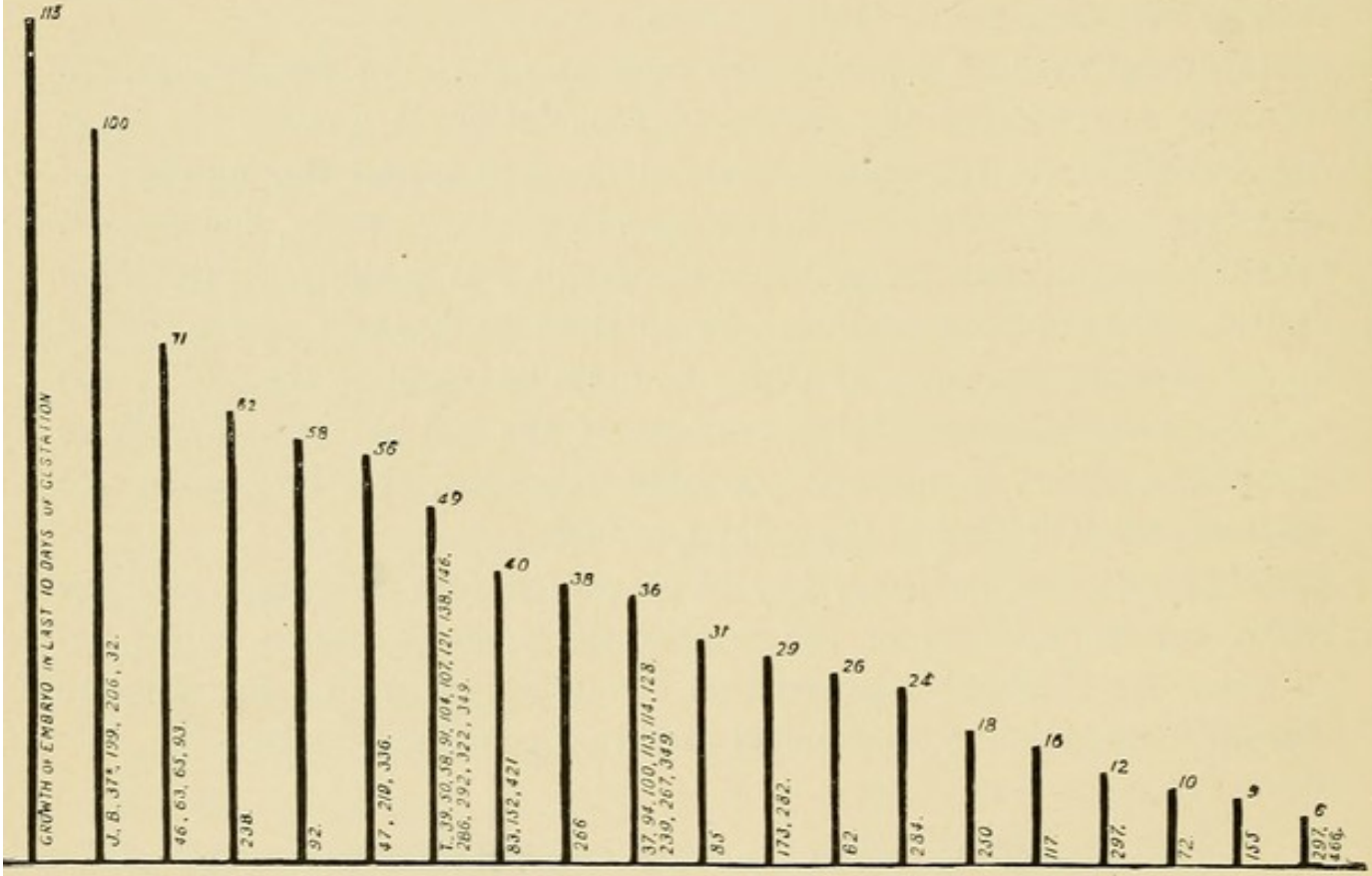
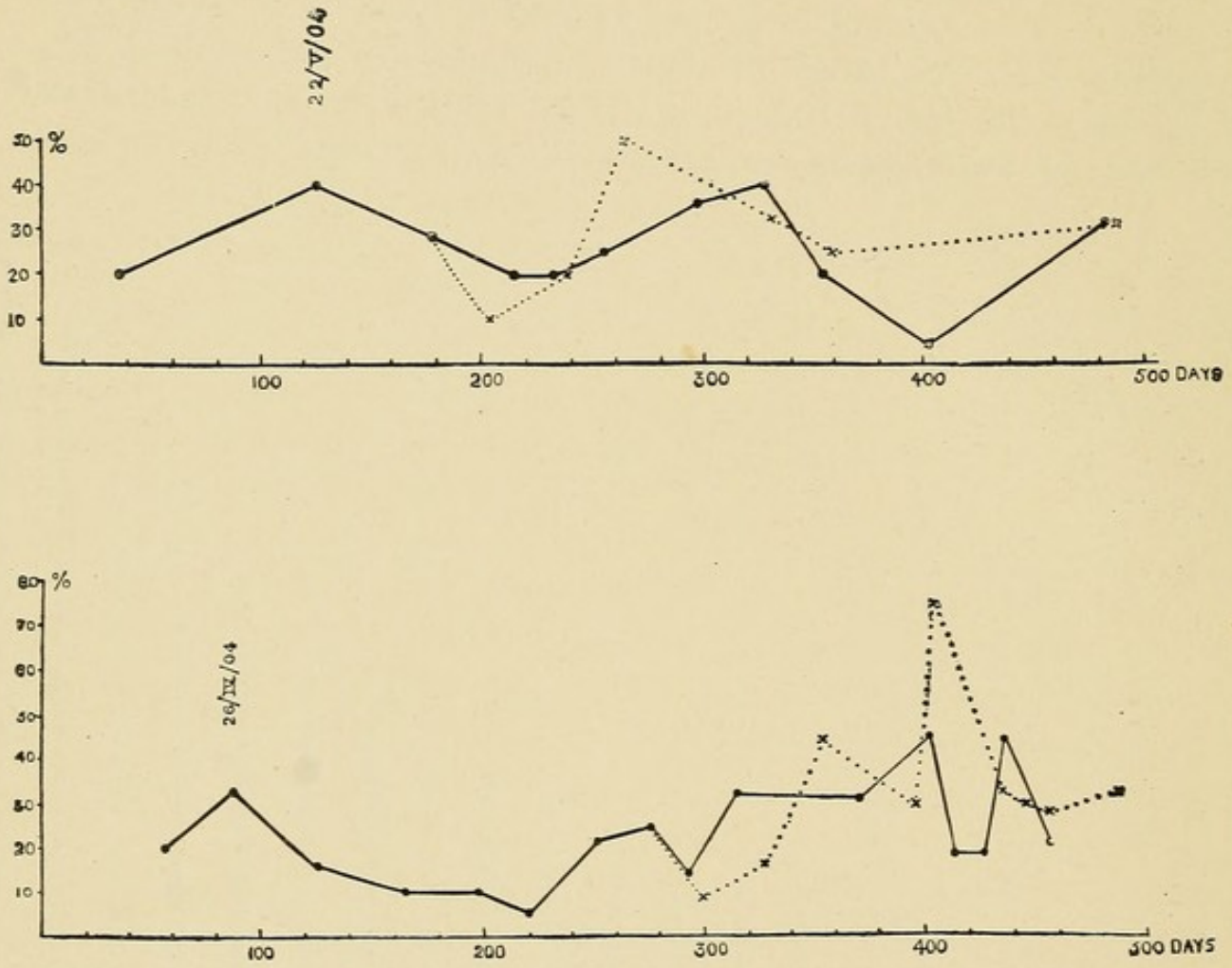


FIG. 3.—Diagrammatic comparison of rate of growth of various tumor strains on the basis of the number of days required to produce 1 gram of tissue from a measured dose (0.02–0.03 gram) inoculated. The rate of growth of embryonic tissue is indicated on the basis that the mouse embryo weighs 0.02–0.03 gram at the eleventh day of gestation and at birth 1–1.5 grams.

weight (0.02 to 0.03 gram) corresponding to the amount of tumor usually inoculated. When he compared the proliferative activity of the two tissues it became evident that, while some tumors might attain the rate of growth possessed by the embryo, most of them fell far below it.

[1] *The Problem of Age, Growth, and Death*, New York and London, 1908, 125.
[2] *Fourth Sci. Report, Imperial Cancer Research Fund*, London, 1911, 199.



Rise and fall in success of transplantation in four strains of Jensen's mouse carcinoma. The abscissæ represent the number of days during which the propagation of the several strains was continued. The ordinates show the percentages of success in consecutive series of sub-inoculations. In each figure the curve bifurcates at the time when two separate tumors of the original strain were used to start new strains. The time when growth was artificially interrupted by transplantation is represented by points and crosses for the two daughter strains of each figure. These curves represent experiments lasting for nearly 500 days, and carried out upon nearly 8000 mice. The results of the first transplantation of Jensen's tumor into English mice are not included.

The large fluctuations in the success of transplantation are similar in all four curves. Success is seen to increase through a series of successive inoculations until a maximum is reached. From this point onward the result diminishes until a minimum is arrived at, from which point the curve again rises to a maximum. The general correspondence in the four series can hardly be pure coincidence, and the conclusion appears justified that the power to establish themselves in new hosts varies periodically in the cells of this tumor, from inherent causes.

The occurrence of such periodic variations in the growth of tumors must be borne in mind in appraising the value of therapeutic measures.

FLUCTUATIONS IN GROWTH ENERGY

When tumor growth was studied over long periods of time in succeeding series of mice by Bashford, Murray, and Cramer,[1] it was found that:—

"The experimental propagation of malignant new growths leads to an apparently continuous proliferation which is merely artificially divided up by the process of transference to successive hosts. The limits of growth are not attained in any one animal, and transplantation again becomes necessary after intervals which vary according to the rate of growth of the tumour or to the degree in which the animal suffers from the presence of the tumour or from intercurrent disease. Thus the time of transplantation does not possess the importance of a natural starting-point for the growth of the tumour which follows it, neither does it coincide with a terminal stage of the growth with which transplantation is effected. . . . While for any one sporadic tumour the average percentage of success of a large number of experiments is fairly constant through many generations, considerable variations in success attend the inoculations of one and the same tumour at different times. The variations in success are frequently accompanied by differences in the rate of growth of the tumour and do not remain constant for their descendants in further transplantation. Thus one series of inoculations may give a small percentage of slow-growing tumours which at a subsequent period may begin to grow rapidly, or on transplantation while still growing slowly give a high percentage of quickly growing tumours.

"These variations in the behaviour of different sporadic tumours, therefore, indicate differences in degree rather than in kind, comparable to those which may obtain in the different descendants of the same tumour. . . ."

In addition to variations in the histological characters of transplanted tumours, gradual fluctuations in the rate of growth and percentage of success were found when the results of long-continued experiments were compared.

"These curves represent experiments lasting for nearly 500 days, and give the results obtained on nearly 8000 mice. . . .

[1] *Sci. Reports, Imperial Cancer Research Fund*, London, 1905, No. 2, Part ii, 48 *et seq.*

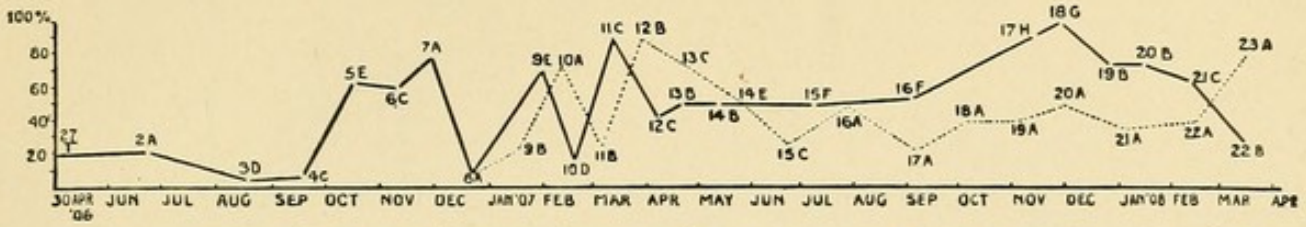
"The large fluctuations in the success of transplantation are similar in all four curves. Success is seen to increase through a series of successive inoculations till a maximum is reached. From this point onwards success diminishes till a minimum is arrived at, from which the curve again rises to a maximum. The fall in the curves coincided with the occurrence of numerous negative series of transplantations in daughter strains. . . .

"The conclusion appears justified that the power of establishing themselves in new hosts varies periodically in these tumour cells from inherent causes."

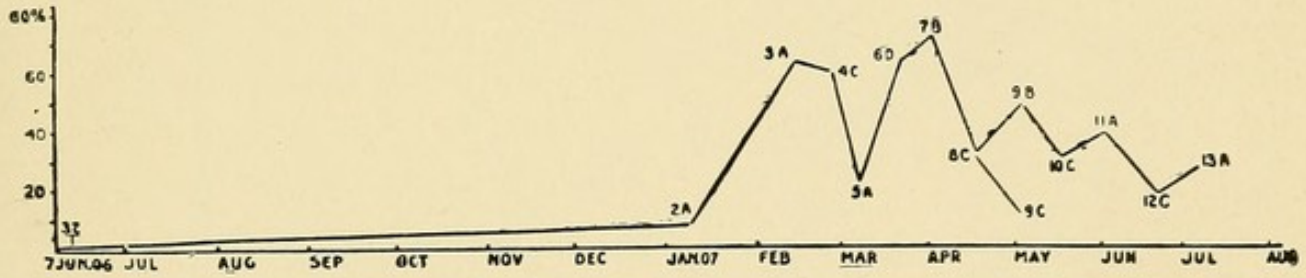
In a later paper, Bashford, Murray, and Bowen[1] analyzed more minutely the fluctuations in growth energy. When all precautions were taken to insure accuracy of dosage, similarity of soil, etc., fluctuations independent of these factors still appeared, and the authors believed that they were, in all probability, natural features of proliferation. They repeatedly saw the inoculation percentage rise to a maximum which could not be maintained, to be followed by a fall, which also was not permanent. They had encountered no exception to this rule in more than six hundred series of inoculations with Jensen's tumor, and the rise to a maximum with the subsequent fall had been repeated fifty times in simultaneous series of experiments. When the subsequent behavior of the descendants of several of the daughter tumors from any one batch of inoculations was followed, successive maxima were seen to arise, one after another, at short intervals. The maximum percentage of success of the experiments as a whole, was maintained continuously at a high level between seventy and ninety. Each strain, after reaching its maximum, fell and made way for another which had previously presented a lower percentage; and this latter fell in turn after having attained its maximum.

The behavior of the component parts of the Jensen tumor, when this growth was propagated in a large number of mice, represented, in the opinion of the authors, what might be regarded as occurring simultaneously in different parts of a single tumor when it was allowed to proliferate for a long period in one mouse. After a time any single tumor

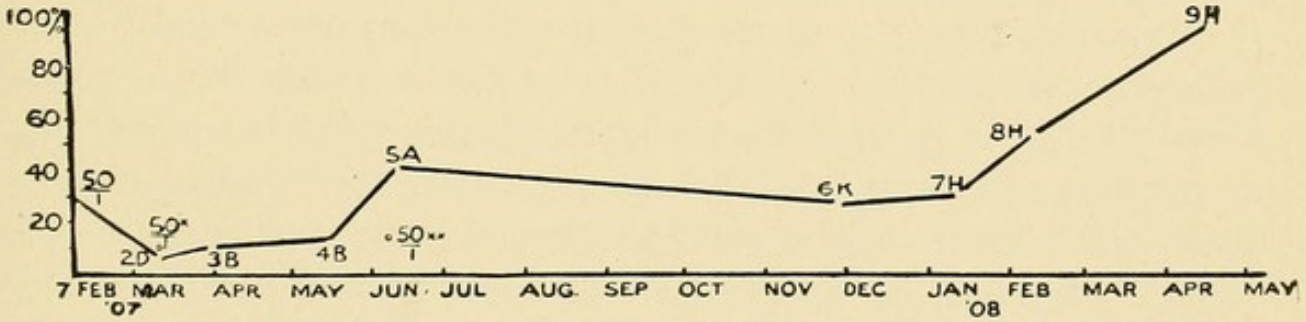
[1] *Proc. Roy. Soc.*, Series B, 1906, lxxviii, 195.
Third Sci. Report, Imperial Cancer Research Fund, London, 1908, 284.



Percentage curve of a mammary adeno-carcinoma of the mouse. After a short period of depression the curve rises rapidly till the 7th generation, and then falls. A second and third rise then follow in two strains propagated separately. While one (———) falls again and then rises slowly to a maximum, the other (·····) fluctuates between 35 and 50 per cent for 9 months, after which it also rises.



Squamous cell mammary carcinoma of the mouse. Rapid rise in percentage of success at the third transfer (3 A), followed by a fall and a second rise.



Hemorrhagic mammary alveolar carcinoma. Slight drop, followed by a gradual rise.

Fluctuations of growth energy in three tumor strains.

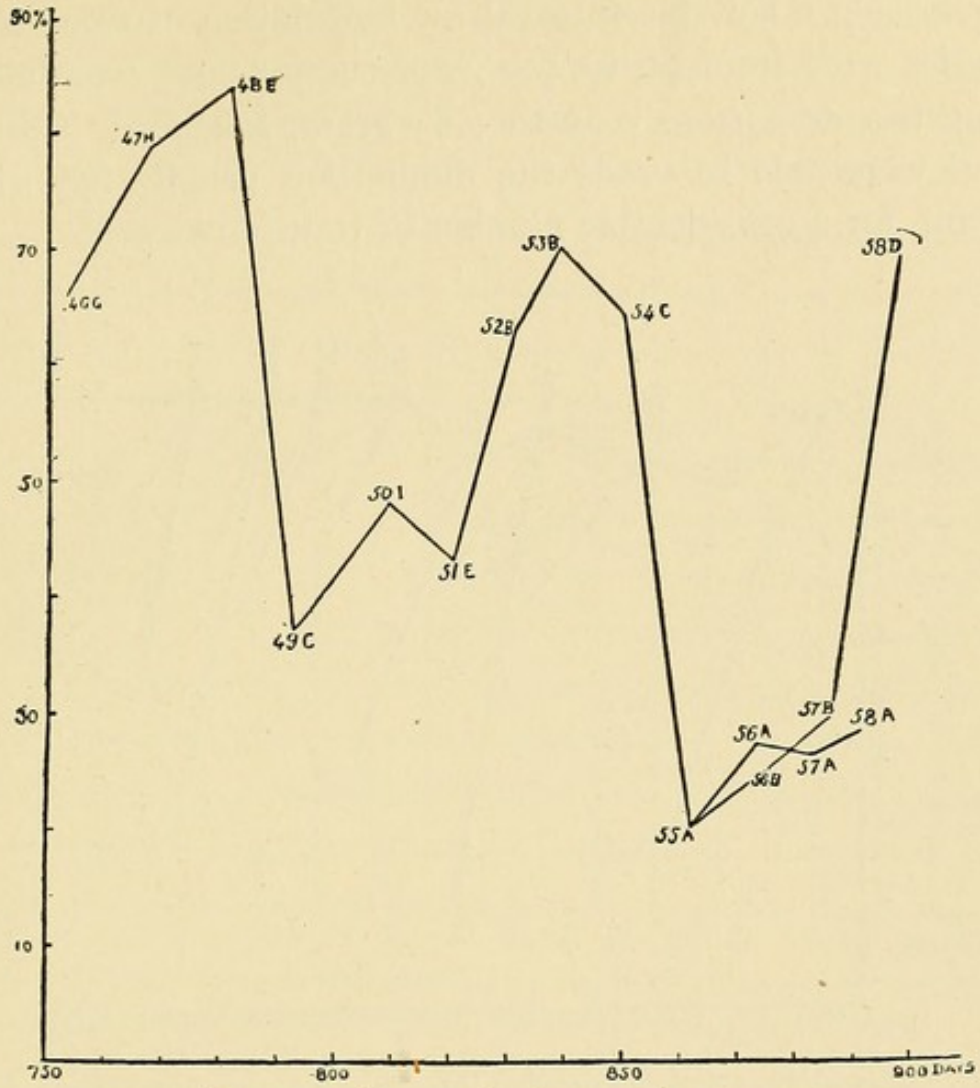


FIG. 4. — Fluctuations in growth energy.

could no longer be regarded as consisting of cells with equal proliferative power, for just as a composite chart of all the strains propagated indicated their very different behavior at any one date, so, in any single tumor, growth was proceeding actively in one part while at another it was going on slowly, or had actually ceased. The same heterogeneity was postulated for spontaneous tumors which, in all probability, owed their apparently continuous growth to the simultaneous presence in different areas of numerous growing centers which masked the effects of concomitant immunization, thus accounting for the rarity of spontaneous absorption among sporadic, as compared with transplanted, tumors. The greater frequency with which growth ceased in propagated tumors, to be followed by spontaneous absorption, seemed to be due to the greater homogeneity resulting from the limited number of growth centers represented in any one implantation.

By choosing a suitable interval for inoculation of tumors selected from series with from 90 to 100% of success, and especially by increasing the dose from 0.01 to 0.05 gram, Bashford, Murray, and Cramer[1] were able to evade the diminution usually following each maximum, for a considerable number of transferences.

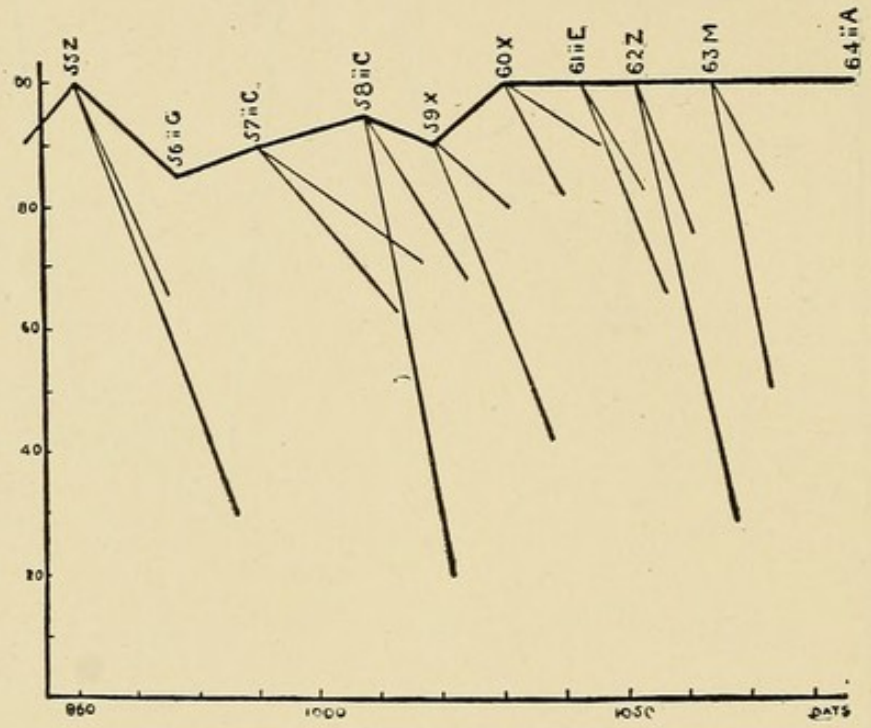


FIG. 5.— Graphic record of propagation through ten *passages* of a single strain of Jensen's carcinoma in which success has not fallen below 85%.

Calkins[2] could not accept the interpretation of these fluctuations advanced by Bashford and his colleagues, and argued that rhythms of growth, to be comparable with those of a free living, or of a cleavage cell, should be looked for in the individual mouse rather than in successive batches of mice. Again, curves such as had been published by the English school introduced two factors, one the percentage of "takes" and the other, the time required for the tumor to develop. It was the time factor which really measured growth energy, indicating roughly, as it did, the rate of division of the cells, and what the curves of Bashford had reproduced were merely recurring alternations of infec-

[1] *Proc. Roy. Soc.*, Series B, 1907, lxxix, 174.
Third Sci. Report, Imperial Cancer Research Fund, London, 1908, 326.
[2] *Jour. Exp. Med.*, 1908, x, 283.

tivity (percentage of "takes"). Calkins constructed a curve to represent the variations in infectiveness, and another to indicate the number of days required for the tumors to reach a point where they would kill the mice. His figures showed that the curves were by no means

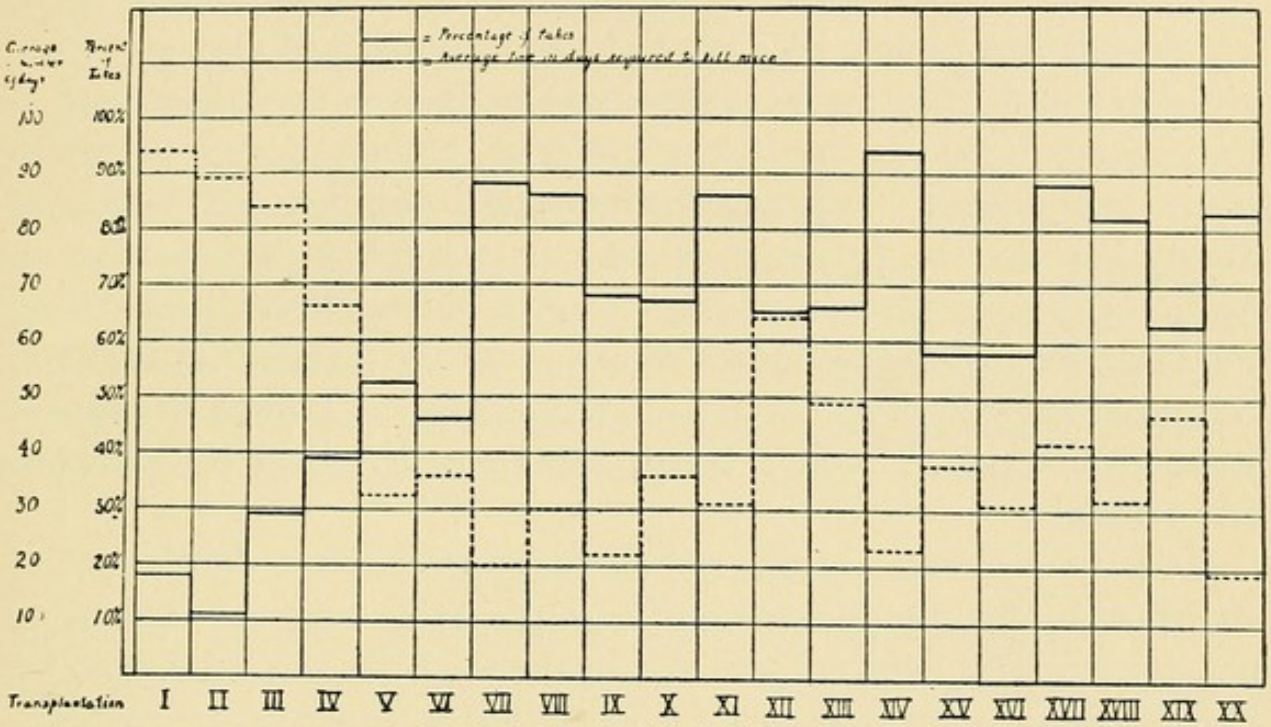


FIG. 6. — The waves of growth described by Bashford are shown by the regular alternations of infectivity, not in the line showing growth energy.

equivalent, and that too much was assumed in choosing the percentage of "takes" as the measure of growth energy.

Bashford, Murray, Haaland, and Bowen [1] replied that, as they had always been aware, the percentage of success could never be regarded as anything but an arbitrary standard by which to measure growth energy. And while, in the comparison of different strains the percentage of success varied independently of the energy of growth, within the limits of a single strain there was a very definite, although not absolute, correspondence between them. Either by measuring the area of tumors drawn in silhouette, or by killing large numbers of animals at regular intervals and weighing the amount of tumor produced, it was possible to get a more correct estimate of growth energy than by means of the plan adopted by Calkins; this was subject to uncontrollable fallacies because the death of the animals was only a secondary result of growth. Nevertheless, the curve constructed by that investigator

[1] *Third Sci. Report, Imperial Cancer Research Fund*, London, 1908, 281.

showed that the higher was the percentage of takes the lower was the interval within which the mice died, and the authors were, therefore, at a loss to understand how he had come to a different conclusion.

SPONTANEOUS ABSORPTION

Transplanted tumors which have become established may grow progressively until they attain a size equal to or even exceeding that of the animals in which they are growing or, having reached a certain point, may undergo a gradual spontaneous absorption. Spontaneous absorption may be entire or it may be partial, growth setting in again before complete disappearance has been effected. A strain may exhibit among the tumors of one series the phenomena of uninterrupted growth, retardation and resumption of growth or, finally, spontaneous cure. Such a tumor has been described by Bashford and Russell.[1]

Loeb [2] was the first to observe and to appreciate the significance of the spontaneous disappearance of transplantable tumors. The same phenomenon had been encountered by Jensen,[3] but was attributed by him to certain therapeutic experiments then in progress, erroneously, however, as he [4] said in a later article.

Michaelis [5] described three tumors of the Jensen strain which had remained stationary or receded after reaching the size of a pea, and Apolant,[6] in an article on the effect of radium upon mouse carcinoma, wrote that in two out of eleven controls the tumor had been absorbed spontaneously. Commenting upon the experiment, he said that cessation of growth, or even the entire disappearance of nodules, had been observed in every series, although in only a small percentage of the animals.

Clowes [7] recorded the complete absorption of Jensen's tumor in at least 15 to 20% of the cases and, in a more detailed article with

[1] *Proc. Roy. Soc.*, Series B, 1909–1910, lxxxii, 298.
Lancet, 1910, i, 784. [2] *Jour. Med. Research*, 1901, N.S., i, 34.
[3] *Centralbl. f. Bakt.*, etc., erste Abt., Orig., 1903, xxxiv, 30.
[4] *Zeitschrift f. Krebsforsch.*, 1908–1909, vii, 281.
[5] *Verhandl. d. Komitees f. Krebsforsch.*, 1903–1904, iii, 38. See *Deut. med. Woch.*, 1904, xxx, 1264.
[6] *Deut. med. Woch.*, 1904, xxx, 456.
[7] *Johns Hopkins Hosp. Bull.*, 1905, xvi, 130.

Baeslack,[1] in about fifty mice. To exclude early inflammatory swellings as a source of error the authors had adopted for a standard the absorption, not less than three weeks after inoculation, of a tumor which had at some period reached the volume of twenty cubic millimeters. Gaylord and Clowes[2] later described spontaneous recovery in one hundred and one cases, or about 23% of the inoculated animals, and concluded that the chances of recovery were inversely proportional to the size of the tumor.

Bashford, Murray, and Cramer,[3] in their earlier experiments, had seen only one case of spontaneous absorption among three thousand Jensen tumors of over fourteen days' growth, but Bashford[4] was later able to confirm the findings of other investigators with the statement that in some series as many as 50% of animals with large tumors were able finally to rid themselves of their growths.

Histology of Receding Tumors

Bashford, Murray, and Cramer[5] examined microscopically a receding tumor of the Jensen strain and found necrosis and a great overgrowth of connective tissue, coupled with the presence of characteristic cells containing small nuclei and a coarse protoplasmic reticulum, a type which they had encountered in growths exposed to radium, and especially about those of animals that had received toxic doses of adrenalin. In some areas a few isolated tumor cells were found lying in a dense connective tissue, and associated with multinucleated cell masses like those common in the reaction zone around foreign bodies. As even an extensive necrosis of the parenchyma had never of itself been sufficient to excite such an active connective tissue proliferation in transplantable tumors, the authors were inclined to connect this fibrosis with the occurrence of hemorrhages in the tumor. Bashford, Murray, and Bowen[6] described a cell with darkly staining

[1] *Med. News*, 1905, lxxxvii, 968.
[2] *Surgery, Gynecology, and Obstetrics*, 1906, ii, 633.
[3] *Sci. Reports, Imperial Cancer Research Fund*, London, 1905, No. 2, Part ii, 63.
[4] *British Med. Jour.*, 1906, ii, 209.
Lancet, 1906, ii, 315.
[5] *Sci. Reports, Imperial Cancer Research Fund*, London, 1905, No. 2, Part ii, 63.
[6] *Proc. Roy. Soc.*, Series B, 1906, lxxviii, 212.
Third Sci. Report, Imperial Cancer Research Fund, London, 1908, 302.

nucleus and protoplasm that was often present in spontaneously regressing tumors, and Bashford, Murray, and Cramer,[1] the occurrence of energetic phagocytosis.

The histological data recounted and pictured by Gaylord and Clowes[2] consisted of retrogressive changes in the epithelium accompanied by the occasional fusion of some of its cells to form pseudo-giant cells, the appearance of hemorrhagic areas, an ingrowth of connective tissue splitting alveoli into smaller cell groups, and the accumulation of small round cells.

CLINICAL COURSE OF THE TRANSPLANTED TUMOR

Bashford, Murray, and Cramer,[3] after two years' observation of almost three thousand mice with propagable tumors, concluded that the presence of such a growth, even when it was of greater weight than the mouse itself, did not necessarily involve any disturbance of the normal nutrition which could be regarded as comparable to the cachexia frequently associated with malignant new growths in the human subject. The animals were able to support large growths for several months, and even massive tumors in the abdominal cavity and extensive metastases in the lungs, without visible inconvenience. But when abrasions occurred in the skin overlying a tumor, the hemorrhage and septic infection which followed speedily produced marked constitutional effects, among which emaciation was almost constantly present, and the mice seldom survived for many days. Taken as a whole, the condition of animals with ulcerated tumors closely reproduced that of cachexia in man, but in the case of subcutaneous tumors which did not involve any important organ, cachexia could be definitely assigned to a position of secondary importance as an occasional accompaniment of malignant growths, to which it had no essential relation whatsoever.

Moreschi[4] investigated the association between tumor growth and nourishment, using for the purpose a mouse sarcoma of great prolifera-

[1] *Proc. Roy. Soc.*, Series B, 1907, lxxix, 187.
Third Sci. Report, Imperial Cancer Research Fund, London, 1908, 340.
[2] *Surgery, Gynecology, and Obstetrics*, 1906, ii, 633.
[3] *Sci. Reports, Imperial Cancer Research Fund*, London, 1905, No. 2, Part ii, 40.
[4] *Zeitschrift f. Immunitätsforsch.*, etc., Orig., 1909, ii, 651.

tive energy. He found that a moderate restriction of the amount of food was followed by a retardation of tumor growth and a lengthening of the life of the animals as compared with controls fed in the usual way, while an extreme condition of malnutrition preceding inoculation might cause a great decrease in growth, or even prevent it entirely. The death of a tumor-bearing animal Moreschi referred, not to a lack of the ordinary food-stuffs, but to withdrawal by the tumor of a specific, autogenous, nutrient material essential to life.

Following up these experiments, Rous [1] inquired whether it might not be possible to delay the course of inoperable tumors, or to hinder the development of metastases after the excision of a primary growth, by restricting the diet. In an attempt to answer these questions a series of experiments was undertaken with the Flexner-Jobling carcinoma of the rat. Contrary to expectation it was found that large tumors continued to grow with the same rapidity in hosts emaciating on an insufficient diet as in controls fed on a full one, many of which were gaining weight. With still larger tumors, in hosts already cachectic, the withholding of sufficient food had as little effect upon the tumor as it had upon the frequency of metastasis formation. Moreschi's findings were thus corroborated, so far as concerned the influence of limited nourishment upon the development of tumor grafts. When food restriction was begun four days after the introduction of the grafts it was noted that the tumors developed a little more slowly than in the controls.

Medigreceanu [2] estimated the weights of various organs in rats and mice bearing transplantable or spontaneous tumors. The ratio of the alimentary canal to the body weight (less that of the tumor) was within the normal limits, while that of the lungs and spleen was variable. The relative weights of the kidneys of tumor-bearing (and pregnant) mice showed only such slight variations as were met with in normal animals, but a transplantable rat sarcoma, on the contrary, induced constantly an increase in the size of these organs which was directly proportional to the size of the tumors. However, as mouse sarcomata did not reproduce this phenomenon in mice, the change

[1] *Proc. Soc. Exp. Biol. and Med.*, 1910–1911, viii, 128

[2] *Proc. Roy. Soc.*, Series B, 1909–1910, lxxxii, 286.
Berl. klin. Woch., 1910, xlvii, 588.

was not thought to be characteristic of the sarcomata. The heart was enlarged in rats and mice bearing tumors, generally in proportion to the weight of the growth, but rapidly proliferating neoplasms did not lead to such marked hypertrophy as did those which were increasing more slowly and were richly supplied with blood. Hypertrophy of the heart in tumor-bearing animals the author was inclined to refer to the action of mechanical factors. The pregnant normal animals examined did not present a corresponding cardiac enlargement.

The important result of the experiment was, however, the demonstration that the liver was hypertrophied in all animals bearing either transplanted or spontaneous tumors, and a general parallel subsisted between the weight of the tumor and the weight of the liver, whether comparison were made between different growths of the same strain, or tumors of different strains. This disturbance of the normal ratio was due neither to a loss of weight in the other organs nor to the attainment by the liver of a weight equivalent to what it would have reached in the same animal during the natural augmentation of the body weight. There were, moreover, differences between the livers of normal and tumor animals, such as the increased percentage of water in the latter (as high as 4 to 5% more than in normals), which indicated the presence of qualitative differences as well. Histological studies had not indicated any anatomical changes. In a rat in which a tumor of ten to twelve grams had disappeared by spontaneous absorption, the liver was still enlarged when examined twenty days after complete recession of the growth, a finding which the author contrasted with the fact that the hypertrophy of the liver found in pregnant mice vanished a short time after the birth of the young.

As a corollary to these experiments Medigreceanu[1] undertook to determine whether augmentation in weight were due to an increment in the diet. The simplest explanation of the circumstance would be that the growing tumor attracted nourishment to itself and left the host in a condition of partial starvation, as a logical consequence of which there would follow an increased appetite and the ingestion of a larger amount of food to satisfy the demands of the organism and its growing tumor. This hypothesis, if it were justifiable, would account for the hypertrophy of the liver, which would have more work to per-

[1] *Berl. klin. Woch.*, 1910, xlvii, 772.

form. Experiments directed toward the solution of this question demonstrated that there was no more food ingested by tumor-bearing rats than by normal ones, that the body weight (less tumor) increased for a time after transplantation, as in normal controls, and then began to diminish, and that during this period of loss in weight the appetite was decreased. The hypertrophy of the liver in tumor rats could not, therefore, be explained by an increased intake of food.

Amyloid degeneration has been described by several authors in the spleens of mice bearing transplanted tumors. Thus, Albrecht and Hecht,[1] in discussing the enlargement of the spleen so commonly found in tumor mice, ascribed it to bacterial infection, and said further that amyloidosis finally made its appearance in these organs.

Lubarsch [2] found amyloid degeneration of the spleen, liver, kidney, adrenal, and pancreas, in the order of frequency named, among forty-two mice with transplantable tumors. While the presence of amyloid was not dependent upon the histology of the growths, occurring as it did in animals bearing sarcoma, carcino-sarcoma, and carcinoma, it was of more frequent occurrence and of earlier appearance among mice with sarcoma or carcino-sarcoma than among those with pure carcinoma. No relation could be demonstrated between amyloid degeneration and the size of the tumor. Lubarsch did not believe that the presence of amyloid could always be referred to an antecedent bacterial infection, and suggested its possible association with the products of the necrosis of tumor tissue.

Freytag [3] examined forty mice with non-ulcerated tumors in which bacteria were not microscopically demonstrable. In most of the cases the spleen was enlarged, and in thirty-three it was the seat of amyloid degeneration. The livers in twenty-six showed the same lesion, and the kidneys a trace of it in eleven. Among sixteen mice which had been inoculated unsuccessfully, amyloid degeneration could be demonstrated in the spleen in nine and in the liver in five, but in only two was it present in the kidneys, and in both of these the amount was very limited. It thus appeared that the cell destruction resulting from one or two fruitless inoculations might inaugurate an amyloid degenera-

[1] *Centralbl. f. allg. Path.*, etc., 1909, xx, 1039.
[2] *Centralbl. f. allg. Path.*, etc., 1910, xxi, 97.
[3] *Zeitschrift f. Krebsforsch.*, 1910–1911, x, 164.

tion, although it was possible that in an animal like the white mouse which, as Davidsohn [1] had already shown, was very prone to amyloid degeneration of its organs, this lesion might occur spontaneously with relative frequency. Twenty normal mice were, therefore, examined, but without anything of moment being found in either spleen or kidney; although the liver often showed a slight degree of fatty degeneration, the amyloid reaction was always negative. Still, these mice were all very young, and experiments were in contemplation which should deal with normal animals that had been kept as long in confinement as the tumor mice, and upon a similar diet.

Price-Jones [2] studied the blood of sixteen mice with transplantable carcinoma, and found a slight degree of anemia, indicated by a lowered hemoglobin content and a diminution of the number of red blood cells per cubic millimeter. A well-marked leucocytosis was present, due to a great relative and absolute increase in the large mononuclear lymphoid cells and the polymorphonuclear leucocytes. Little change could be found in the marrow of these mice for, owing to the augmented myeloid activity of their spleens, any increased vigor on the part of the marrow was either masked or obviated. Thirteen normal mice served as controls.

A diminution in oxygen capacity with a fall in the percentage of hemoglobin parallel to the decrease in number of red cells per cubic millimeter, was found by Chisholm [3] in the blood of rats bearing transplanted sarcomata. The anemia, which was usually accompanied by a wasting of the body tissues, did not depend on the presence of ulceration nor was it in all cases proportional to the amount of necrosis in the tumor. Signs of regeneration present in the blood smears indicated that the anemia was due, at least in part, to blood destruction, but the mechanism of its production was uncertain. The presence of the tumor was associated with an increase in the total volume of the blood. In certain exceptional cases there was reason to believe that the animal increased both its oxygen capacity and blood volume to meet the needs of the tumor, but this occurred only in the case of small growths.

[1] *Verhandl. d. deutschen path. Gesellsch.*, 1904, 7te Tagung, 41.
[2] *Arch. Middlesex Hosp.*, 1911, xxiii, 56.
[3] *Jour. Path. and Bact.*, 1911, xvi, 152.

In a review, Cramer [1] said that he had discovered no diminution in the total acidity of the gastric contents in mice that had been successfully inoculated with the Jensen tumor.

Copeman and Hake [2] found an increase of the physiologically active hydrochloric acid (including both free acid and that combined with proteids and nitrogenous organic bases) in the gastric contents of mice and rats bearing transplanted tumors, and in mice with spontaneous growths. The stomachs of one hundred and fifty normal mice, where no account was taken of the period of digestion, gave an average of 0.1121% hydrochloric acid, while in one hundred and seventy-eight mice with transplanted tumors the average was 0.1752%. An average of two hundred and forty-five stomachs from normal mice for periods of digestion varying from one to one and one-half hours gave 0.1456% hydrochloric acid, and of two hundred and ninety stomachs from mice with transplanted tumors, 0.1673%. Fifteen stomachs of mice which were the subjects of spontaneous neoplasms showed an average of 0.1929% hydrochloric acid during different uncertain periods of digestion. An average of the stomachs of six normal rats after one hour's digestion gave 0.1427% hydrochloric acid, and of seven rats with transplanted growths (varying from 0.3 to 15 grams) 0.1837% after the same period.

Regarding experiments on animals bearing transplanted tumors Cramer [3] indicated the importance of recognizing the difference between an animal with a propagable tumor and one the subject of a spontaneous growth, pointing out that the question whether the increase in hydrochloric acid in animals bearing transplanted tumors was connected in any way with the decrease of this substance observed clinically in man should still be considered, therefore, an open one.

The same author,[4] as the result of an inquiry into the gaseous metabolism of rats bearing the Jensen rat sarcoma, found that the growth of a tumor in a normal organism led at first to physiological changes only, tumor growth causing a distinct increase in the weights of the

[1] *Biochem. Centralbl.*, 1905, iv, 65.

[2] *Third Sci. Report, Imperial Cancer Research Fund*, London, 1908, 398. *Proc. Roy. Soc.*, Series B, 1908, lxxx, 444.

[3] *Metabolism and Practical Medicine*, v. Noorden, London, 1907, vol. iii, 824.

[4] *Third Sci. Report, Imperial Cancer Research Fund*, London, 1908, 427.

animals, and that pathological changes in the general metabolism did not ensue until the physiological resources had become exhausted. He compared the effect exerted on the body by a growing tumor, in so far as it was a problem of nutrition, to that of the growth of a fetus in a pregnant animal. It could not be explained by attributing to the cancer cell the formation of pathogenic substances of a hypothetical nature, such as a "cancer ferment" or a "cancer toxin."

Cramer's findings in the case of rats bearing tumors, and his comparison of such rats to pregnant animals, form an interesting supplement to some experiments conducted many years ago by Edelfsen and Hensen.[1] These authors demonstrated that female guinea-pigs grew at about the same rate whether they had young or not during their own period of growth. Minot[2] was able fully to confirm these statements, and concluded from his own experiments that gestation did not represent a tax on the mother, but a stimulus — that it favored, rather than impeded, growth.

Cramer and Pringle[3] determined the nitrogenous metabolism in three rats before and after the implantation of the Jensen rat sarcoma. Their conclusions were applied only to animals bearing tumors of sufficient size to warrant the assumption that they would reveal any specific property or function which might be possessed by the cells of a neoplasm. The effects which a large tumor must necessarily produce, by virtue of its mere mass, were not considered. The main outcome of the experiments was stated in the following conclusions: —

"1. Less nitrogen is necessary to build up a certain weight of tumour tissue than is necessary to build up an equal weight of the somatic tissues of the host.

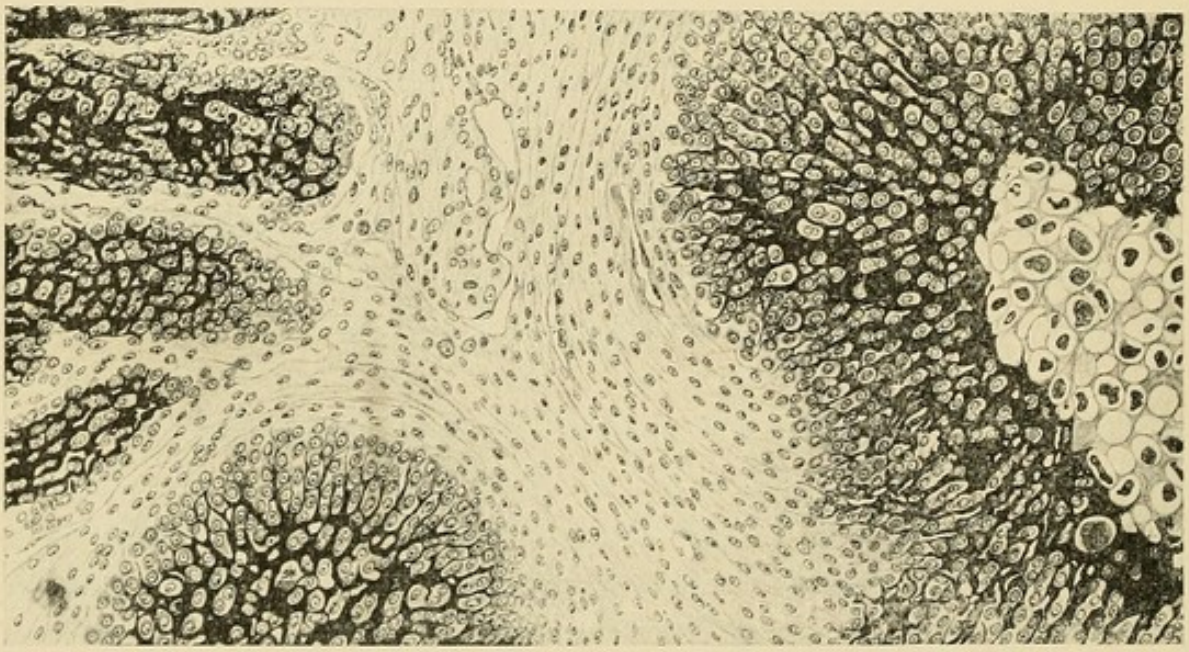
"2. Animals bearing tumours maintain their positive nitrogen balance, and the nitrogen retention actually increases with the size of the tumour.

"3. In our experiments the cells of the new growth derived their nitrogenous material necessary for the building up of new tissue by a sparing action on the protein metabolism. The tumour cells do not proliferate at the expense of the tissues of the host, nor is there any

[1] *Arb. a. d. Kieler Physiol. Inst.*, 1868. Cited by Minot.
[2] *Jour. Physiol.*, 1891, xii, 141.
[3] *Proc. Roy. Soc.*, Series B, 1909–1910, lxxxii, 307.

J. R. Ford, del.

Mammary chondro-osteo-sarcoma of mouse. Primary tumor. Spindle-cell sarcoma; at left of figure an acinus lined by columnar cells — mouse nearing end of pregnancy. $\times \frac{500}{1}$.



J. R. Ford, del.

Transplanted tumor from first generation of growth pictured above. Illustrates also the structure of the recurrent spontaneous tumor, which contained osteoid nodules, spindle cells, and cartilage.

evidence that they have a higher affinity for nutritive material than the growing cells of the host.

"4. There is no evidence of the existence of substances secreted by the tumour disturbing the nitrogenous metabolism by means of a toxic action on the tissues of the host."

If now, said the authors,[1] it were true that less nitrogen was needed to build up a given weight of tumor tissue than was necessary to build up an equal weight of the somatic tissues of the host, it would follow that cancer would have a lower nitrogen percentage than the somatic tissues. The estimation of the total amount of nitrogen (protein and non-coagulable nitrogen) in various tissues of the three rats mentioned in the previous article was, therefore, undertaken, and the authors were able to show that, weight for weight, the malignant cells contained only about three-fourths of the protein nitrogen present in the tissues of the host. In other words, with the same amount of protein a larger mass of tumor than of host tissue could be evolved. In a former paper Cramer had indicated the similarity which existed between the growth of cancer and the growth of the fetus, and preliminary experiments by Dr. Lochhead had shown that a rapidly growing normal tissue (the fetus) had a lower nitrogen value than the maternal organism.

HISTOLOGICAL VARIATIONS OCCURRING DURING TRANSPLANTATION

In the Parenchyma

Distinct variations from the tumor type originally transplanted may occur under extended propagation, and it is possible for these to be so marked that, as pointed out by Bashford, Murray, and Cramer,[2] if growths with the characters of various transplanted tumors in the same series had occurred sporadically they might well be regarded by pathologists as totally distinct conditions. Thus an acinous arrangement of the parenchyma was a nearly constant feature of tumors examined within the first week after transplantation, while another characteristic was the loose columnar arrangement of tumor cells in large alveoli. At a subsequent stage the central parts of certain al-

[1] *Proc. Roy. Soc.*, Series B, 1909–1910, lxxxii, 315.

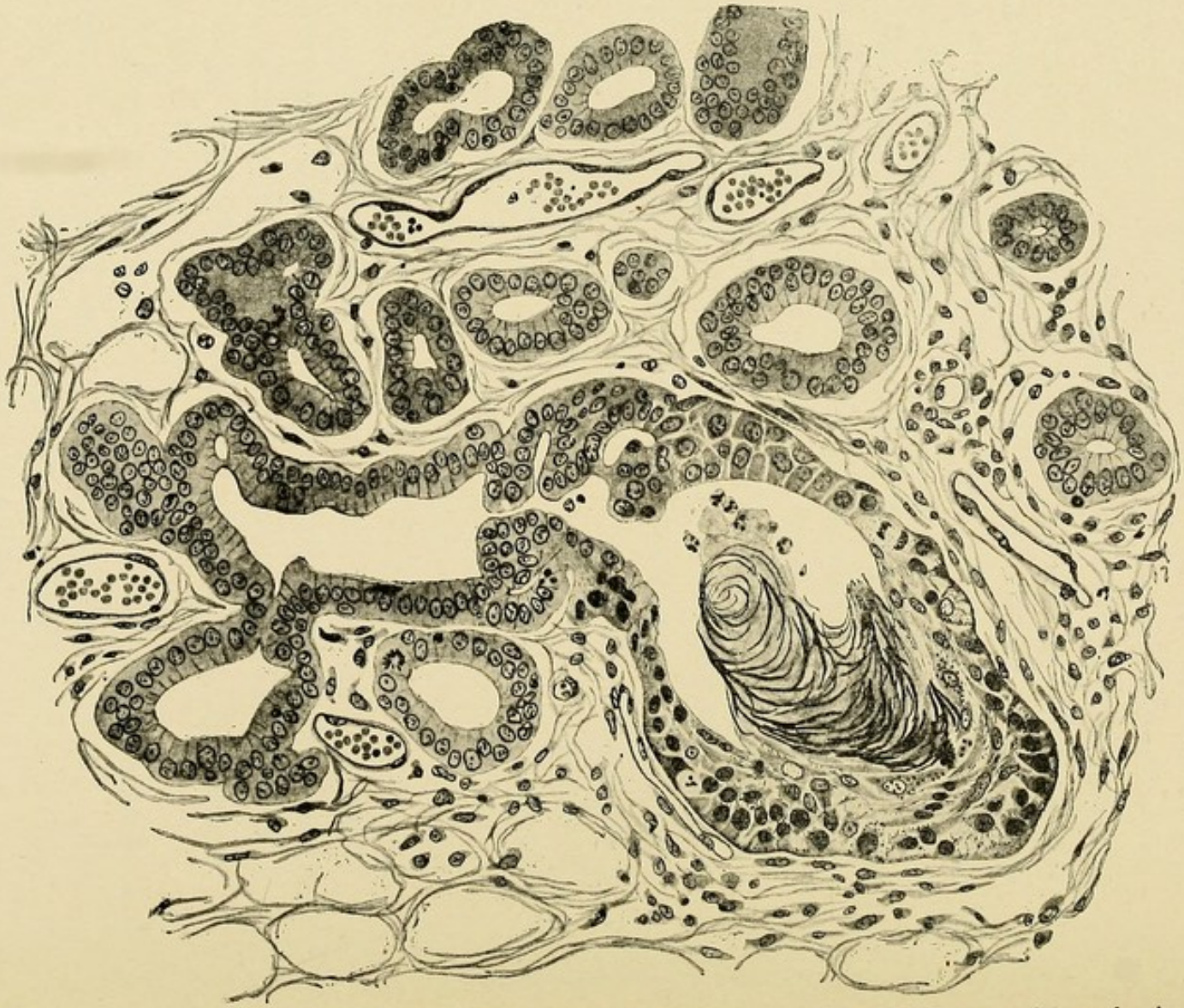
[2] *Sci. Reports, Imperial Cancer Research Fund,* London, 1905, No. 2, Part ii, 52.

veoli underwent a necrotic change and, indeed, in advanced states of this process the alveoli might consist only of a thin layer of healthy cells and completely necrotic granular central areas. On the other hand, equally large alveoli occurred which were completely filled by healthy cells showing no traces of necrosis. All the conditions just discussed might be discovered in different areas of the same growth. The authors had been able to determine that these different histological appearances were not demonstrable at uniform fixed periods after the date of inoculation, whence they concluded that the time of their production was not determined by the disturbance of nutrition following transfer from one host to another.

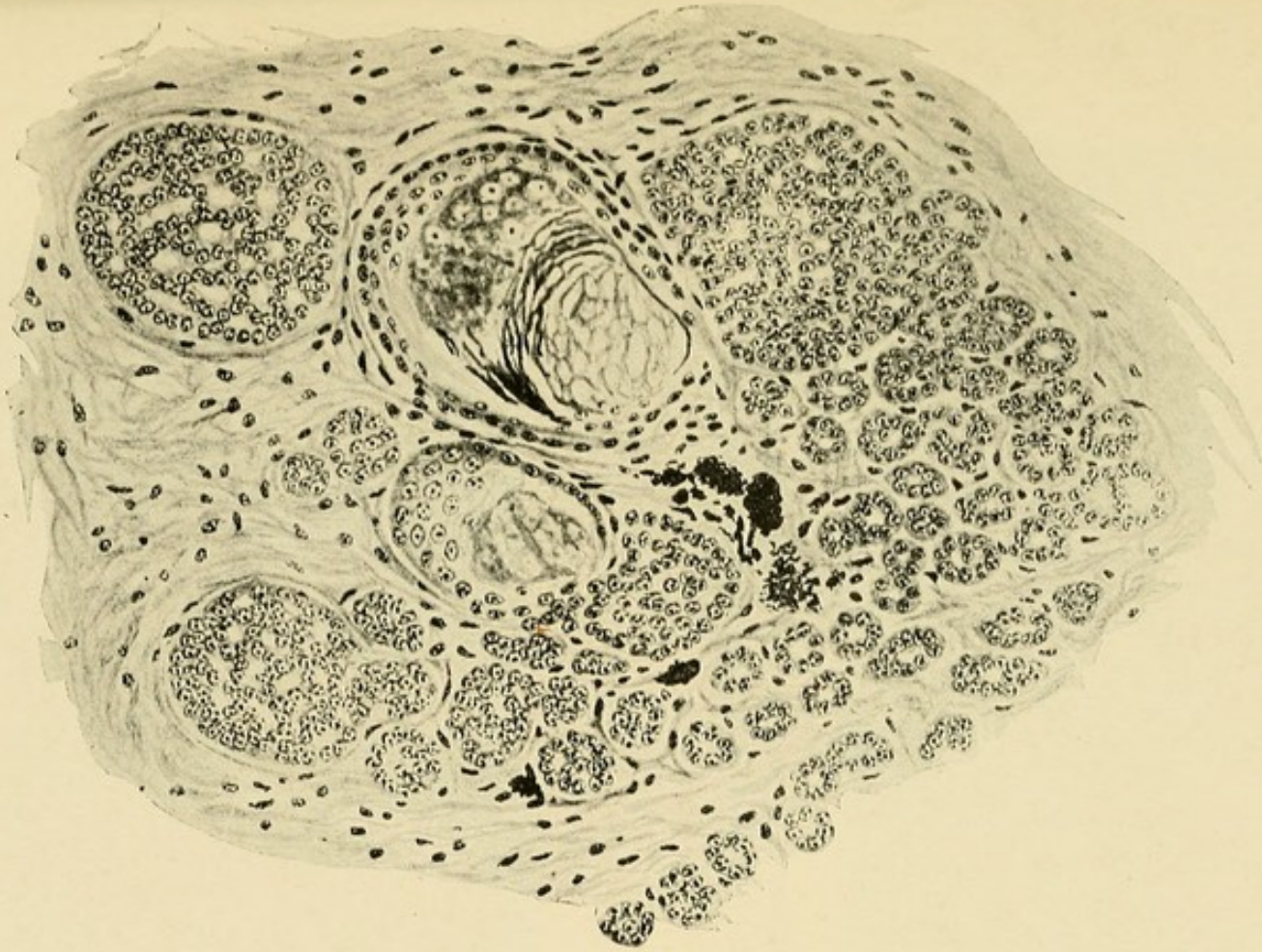
Murray [1] encountered a tumor which he at first regarded as a spindle cell sarcoma, recurrences of which contained hyaline cartilage and uncalcified bone. Daughter tumors propagated from recurrences showed the same great variability of structure, some consisting of spindle cells, others showing large masses of cartilage either associated with osteoid tissue or alone, while in one instance there had been a deposition of lime salts in the osteoid matrix, so that true bone had actually been formed. The subsequent history of this tumor as given by Bashford [2] was that, after the fourth generation, cartilage and osteoid tissue disappeared and two strains with divergent characters were obtained, in one of which the growths were soft, consisting of closely packed spindle cells with very little interstitial collagenous tissue and large necrotic areas. The second, derived from a sclerotic tumor of the eighth generation, differed in several particulars. Its tumors were firm, strongly collagenous, with little or no necrosis even when they had reached a large size, and these differences had been maintained, at the time of writing, for over two years.

Murray [3] reported, further, a mammary tumor of the mouse containing three distinct histological structures — keratinized alveoli, solid alveoli, and adenomatous areas. In the first few generations of transplanted tumors the growths generally presented a solid structure with only minute areas of keratinization, a condition which continued with a slight progressive increase in the amount of keratin until

[1] *Third Sci. Report, Imperial Cancer Research Fund*, London, 1908, 78.
[2] *Fourth Sci. Report, Imperial Cancer Research Fund*, London, 1911, 178.
[3] *Third Sci. Report, Imperial Cancer Research Fund*, London, 1908, 159.

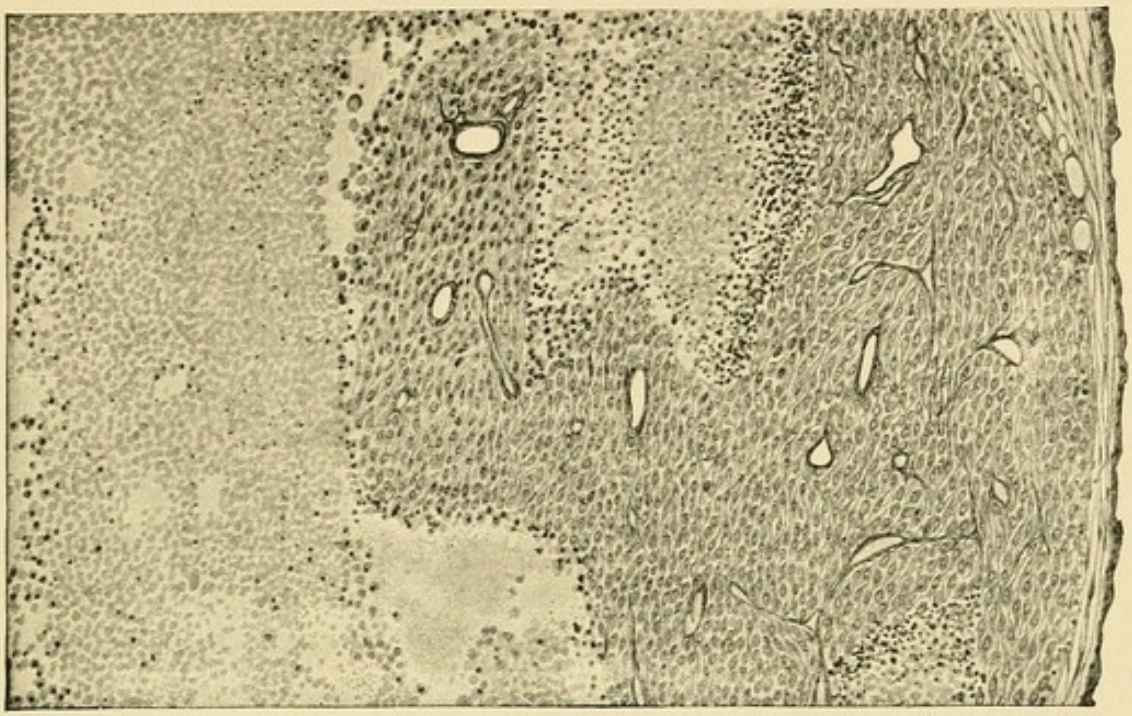


Part of a spontaneous mammary adeno-carcinoma containing keratin, showing juxtaposition of acinous



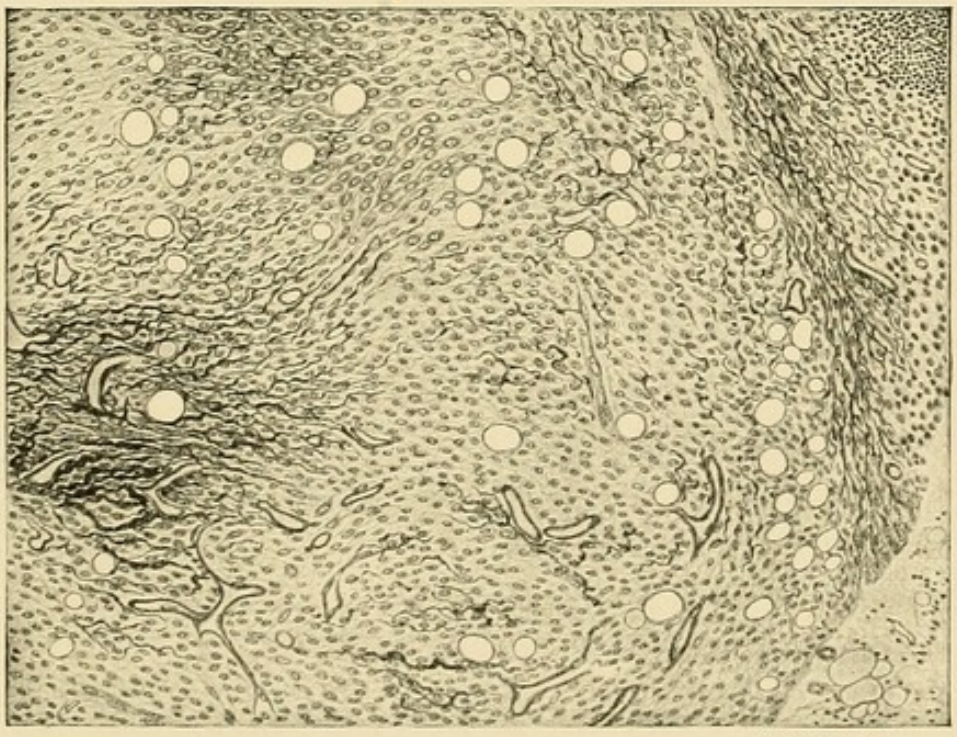
R. Muir, del.

Transplanted tumor of the same strain. The nodule contains keratinized, adeno-carcinomatous, and adenomatous areas. $\times \frac{200}{1}$.



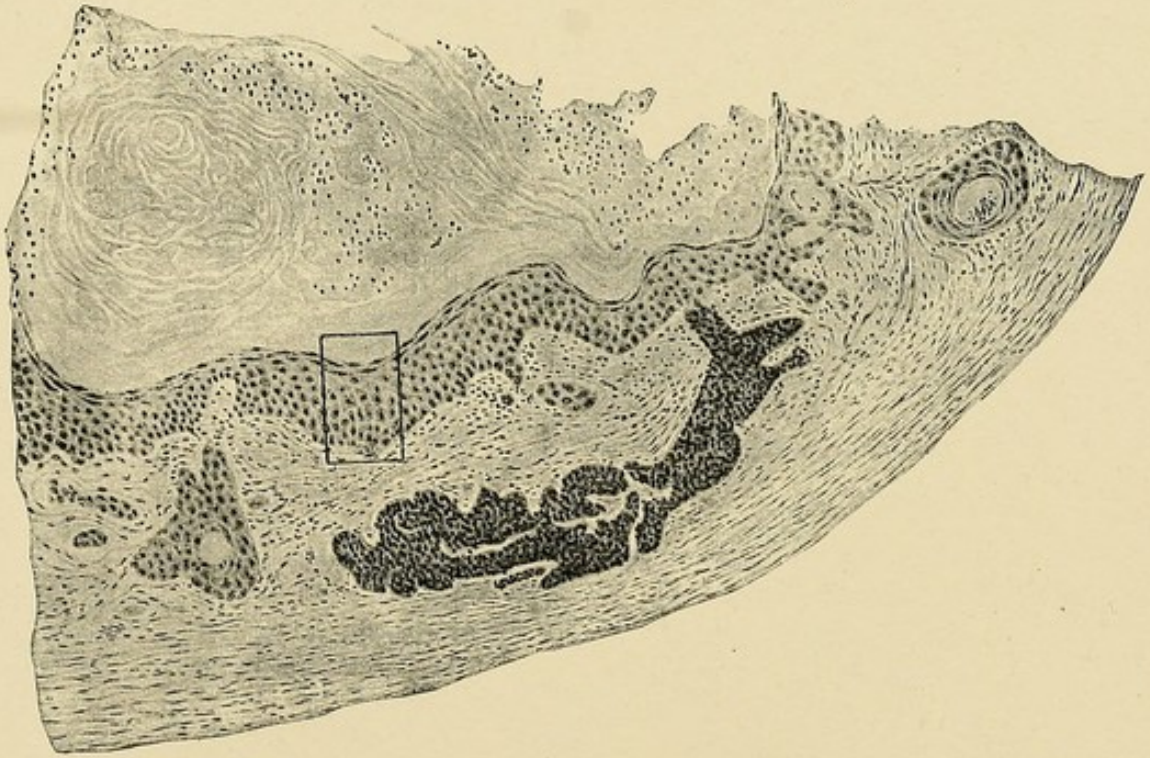
J. R. Ford, del.

Osteo-chondro-sarcoma of the mouse. 38-day-old tumor from 36th generation of a soft, necrotic strain in which very little collagen is produced. $\times \frac{87}{1}$.



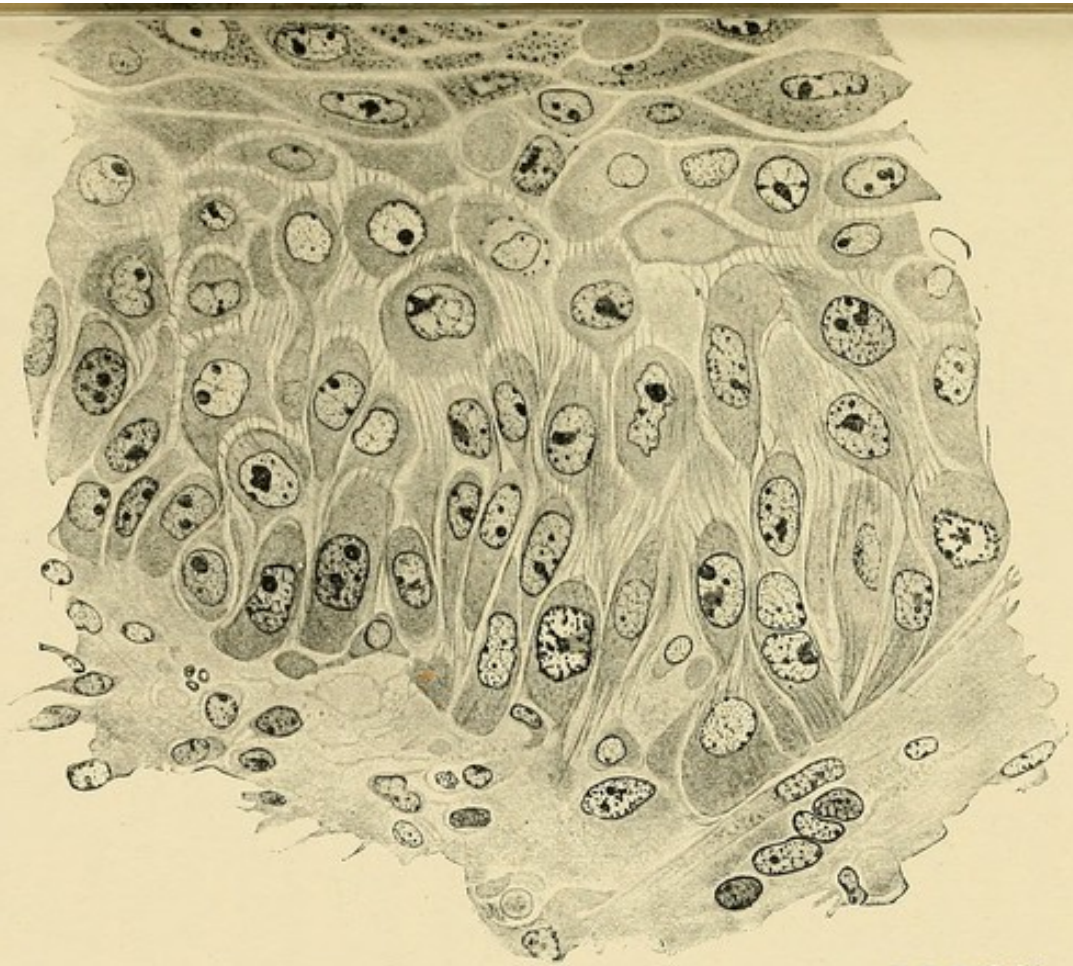
J. R. Ford, del.

36-day-old growth, 31st generation of a sister strain of above. The tumors of this series are firm, and produce an abundant amount of collagen. $\times \frac{87}{1}$.



J. R. Ford, del.

Tumor from 8th generation of a mammary adeno-carcinoma containing areas of keratinization. Perfect reproduction of structure of stratified squamous epithelium in the wall of an epithelial cyst. $\times \frac{60}{1}$.



J. R. Ford, del.

High power view of area marked in above figure. Malpighian layer, prickle cells, eleidin granules, and keratinization, as in normal skin. $\times \frac{750}{1}$.

the sixth generation. In the seventh, however, keratinization appeared to an even more pronounced degree than in the primary tumor and endured for three or four generations, after which it diminished. At the conclusion of this period the tumors consisted of large alveoli of closely packed, small cells, interrupted only by an occasional capillary. Upon this medullary condition of the parenchyma, and after it had persisted through several generations, there intruded a well-marked adenomatous structure, which involved some tumors to such an extent that considerable portions of them presented the appearance of an adenoma. Others exhibited the formation of adeno-carcinoma, while still others had preserved their alveolar arrangement. Murray pointed out that separation of the keratinized and adenomatous types by a long interval of time, during which there had been many successive transferences from animal to animal, proved that the two differentiations, however distinct they might appear, were inherent in cells of one kind. It was of merely academic interest to argue whether the original growth should be considered a squamous cell carcinoma capable of growing as an adeno-carcinoma, or, conversely, an adeno-carcinoma of the mamma in which excessive keratinization had occurred. Bashford [1] has described the subsequent history of this strain, which at the time of writing had been propagated through eighty-two generations. The acinous structure disappeared after the twelfth generation and since then the tumor had been cultivated as a pure alveolar carcinoma without any differentiation whatsoever, except in one very old tumor (eight months) of the fifty-sixth generation, which showed areas of keratinization.

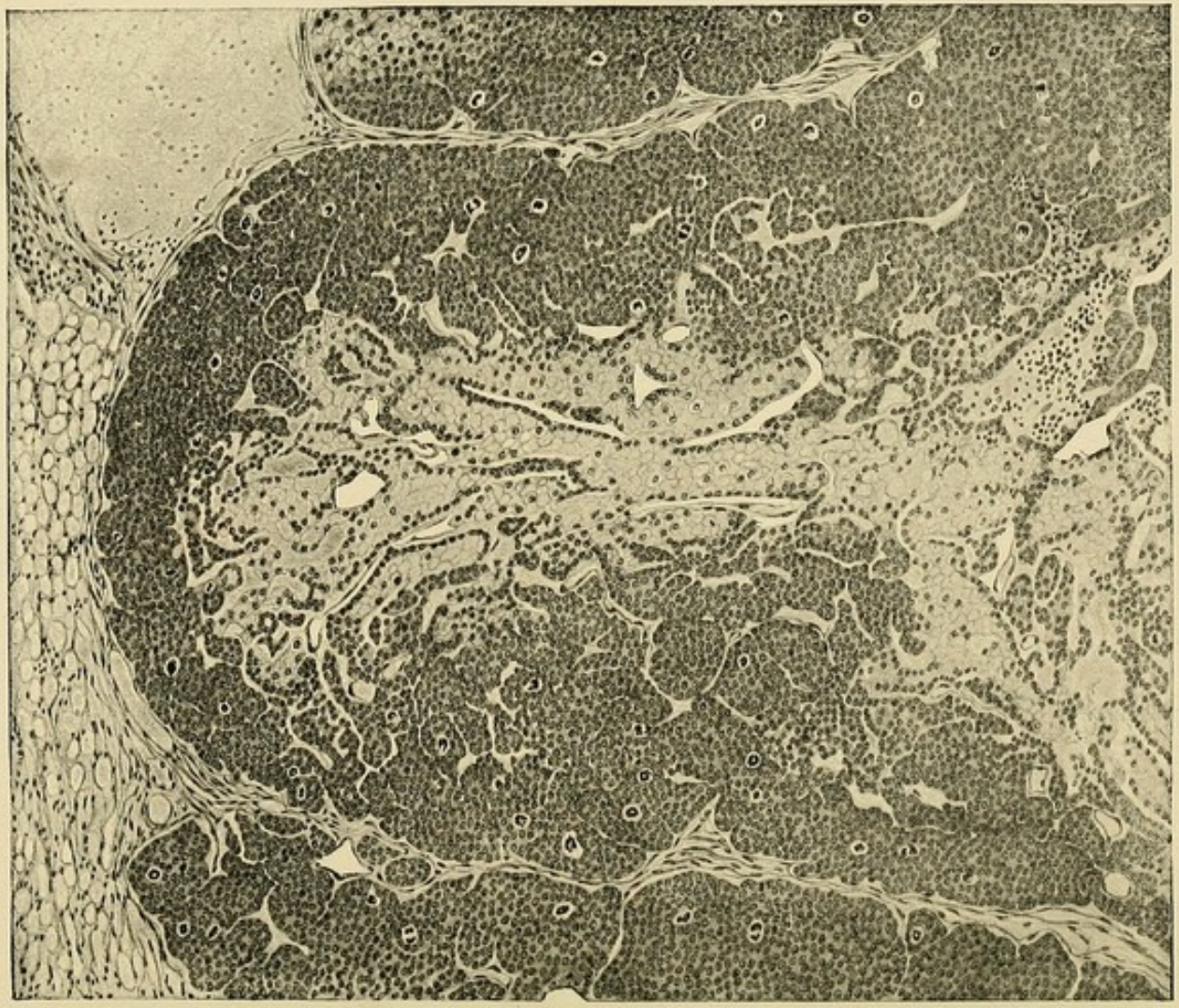
A most comprehensive review of the behavior of the tumor cell during extended propagation has been written by Bashford,[2] based upon observation of spontaneous tumors in over six hundred and fifty mice, as well as eighty-five propagable tumors, thirty-five of which had been in cultivation for more than three years.

Generally speaking, the results following the continuous propagation of an extremely varied series of tumors showed a remarkable average constancy over long periods among both the carcinomata and sarcomata. The morphological variations which had been observed

[1] *Fourth Sci. Report, Imperial Cancer Research Fund*, London, 1911, 155.
[2] *Fourth Sci. Report, Imperial Cancer Research Fund*, London, 1911, 131.

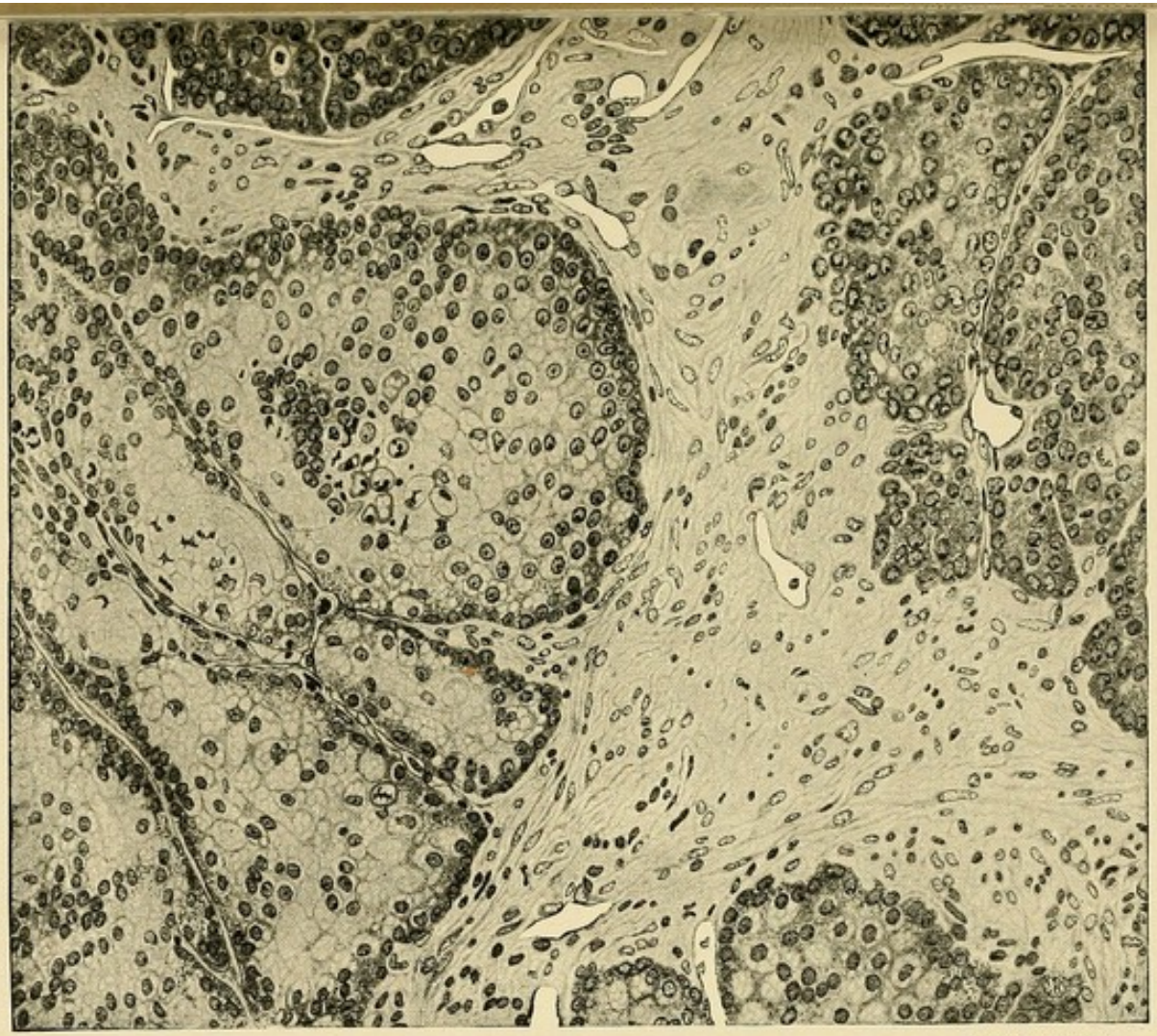
were but slight and, with few exceptions, served only to emphasize this statement. The apparent loss of acinous structure in the later generations of many adeno-carcinomata of the mamma was hardly to be considered as evidence of a real change. In some tumors, examination at a short interval after transplantation revealed a purely alveolar condition, which gradually gave way to a typical acinous structure as the tumors grew older, and which might even be entirely replaced by perfect acini in very old tumors. This fact showed the necessity for examination of old tumors in all cases where normal histological differentiations present in the primary growth or in the earlier generations of daughter tumors were absent in young tumors of later generations used for transplantation. When this precaution was observed, adeno-carcinomata primarily hemorrhagic might be found to reproduce their original hemorrhagic character, even though it had been absent in the periods immediately succeeding transplantation. The same statement could be applied, also, to certain of the squamous cell carcinomata. The differences in histological structure between the spontaneous tumor and its early daughter tumors on the one hand, and the later transplanted growths on the other were, therefore, not necessarily final evidence of a real change in the character of the parenchyma in every case in which they were found.

As already stated, the general conclusion was drawn that the cells of many tumor parenchymata preserved unaltered and in the most persistent manner the majority of the characters with which they were endowed from the earliest period at which they had come under observation. The various strains did not approximate a common type either in structure, rate of growth, or inoculation percentage; and while a number of strains might be selected as forming a group homogeneous in naked eye structure and in histology, it could be demonstrated that the similarity was primary and already associated with the spontaneous tumors, rather than a secondary convergence from an initial heterogeneous condition. The conclusion seemed warranted that the relative permanence of the distinctive characteristics in typical tumor strains had its foundation in the cellular changes by which the non-cancerous tissue cells had passed over into the cancerous state, and this persistence suggested how radical must have been the alterations of which cancerous transformation was the expression.



J. R. Ford. del.

Sebaceous adeno-carcinoma of the right groin. Tumor of the 6th generation, showing the typical differentiation into sebaceous cells in the center of the lobe, and the undifferentiated condition of the



J. R. Ford, del.

Higher power view of a tumor belonging to the 2d generation of the same strain. $\times \frac{230}{1}$.

Bashford arranged his tumors in groups according to whether they showed only minor alterations from the primary growth, definite temporary, or permanent, alterations. In the first there were fourteen mammary adeno-carcinomata, five of which were hemorrhagic and four papilliferous, two sarcomata, a sebaceous adeno-carcinoma of the axilla, two carcinomata of the preputial gland, and a strongly keratinizing squamous cell carcinoma of the orbital region. The second class differed but slightly from the first in that certain definite changes in structure or behavior appeared, persisted for a time, and then vanished. Its members were all mammary carcinomata, and the main variations affected the relative amounts of alveolar or acinous structure or, as in a few cases, were concerned with the appearance in the stroma of spindle shaped cells undoubtedly of parenchymatous origin, although they were strikingly like those of a sarcoma.

Placed in the third group were those tumors among which changes in structure or behavior had been maintained. This division comprised seventeen mammary carcinomata, two adeno-cancroids, a sebaceous carcinoma, a squamous cell carcinoma, an osteo-chondro-sarcoma, and an osteo-sarcoma, and the mutations which had occurred in them were mainly the assumption of a more alveolar type, the disappearance of keratin, or of both sebaceous differentiation and keratin, a diminution in the amount of sebaceous differentiation with increased keratinization, and the disappearance of epithelial cells growing out diffusely into the stroma.

His experience of spontaneous and transplanted tumors enabled Bashford to approach certain theoretical aspects of oncology, among them v. Hansemann's assertion that metastases in the human subject were less differentiated than the primary growth because they were more anaplastic. They were often undifferentiated, it was true, but merely because they had not had time in which to differentiate. The doctrine of anaplasia as a progressive, irreversible change in cancer cells had accordingly lost its objective basis, and had become a matter of subsidiary importance.

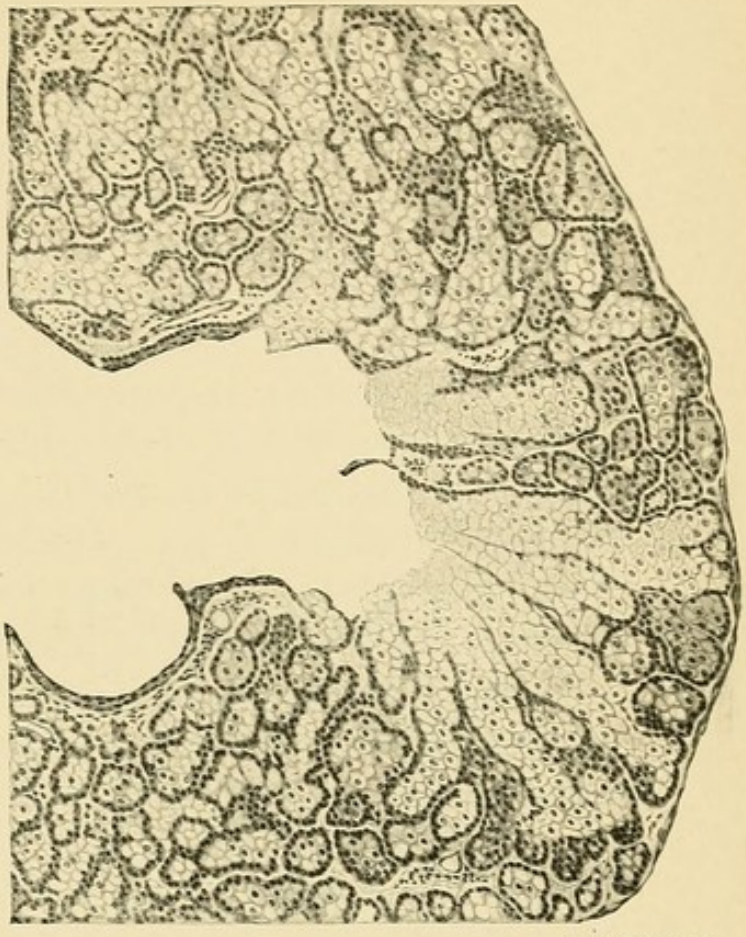
The artificial propagation of a number of tumors over considerable periods of time had given an answer to a conundrum in the domain of metaplasia that had hitherto been insoluble: Of what differentiation would metaplastic cells be capable if they continued to proliferate?

The evidence was quite clear that the occurrence of one differentiation did not preclude the later development of another, for the parenchymata of such tumors as adeno-cancroids, osteo-chondro-sarcomata, and adeno-carcinomata with spindle shaped epithelium were capable of either differentiation over long intervals, and presented one or the other for reasons independent of the conditions of growth. It was probably wrong to regard as evidence of lost differentiation periods during which this faculty was absent, and they might be interpreted more properly as latent periods. No other assumption was compatible with the reappearance of differentiation in spite of the repeated subdivisions to which a tumor parenchyma had been subjected during propagation throughout long periods of time, for after a few transfers all the cells of a growth in any one animal would be the descendants of a single cell in a preceding generation not very remote. The restoration of two distinct differentiations in later generations in the same form and association in which they had been encountered in the primary tumor could accord only, therefore, with the assumption of a homogeneous parenchyma possessed of dual powers of differentiation.

Bashford's paper contained, furthermore, certain important suggestions on the relations existing between structure and malignancy. While most of the transplantable strains had shown marked deviations from the normal structure of the tissue which had given them origin, it was not an invariable rule for such mutations to progress further during continued propagation. Hence complete loss, or even latency of differentiation, was not at all necessary for unlimited growth and, indeed, the purely glandular structure of several adeno-carcinomata of the mamma afforded evidence that the capability for proliferation was not necessarily dependent upon, or accompanied by, marked changes of structure. Other tumors also, with a high or almost complete degree of differentiation, showed this capability for propagation, among these being squamous cell carcinomata and adenomata of the sebaceous and preputial glands which, having retained their characteristic differentiations and secretory functions, reminded one strongly of a type of malignant growth of the thyroid in man and animals. It had thus been demonstrated that tumors distinguishable under the microscope only with difficulty from their normal mother

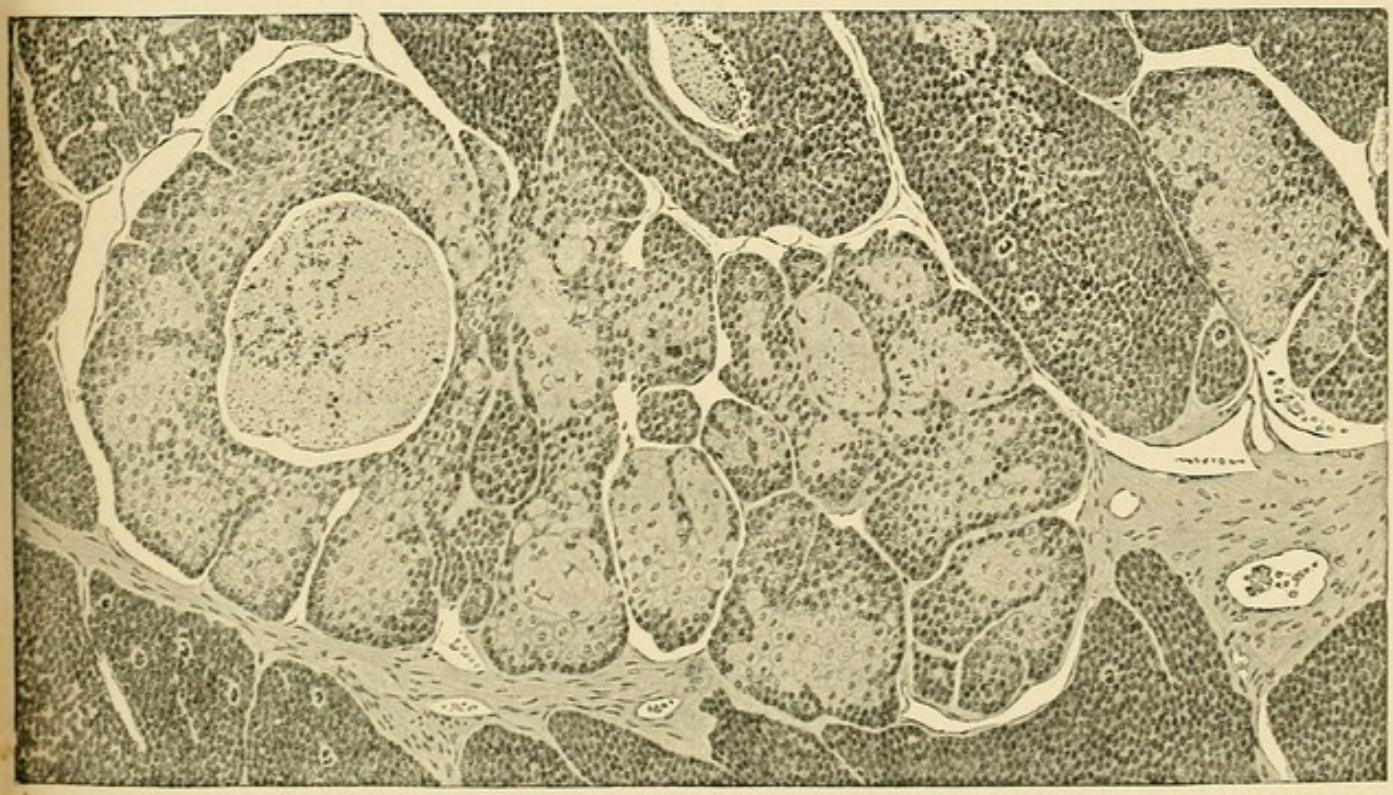
J. R. Ford, del.

ıdeno-carcinoma of the preputial gland, male mouse, showing its close resemblance to the normal gland in spite of its definitely malignant character. The propagated tumors retain the same histological picture. $\times \frac{87}{1}$.



J. R. Ford, del.

Normal preputial gland from adult male mouse, for comparison with tumor in left-hand illustration. The larger ducts of the gland are lined with cells resembling squamous epithelium. $\times \frac{87}{1}$.



J. R Ford, del.

Sebaceous adeno-carcinoma. Spontaneous tumor in left groin, showing the same typical differentiation as the cells of a sebaceous gland. For comparison with the upper left-hand figure. $\times \frac{135}{1}$.

tissues, were capable of unlimited propagation — a fact of the highest theoretical importance in showing the independence of biological behavior (malignancy) and structure. So far as the retention of normal histological build was a criterion of innocence, its significance had been lost, and at the time of writing only the biological properties of some tumors, that is, their power of unlimited growth, distinguished them from the corresponding normal tissues.

Apolant [1] believed that characteristics which were not present in the spontaneous tumor could never arise during transplantation, but that altered biological conditions surrounding the transplanted growth allowed certain properties to appear with more clearness than they had in the primary tumor. Such changes proceeded hand in hand with increased proliferative power. While in general the propagable neoplasms thus remained true to the type of the spontaneous growth from which they had been derived, certain variations might, nevertheless, occur from time to time.

It was among the alveolar, those most rapidly growing of all the transplantable carcinomata, that the characteristics of propagable tumors appeared in their most salient form. Here necrosis supervened in the centers of the alveoli and spread progressively with the increase of the tumor, because nutrition was unable to keep pace with growth, the malignant tissue meanwhile continuing its proliferation in the form of a relatively narrow surrounding band. Even when the proliferative energy was intermediate, the alveolar type was retained; but coincident with a decrease in growth power isolated cell nests emerged, the stroma became more vigorously developed, and the degeneration receded very perceptibly. While this process might even go so far as to produce occasionally an acinous structure, such a condition never progressed to any considerable degree.

Appearance and disappearance of cysts had also been observed among the transplantable alveolar carcinomata, but to a very variable extent. In some strains, cysts were almost never encountered, while in others they might be surprisingly numerous.

The histological expression of augmented growth energy among the fissure-forming carcinomata was a tendency on the part of the epithe-

[1] *Arb. a. d. Königl. Inst. f. Exp. Therap.*, 1906, Heft i, 41.
Verhandl. d. deutschen path. Gesellsch., 1908, 12te Tagung, 7.

lium, more marked than in the primary tumors, to arrange itself in several layers. But the characteristic property of this type of carcinoma, the formation of pleomorphous, although usually fissure-like lumina, never disappeared entirely.

Papillary carcinomata also preserved their type during transplantation, but to be convinced of this fact, it was necessary to examine older tumors, because growth was so extraordinarily slow that the process required a long time for its completion. In the first one or two months there was produced only the alveolar pre-stage, marked by the presence of small cell nests, most of which contained distinct lumina. With the enlargement of these lumina, cell processes began to be intruded from the walls, and thus in the course of many weeks or months a typical papillary carcinoma was evolved.

Apolant[1] had under observation a fissure-forming carcinoma which gradually developed into a growth of the solid type with a scanty stroma, while later still, connective tissue no longer sharply separated the single alveoli. The cells toward the periphery of the alveoli gradually assumed a spindle shape, which could not be accounted for by the effects of mechanical pressure. A further peculiarity of this strain was an isolation of the cells — a resolution of the tumors into their elements, which made it hardly justifiable, from a purely histological standpoint, to describe the growths as carcinomata. Apolant was not certain of the nature of the change, and was in doubt whether it would be permissible to explain the alteration as the beginning of a sarcomatous transformation solely on the basis of this histological picture. Against such an explanation was arrayed the observation that developing sarcoma was always separated by a sharp boundary from the carcinoma, while in the present tumor the transition was gradual. It was, therefore, very probable that the spindle shaped structures were merely carcinoma cells which had undergone a morphological change.

That this opinion was justified was shown by a subsequent article, in which Apolant[2] wrote that at the one hundred and thirty-ninth generation of the tumor all the spindle elements had disappeared and the growth had reverted to a typical adeno-carcinoma.

[1] *Verhandl. d. deutschen path. Gesellsch.*, 1908, 12te Tagung, 11.
[2] *Arch. f. mikroskop. Anat.*, 1911, lxxviii, 144.

During the propagation of a mammary adeno-carcinoma of the rat, first described by Michaelis and Lewin,[1] Lewin [2] observed the appearance of an alveolar structure in the second and third generations, with the gradual transformation of the growth into one of solid type, and almost complete regression of the stroma. In the third generation keratin was found. In some tumors of the fourth, there was a considerable increase in the amount of stroma, while others had a scirrhous appearance. One substrain grew as a pure adeno-carcinoma, and in the other there were transitions between adeno-carcinoma and cancroid. In the fifth generation there appeared a pure cancroid with no admixture of glandular elements, and in the sixth, a pure adeno-carcinoma, a solid carcinoma with mucous degeneration, and a pure cancroid. At the time of writing the growth had reached its twelfth *passage*, in which no tumor of a pure type had occurred but, on the contrary, mixtures of keratinized adeno-carcinoma and solid carcinoma, which could not be separated.

Apolant[3] was convinced that the histological structure of transplanted tumors depended upon certain conditions in the host, for in a strain which through more than fifty generations had been propagated as a solid reticular carcinoma he encountered a distinctly adenomatous build. This transformation, however, was discoverable only in animals that had been partially immunized by the inoculation of either tumor or normal tissue. He looked upon the transmutation as a proof either that the lost inhibition to cell growth had been replaced by artificial immunization, or else that the normal inhibitory powers of the organism might be increased to such a degree that the lawlessly growing cells of a carcinoma could be led back again to the more orderly growth of an adenoma.

But Gierke,[4] on the basis of a research carried out upon tumors of the thyroid gland, had already expressed the opinion that the histological appearance of a growth did not of itself permit one to decide whether the neoplasm were benign or malignant. There occurred

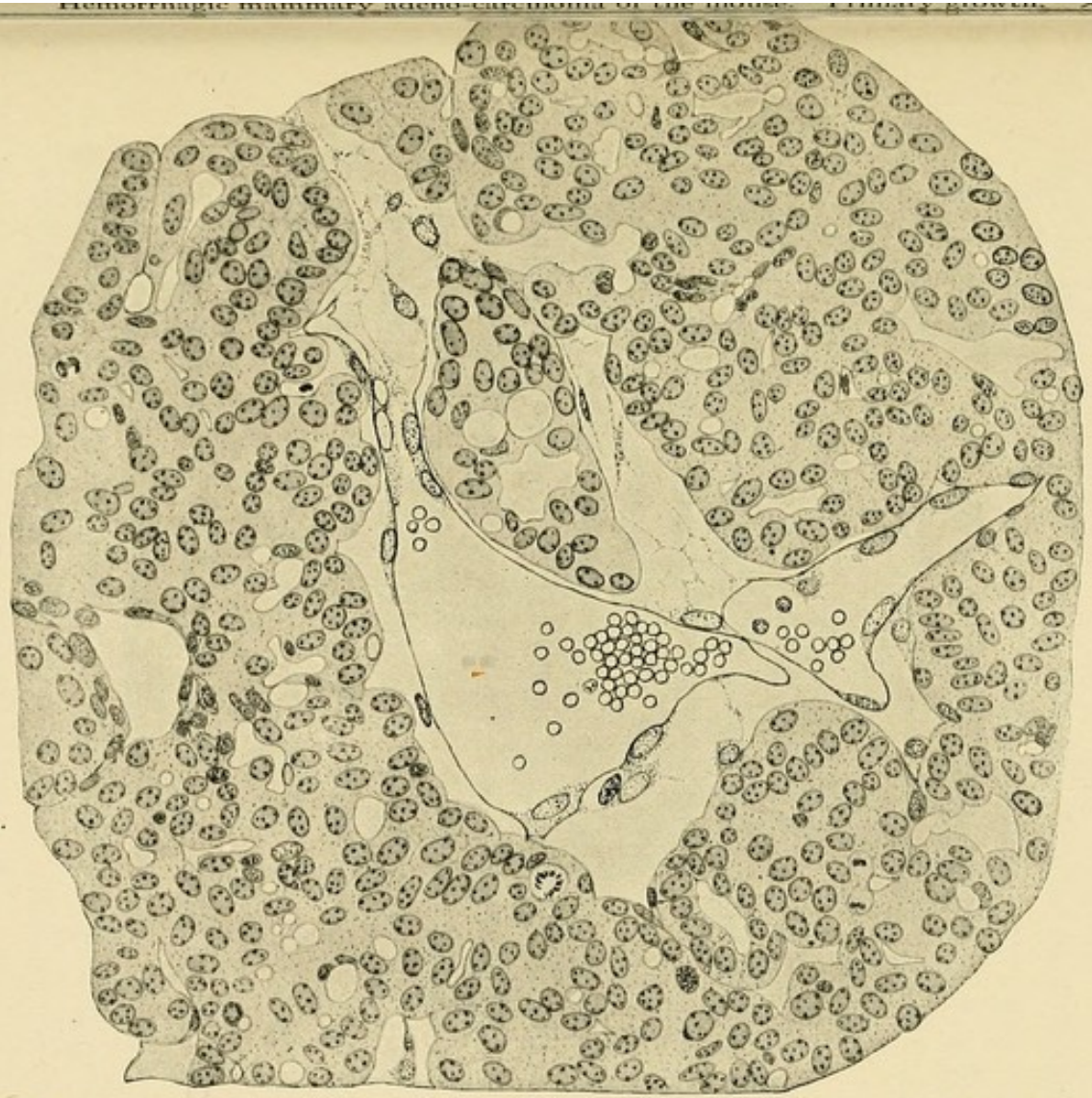
[1] *Berl. klin. Woch.*, 1907, xliv, 419.
[2] *Berl. klin. Woch.*, 1907, xliv, 1602.
Zeitschrift f. Krebsforsch., 1907–1908, vi, 270.
[3] *Münch. med. Woch.*, 1907, liv, 1720.
[4] *Beitr. zur path. Anat.*, etc., (Ziegler), 1908, xliii, 338.
Third Sci. Report, Imperial Cancer Research Fund, London, 1908, 126.

thyroid tumors exhibiting a structure absolutely benign when considered from the histological standpoint which, nevertheless, were not surpassed in malignancy by any of the carcinomata. Such tumors must be acknowledged as carcinomata whose cells had retained the capacity for more advanced differentiation. Transitions were provided by tumors possessing in some parts a well-marked carcinomatous structure and in others that of a simple goiter as, for example, in metastases in the lungs or lymph nodes. Eberth had already characterized this condition as a "change for the better" but, unfortunately, the histological "improvement" did not necessarily correspond to a biological one; and it was their biological behavior alone which lent to malignant tumors their chief significance and necessitated, at least in principle, the maintenance of a distinction between them and the benign growths. Exactly the same conditions were to be found in the mammary tumors of the mouse, and hence Gierke concluded that there were certain interchangeable relations between the histological structure and the biological behavior of a tumor, but that any direct inference from one to the other could be made only with great caution.

Murray[1] believed that an experimentally induced increase of resistance, in so far as it did have any effect on the structure of a tumor, exerted it in a direction opposite to that indicated by Apolant — in the direction, that is, from adenomatous toward alveolar build. He was unable to subscribe to the far-reaching conclusions of Apolant, which, as an explanation of increased resistance, postulated a direct influence upon fundamental properties of the tumor cell in the sense of decreasing its malignancy. The histological study of many strains during their growth in normal and resistant animals had shown that the malignant cell possessed an inherent cyclical variability of histological differentiation, which, however, was not expressed to the same degree in all cases. This property was as much an expression of the cyclical growth of cancer as were spontaneous variations in proliferative energy, but since no connection between these two phenomena had yet been discovered they must, at least for the present, be looked upon as two independent properties of the tumor cell. To the cyclical variations in histological appearance there could be superadded, through

[1] *Berl. klin. Woch.*, 1909, xlvi, 1520.

Hemorrhagic mammary adeno-carcinoma of the mouse. Primary growth. × 400



Transplanted tumor from second generation of growth reproduced above, showing the retention of character by connective tissue and blood vessels. The difference in the size of the cells is due to different fixation. $\times \frac{400}{1}$.

the induction of experimentally increased resistance, structural changes of slight degree, such as a tendency to assume the alveolar type with a loss of the characteristic adenomatous differentiation. This relationship could be explained by the inhibition, in resistant animals, of the stroma reaction induced by the cancer cell in normal ones. It was clear that the construction of a perfect adenomatous condition required an intimate relationship between parenchyma and connective tissue, and that if the stimulus of the cancer cells upon the stroma were to be decreased or abolished, they would remain in masses and the growth would contain a minimum of connective tissue.

The reappearance of acini in Apolant's experiment may have been due, according to Bashford,[1] to the comparison of an old, slowly growing tumor with more rapidly growing and younger controls.

In the Stroma

Bashford, Murray, and Cramer,[2] and Apolant[3] found that, as a general rule, the character of the stroma in hemorrhagic tumors was preserved during propagation, a fact which Ehrlich[4] attributed to a chemotactic power resident in the cells of the parenchyma, by virtue of which they were enabled to attract angioblasts. On the other hand, as Bashford[5] has observed, the hemorrhagic character of the stroma may be lost or diminished during continued transplantation.

An extended consideration of such changes as this may, however, be dismissed as unprofitable while there remains to be reviewed the assumption of sarcomatous properties by stroma cells previously benign.

This significant deviation was first observed by Ehrlich and Apolant[6] in a transplantable adeno-carcinoma of the mouse, in the tenth generation of which there were found two growths containing both carcinomatous and sarcomatous areas. Exactly at what point the change had occurred the authors were unable to say, but it must have

[1] *Fourth Sci. Report, Imperial Cancer Research Fund*, London, 1911, 170.
[2] *Sci. Reports, Imperial Cancer Research Fund*, London, 1905, No. 2, Part ii, 30.
[3] *Verhandl. d. deutschen path. Gesellsch.*, 1908, 12te Tagung, 7.
[4] *Arb. a. d. Königl. Inst. f. Exp. Therap.*, 1906, Heft i, 69.
[5] *Fourth Sci. Report, Imperial Cancer Research Fund*, London, 1911, 154.
[6] *Berl. klin. Woch.*, 1905, xlii, 873.

been present in the ninth generation, because two of the descendants were of a mixed character. Those of the first, second, and sixth generations, however, were pure adeno-carcinomata.

The mixed growths contained carcinoma cell nests separated from one another by strands of large spindle cells in which mitosis was actively progressing, while in some parts of the sections the process was so advanced that the carcinomatous elements had disappeared entirely. In one tumor of the thirteenth, and in all those of the fourteenth and subsequent generations, no trace of carcinoma was discoverable, pure sarcoma having been evolved through the gradual elimination of the epithelial components. The growth had been cultivated as far as the twenty-sixth generation without showing the slightest variation from the characteristic structure of a pure spindle cell sarcoma.

Two explanations were advanced to account for the transformation. An alteration in the chemistry of the cancer cell was conceivable, in the course of which there was elaborated a material possessing the power to incite connective tissue cells to malignant growth. This hypothesis was later extended by Ehrlich [1] to include and explain a difference that had been observed between the grade of sarcoma development attained in individual mice of one and the same series. He suggested that this was best elucidated by assuming that the predisposition to the growth of connective tissue varied in different animals as, for instance, certain persons were more prone than others to the development of keloids. To this amendment, however, Russell [2] objected that the tumor with which he himself had been working was able to initiate the sarcomatous change in nearly every mouse and that practically all of them must, therefore, have been "keloid" mice, while Jensen's carcinoma, which had probably been transplanted into more mice than any other tumor, had never yet happened to be inoculated into a "keloid" mouse.

Ehrlich and Apolant's alternative explanation for the sarcomatous change was that repeated transplantation of cancer cells and connective tissue evoked a proliferative energy in the cells of the latter which advanced to the degree of malignancy.

This latter hypothesis was excluded by Bashford, Murray, and

[1] *Zeitschrift f. Krebsforsch.*, 1907, v, 64.
[2] *Jour. Path. and Bact.*, 1910, xiv, 374.

Cramer[1] on the ground that Jensen, and afterward they themselves, had demonstrated that the stroma of the introduced graft always degenerated. The explanation they therefore considered to be at variance with the facts.

To this criticism Ehrlich and Apolant[2] answered that the hypothesis in question did not in the slightest degree conflict with the commonly recognized fact that the inoculated stroma *usually* died out. In the course of many transplantations, and under the influence of the carcinoma, it might *occasionally* happen that the stroma cells suffered a transformation which allowed them to remain and grow like those of a carcinoma.

Bashford[3] replied that the authors had neglected to eliminate certain sources of error, and until that were accomplished it would be impossible to decide whether the growth had been a mixed tumor from the beginning, whether an infectious granuloma had arisen upon a carcinoma, or whether a true sarcoma had evolved itself from the introduced stroma or the reaction tissue of the host. That a sarcoma might arise during the transplantation of a carcinoma was, *a priori*, not impossible.

Ehrlich and Apolant[4] retorted that Bashford had never had under observation a tumor of really high virulence, and that in this respect his experience was imperfect. As regarded Bashford's first objection, the authors had already stated that neither in the primary growth nor in the daughter tumors immediately following its transplantation had there been any departure from the usual appearance of carcinoma of the mouse.[5] So far as concerned the differential diagnosis between granuloma and sarcoma, the most eminent pathological judgment in Germany had concurred in favor of the latter; and, furthermore, such enormous growth energy as was exhibited by the tumor under discussion never had been seen in an infectious granuloma.

[1] *Berl. klin. Woch.*, 1905, xlii, 1434 (footnote).
[2] *Berl. klin. Woch.*, 1906, xliii, 39 (footnote).
[3] *Berl. klin. Woch.*, 1906, xliii, 477.
[4] *Berl. klin. Woch.*, 1906, xliii, 668.
[5] When Apolant demonstrated two of these primary tumors, however (*Verhandl. d. deutschen path. Gesellsch.*, 1905, 9te Tagung, 168), v. Hansemann said that he had found spindle cells in the stroma of one of them, and therefore suggested that the second growth be subjected to the closest scrutiny, to ascertain whether they might not be present in other areas. But Apolant thought that the existence of the cells described by v. Hansemann would hardly warrant one in calling the growth a mixed tumor.

The diagnosis of sarcoma was once again defended by Apolant,[1] on the double basis of clinical behavior and histology. These tumors grew just as rapidly as they had before the change took place, yielding growths the size of a plum within three weeks, and infiltrating the surrounding tissues just as seriously. The histology permitted no doubt of their sarcomatous nature, for in addition to the typical arrangement of their elements they presented from their very inception a number of mitoses not exceeded by that found in the most rapidly growing mouse carcinomata. Metastatic deposits, however, had not been discovered.

Ehrlich and Apolant [2] reported two further instances, the first of which arose between the twelfth and fourteenth generations of an alveolar carcinoma which had originated from a mixture of four epithelial tumors. Here the growth energy of the new sarcoma was less than in the first case, for the mixed tumor stage persisted through ten generations (six months) without change. The second case occurred during the transplantation of one of the four epithelial growths just mentioned. Little alteration had been observed up to the fortieth generation, but at that point an increased cellularity of the stroma made its appearance, although sarcoma development could not be indubitably established. It was confirmed, however, in the sixty-eighth generation, after two years and a half of propagation. It seemed significant to the authors that the sarcomatous change arose only in the later generations, for although it had occurred relatively early in the first case, after only nine months of transplantation, in the second and third cases it did not supervene until after two and two and a half years respectively. This fact explained why the transformation had not been observed more frequently, for at the time of writing only the Jensen tumor had been cultivated over a period of time at all comparable to these. Its virulence, however, was much lower than that of the Frankfort tumors. In later papers the authors returned to this question of the relation between virulence and sarcoma development, and were inclined to attribute great importance to virulence in the initiation of the transformation.

That the length of time during which a tumor had been under

[1] *Arb. a. d. Königl. Inst. f. Exp. Therap.*, 1906, Heft i, 56.
[2] *Berl. klin. Woch.*, 1906, xliii, 38.

cultivation did not deserve the importance at first ascribed to it they were able to record in the following year, after they[1] had discovered one instance in which sarcoma development had certainly occurred in the spontaneous tumor, and a second where such a change had in all probability taken place there. A similar example was later recorded by Haaland,[2] in which a spontaneous growth, removed by operation, was found to consist of a central carcinomatous part surrounded by a band of spindle cell sarcoma.

Useful reviews of the cases of sarcoma development which have been observed at the Frankfort laboratory have been given by both Ehrlich[3] and Apolant.[4]

Loeb[5] described a primary non-metastasizing adeno-carcinoma in the submaxillary gland of a female Japanese mouse from six to eight months old. The tumor was of uniformly epithelial structure and belonged to the glandular type. It was possessed of ducts and alveoli; the lumina of the latter contained colloid material, and the epithelial elements were supported by a diffuse connective tissue. Inoculation was successful in Japanese mice but failed in those of the white variety. In one of two surviving Japanese mice a few slowly growing nodules were found fifty-eight days after inoculation, one of which, removed a few days later by operation, proved to be a spindle-cell sarcoma. When the animal was found dead ninety days after inoculation and twenty-six days after the excision of the nodule, there was a recurrence which had grown rapidly and which consisted almost exclusively of pure sarcoma, and in addition a large glandular tumor in which sarcoma was developing at one point. The tumors of the second mouse, glandular growths with some sarcomatous elements in them, were used for transplantation ninety-three days following inoculation. They were ingrafted into six mice, one of which was found later to have developed a pure sarcoma, while the growths in four of the other mice were mixed.

[1] *Berl. klin. Woch.*, 1907, xliv, 1399.

[2] *Fourth Sci. Report, Imperial Cancer Research Fund*, London, 1911, 19.

[3] *Zeitschrift f. Krebsforsch.*, 1907, v, 62.

[4] *Handbuch der pathogenen Mikroorganismen*, Kolle and Wassermann, erster Ergänzungsband, 1906, 452.

Zeitschrift f. allg. Physiol., 1909, ix, Sammelreferat, 96.

[5] *Univ. Pennsylvania Med. Bull.*, 1906, xix, 113.

Berl. klin. Woch., 1906, xliii, 798.

The sixth had two, one glandular and one a pure sarcoma. In the second generation the sarcoma was in excess of the carcinoma, as it had been in Ehrlich and Apolant's first case. Because the chances of further transplantation were excluded by a shortage of Japanese mice the experiment was perforce terminated.

For the appearance of the sarcoma Loeb offered three explanations — that the tumor was originally a mixed tumor, that the sarcoma was derived from the connective tissue, or, finally, that a transformation of glandular into spindle cell tissue had taken place. The first he thought improbable, and left open the choice between the second and the third. If one accepted the second it would be possible to eliminate the theory that sarcoma development was the result of repeated inoculation of stroma, because the change had occurred in the first generation. It seemed most probable that the sarcomatous transformation was the result of a stimulus of unknown nature exerted upon the stroma by the carcinomatous moiety of the tumor.

In a later paper he[1] said, in discussing the third explanation, that it had been impossible to find any evidence of transition stages between adeno-carcinoma and sarcoma in those areas where sarcomatous tissue was present. The article concluded with a reaffirmation of the hypothesis that the probable cause of sarcoma development was a stimulus exercised upon the surrounding connective tissue by the cells of the carcinoma.

Haaland,[2] investigating the occurrence of metastases in the second of Ehrlich's sarcoma strains, found that microscopic nodules occurred in the lungs of 60% of his mice with tumors that were from fourteen to forty days old. The importance of this observation lay in the fact that the method by which a tumor spread through the organism was a sound criterion for the distinction between true malignant growths and the infectious granulomata. By examining serial sections Haaland could show that the metastatic emboli reached the lungs through the blood stream, determining their relation to the vessels by the aid of Weigert's elastic tissue stain. For the investigation of the stroma either van Gieson's or Mallory's method was employed.

[1] *Zeitschrift f. Krebsforsch.*, 1908–1909, vii, 101.

[2] *Berl. klin. Woch.*, 1906, xliii, 1126.
Zeitschrift f. Krebsforsch., 1907, v. 122.

It was probable that a considerable number of emboli were destroyed, or at least did not grow, for he found them occasionally, as Schmidt[1] had described them in human lungs, surrounded by collections of round cells and leucocytes, and the seat of regressive changes. The majority, however, were in active growth, filling and distending the lumina of the vessels; in this intravascular stage there was no sign of reaction either in the vessel wall or the surrounding lung tissue. In their further development these emboli assumed an infiltrative growth during which their elements rapidly penetrated the wall and, infiltrating the neighboring tissues, surrounded the vessels with a mantle of tumor cells. Larger nodules were thus evolved which entirely destroyed the lung parenchyma and spared only the elastic tissue. While the stroma of these tumors was sparse, and in most cases hardly recognizable in the early stages, there were a few instances in which Mallory's stain would demonstrate a few very delicate, sinuous, blue-stained fibrils between the individual cells, even before the penetration of the vessel wall. In the later stages, when the wall had been broken through, the histological picture was complicated by the fact that portions of the connective tissue of the lung, and of that accompanying the blood vessels, had persisted and were participating in forming the stroma of the new nodule. But even here stroma development remained within certain bounds, being limited to the reproduction of a few isolated fibrils between the individual tumor cells.

Liepmann[2] had under observation a mouse carcinoma which, at the eighth transplantation, had assumed the character of a carcinoma sarcomatodes, although as far as the fifth generation the typical carcinomatous structure of the spontaneous tumor had been retained.

Bashford, Murray, and Haaland[3] described the appearance of sarcoma during propagation of a mammary adeno-carcinoma ("37") of the mouse. The transformation occurred in the seventh and eighth generations in two strains, one of which had always been transplanted with the needle, and the other, after the fourth generation, with the syringe. Seven tumors out of ten in the seventh generation were transplanted, giving rise to the eighth. The usual systematic examina-

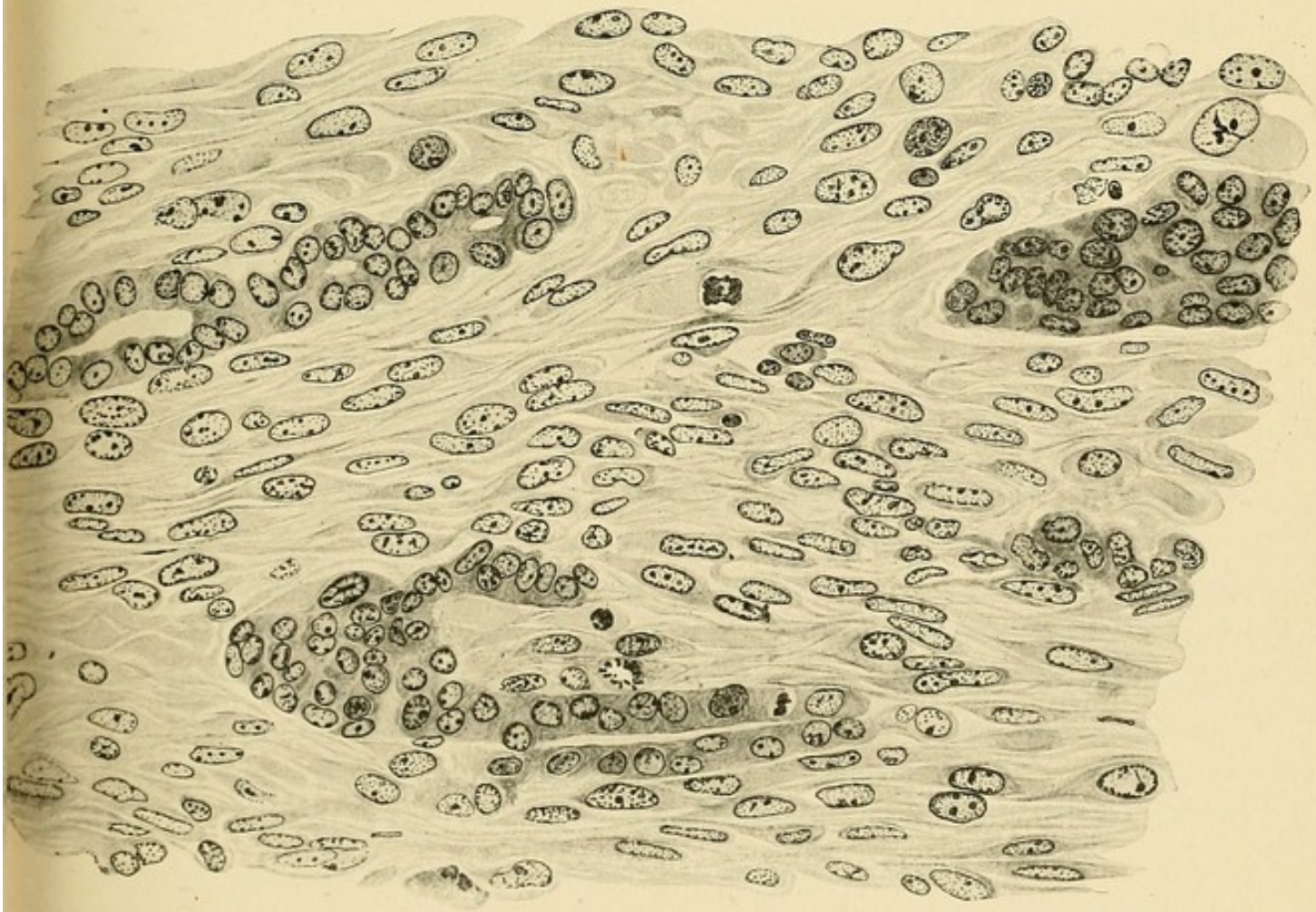
[1] *Die Verbreitungswege des Carcinoms*, etc., Jena, 1903, 41.
[2] *Münch. med. Woch.*, 1907, liv, 1345.
[3] *Berl. klin. Woch.*, 1907, xliv, 1238.

tion of the growths in each generation had revealed no change in the stroma before the seventh. At this point two again were unchanged, while in two others there was a slight increase in the amount and cellularity of the stroma, and one of them contained a small area of spindle cells. In the remaining three the stroma had increased greatly in amount and now formed broad bands of elongated cells lying among the carcinomatous elements. Transplantation of this generation resulted in a yield of twenty-two tumors, in twenty of which there were found spindle cells arranged in bands and undergoing relatively rapid division. The examination of grafts at short intervals after transplantation showed conclusively that the stroma did not die out as it had in the previous generations, but, on the contrary, that it remained alive, and, moreover, possessed the power of independent proliferation. In older tumors of the eighth and in those of the ninth and tenth generations, the carcinomatous portions were surrounded by halos of clear polymorphous cells, larger than those of the carcinoma and distinguished from them by their lightly stained protoplasm. That they were not of epithelial origin was certain. In fact, one could find all transitional stages between them and the typical spindle-shaped stroma cells, which appeared to be a more highly differentiated form of the cell now under discussion. Although the zones just mentioned gave the impression of a reaction tissue composed of elements newly arisen from those of the host, the study of young grafts demonstrated that the clear cells did not die out after transplantation, but were able to proliferate and to produce sarcoma. The authors believed that, although endowed with the power of independent growth, these cells had not yet lost entirely their old relation to those of the carcinoma, but had, on the contrary, remained sensitive to influences emanating from these elements and were accordingly arranged about the alveoli in the manner of the old stroma. Only the stroma cells far removed from the carcinoma were exempt from this influence and able to differentiate characteristically into bands of spindle cells.

As the stroma of three tumors in the same series showed sarcomatous transformation, it seemed more likely that some special stimulus over and above the ordinary stroma-forming stimulus had been exerted upon the fibroblasts by the carcinoma cells, than that the sarcomatous

J. R. Ford, del.

One of four growths (41 days old) of Series D, 7th generation of tumor 37, which showed first cases of sarcoma development. Abundant cellular interstitial connective tissue, consisting of large spindle cells, runs between the islands of acinous parenchyma throughout the whole tumor. $\times \frac{62}{1}$.



J. R. Ford, del.

Mixed tumor of the 10th generation, 23 days old. Broad bands of sarcomatous tissue containing mitoses, and separating the acini of the carcinoma. $\times \frac{350}{1}$.

change had been initiated through any particular attribute of the mice in which it had taken place.

The stroma, now able to proliferate independently, was no longer subservient to the needs of the epithelial parenchyma in the matter of providing nutrition for its cells, and, indeed, it even seemed as though the carcinomatous part of the tumor was isolated from the blood vessels by the halos of clear cells. Whether or not this explained the disappearance of the carcinomatous elements, the contrast in size between the succulent cells of the sarcomatous stroma and those of the epithelial parenchyma was striking. Both the nuclei and protoplasm of the latter appeared to diminish in size, while mitoses became less and less frequent as the transformation of the stroma proceeded, until finally complete disappearance supervened.

An attempt was made, with a fairly successful outcome, to hasten "purification" of the tumor by introducing it into mice resistant to carcinoma. One tumor showing a pronounced decrease in the amount of carcinoma was obtained from ten inoculations. In the next transplantation, which was made into normal mice, only traces of carcinoma could be discovered, and in the next succeeding one a pure sarcoma was found. In none of the controls was there obtained, within the same period, a tumor of purely sarcomatous type.

The quantitative relation between carcinoma and sarcoma varied somewhat from tumor to tumor, but as a rule the progress of the sarcoma was uninterrupted, although in isolated cases the carcinoma appeared to obtain the mastery, so that the regaining of a pure carcinoma seemed possible of achievement.

The authors suggested two ways of accounting for the appearance of sarcoma. Either the tumor was from the start a mixed one, or its cells had the power to exert a specific stimulus upon the connective tissue, causing its elements to become capable of continued proliferation and thereby transplantable. Systematic study of early stages led Bashford and his associates to accept the latter explanation, in doing which they were in agreement with the hypothesis advanced by Ehrlich and Apolant. None of the three factors, length of propagation period, virulence, or histological structure of a tumor, seemed to be of moment in determining whether the growth would or would not be able to induce sarcomatous transformation.

Loeb[1] also was of the opinion that it was not necessary for the carcinoma to be possessed of any particularly high degree of virulence.

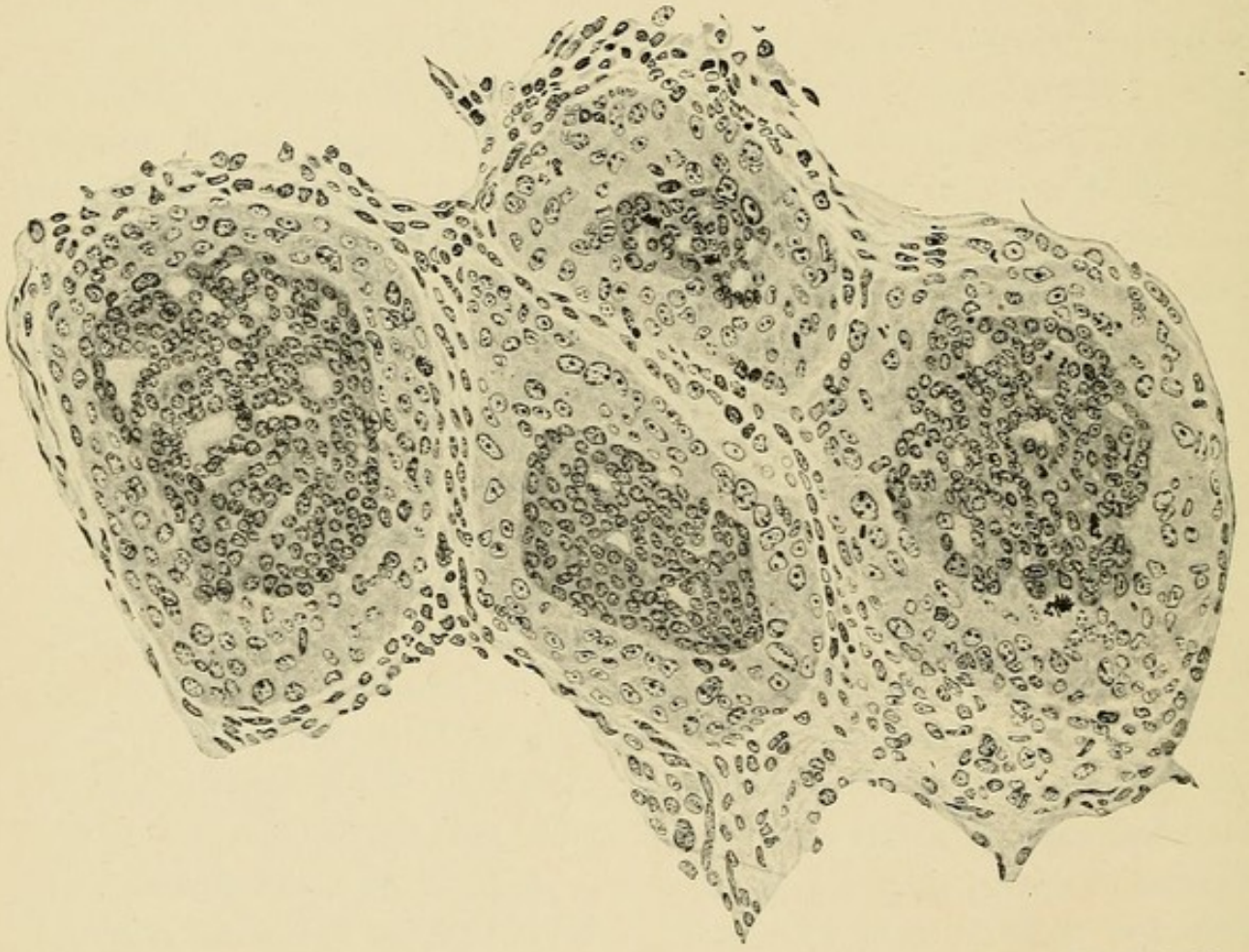
In the rat, Lewin[2] observed sarcomatous transformation in the fifth generation of a keratinizing adeno-carcinoma, and was inclined to consider that endothelial cells were the ancestors of the sarcoma.

Orth,[3] who examined Lewin's preparations, was not convinced that the tumors had actually undergone a sarcomatous change, and expressed the belief that the new cells were those of ordinary granulation tissue.

Sticker[4] advanced the supposition that many cases of so-called sarcoma development were either the issue of the inoculation of a mixed tumor, or else represented the simultaneous occurrence of a transplantable carcinoma and a spontaneous sarcoma in the same animal.

Haaland[5] described the change in other strains of the tumor reported by Bashford, Murray, and Haaland, pointing out that sarcoma development had hitherto taken place so unexpectedly that observations had of necessity been made long after the actual occurrence of the transformation. The material available had, therefore, been incomplete. The present case, however, was encountered under much more favorable auspices, for, as the primary growth had been examined on two occasions and sections from every tumor in the following generations were at hand for histological survey, there were no gaps in the continuity of the earlier material. Moreover, it had been possible to supplement the morphological data thus obtained with a comparison of the biological peculiarities of different strains, because the clinical behavior of all the tumors had been recorded on a series of charts. In addition to this investigation of general histological and biological characters, the processes in play at short intervals after transplantation had been examined, and the behavior of the tumor at any period after the moment of inoculation could thus be compared with its behavior at any later stage. Finally, the carcinoma had not been lost during the development of the sarcoma,

[1] *Deut. med. Woch.*, 1908, xxxiv, 25.
[2] *Zeitschrift f. Krebsforsch.*, 1907–1908, vi, 273.
[3] *Zeitschrift f. Krebsforsch.*, 1907–1908, vi, 431.
[4] *Zeitschrift f. Krebsforsch.*, 1907–1908, vi, 431.
[5] *Jour. Path. and Bact.*, 1908, xii, 437.
Third Sci. Report, Imperial Cancer Research Fund, London, 1908, 175.



J. R. Ford, del.

24-day-old mixed growth from 9th generation of tumor 37, to show halos around *healthy* alveoli. × *circa* $\frac{200}{1}$.

and ten separate strains of purely epithelial tumors had been preserved.

Haaland and his colleagues had been enabled, therefore, to make an exhaustive study of the question from every standpoint—the histology of the primary growth, the progressive advance of the sarcomatous change, the condition of the stroma in old tumors of carcinomatous strains as well as in new grafts, and the biological characteristics of carcinomatous, mixed, and sarcomatous strains.

Although the mother material of the four tumors in which sarcoma development had arisen had been examined in the hope that the route taken by the stroma cells could be retraced, the change seemed to have been a sudden one, and no indication could be discovered of the manner in which it had been initiated.

The progressive advance of the transformation took place by an increase of spindle cell tissue from one generation to the next, and the alveoli became more widely separated from one another in consequence, often showing slight necrotic changes due to impairment of nutrition. The next step was the interpolation of an intermediate period where for several generations the spindle elements were replaced by polymorphous cells, and as a constant and characteristic feature of this stage lightly staining and extremely polymorphous elements collected about the carcinomatous acini, forming halos sharply demarcated from the latter on their inner circumferences, but shading off gradually into the sarcomatous stroma on the outer. The halos varied from a single row of cells to the more usual width of several layers, and the nuclei were very large and sometimes multiple. Often the nuclei were of the true giant type, very rich in chromatin. As long as any epithelial components remained in the tumors, so long did polymorphism persist, but with the entire disappearance of the carcinomatous cells those of the sarcoma resumed their primary typical spindle shape.

What was the derivation of these lightly staining polymorphous cells? The idea of an epithelial origin Haaland felt unable to entertain and, as they proliferated after transplantation, they could not be the elements of ordinary granulation tissue. It seemed most probable that they did not originate from the connective tissue of the new host, but that they were derived from preceding sarcoma cells and

transferred as such with the graft, and this explanation was made even more plausible by the discovery throughout the body of growing metastases made up of similar cells.

The alveoli that were surrounded by halos very often showed central necrosis, ordinarily a rather rare occurrence in the alveoli of the tumor under discussion. These degenerative changes Haaland referred to the fact that no trace of capillaries could be found inside the halos, and the cells of the latter thus seemed to be interposed between the alveoli and the capillaries of the stroma.

The sarcomata of this strain had a greater initial rapidity of growth than the carcinomata and, on the whole, gave a higher percentage of growing tumors, although spontaneous absorption was of very frequent occurrence. Their power of infiltrative growth much exceeded that usually evinced by the carcinomata, and they metastasized readily, producing secondary growths in the lungs, heart, liver, kidney, spleen, and lymph nodes.

Mixed growths tended to become pure sarcomata, but the process required a certain length of time and seemed to occur independently of the rapidity of *passage* from host to host; and while the disappearance of the epithelial part of the tumor was generally accomplished first in the center, the rate at which the process went on in the different portions was variable. To see whether the transformation were a sudden one Haaland investigated old and young growths from strains in which the change had occurred, as well as from others which had been consistently free from it. In all the later cases of sarcoma development he found the first alterations at the center of the carcinomatous tumor, and here sclerotic lesions were habitually present, often accompanied by more or less cellularity of the stroma. The sarcomatous change suggested in some cases a process similar to this, although enhanced in degree. It was not suddenly gained, but appeared to be a gradual acquisition, the power of continuous growth and the rate of proliferation becoming more advanced in its later stages. The condition seemed to be, on the whole, the result of a gradual, fluctuating evolution.

The dependence of sarcoma development upon virulence, as upheld by Ehrlich and Apolant, was not a phenomenon of universal distribution, for in the present case the tumor was one of rather slow growth.

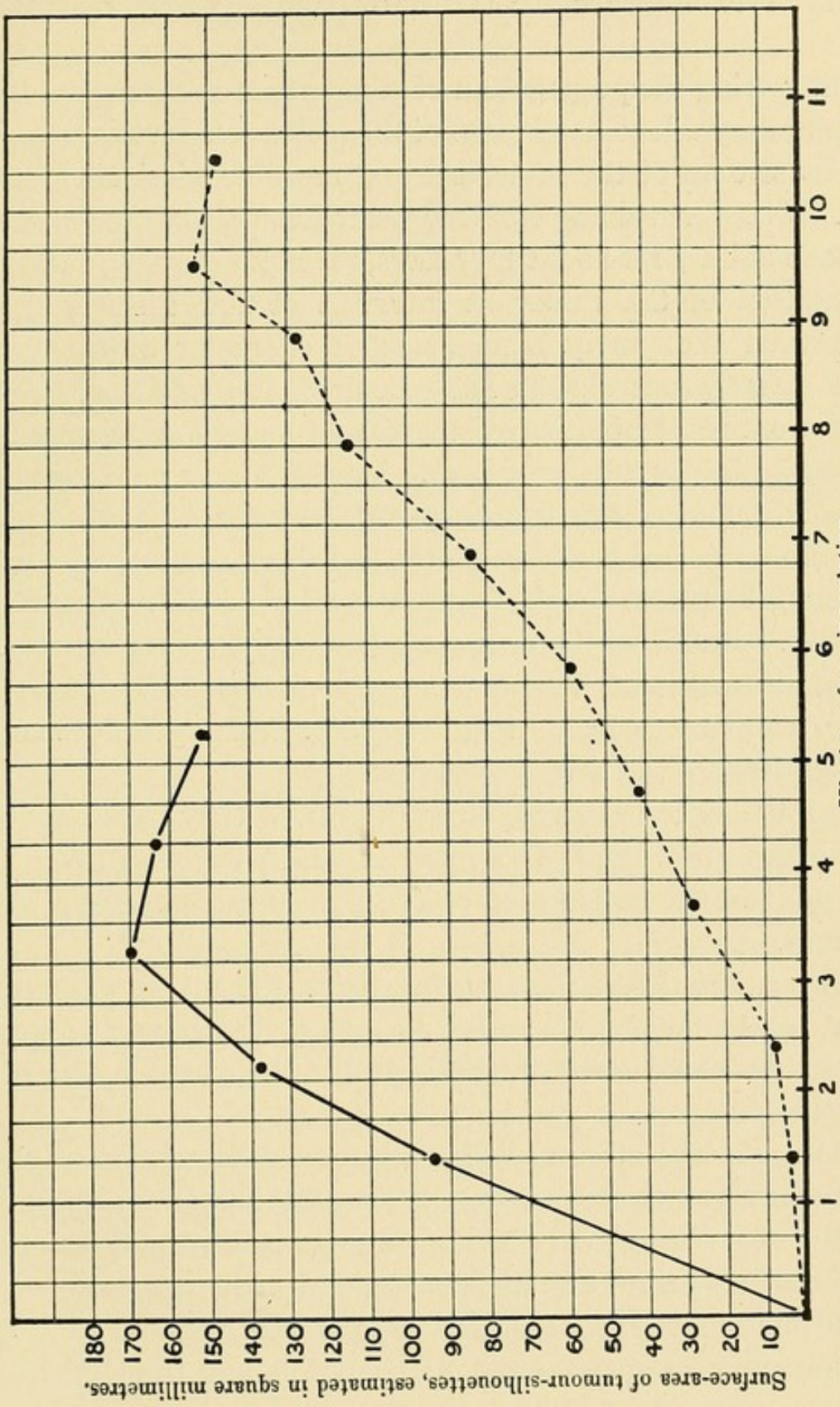


FIG. 7. — Curves showing difference in the average rate of growth of daughter tumors developing in a carcinomatous (- - - -) and in a pure sarcomatous (——) series. The curves depict the greater initial rapidity of growth in the sarcomata.

Albrecht and Hecht[1] confessed that they had been unable either to initiate or to influence the transformation of carcinoma into sarcoma.

Russell,[2] during the propagation of a recurrent spontaneous hemorrhagic mammary adeno-carcinoma of the mouse, observed the assumption by its stroma of the biological and morphological characters of sarcoma. The determining factor appeared to be continuous proliferation in one animal for about fifty days, for rapid *passage*, that is, the transplantation of the tumor at intervals of about thirty days or less, preserved the purely carcinomatous character of the growth. Once the sarcomatous change had occurred it tended progressively toward the entire elimination of the carcinomatous component, and the tumors could then be propagated indefinitely as pure sarcomata. Upon the initiation of the transformation there was no change in the speed of growth, and tumors which had been cultivated through one or more generations as mixed growths retained the rate of the pure carcinomata. But with the disappearance of the epithelial elements the rate of development increased greatly, whence Russell suggested that they might be capable of exerting a restraining influence upon the proliferation of the sarcomatous areas.

Although the sarcomata were of rapid growth they were very prone to spontaneous absorption, and mice in which this had taken place were resistant to the inoculation of the purely carcinomatous strains of the tumor.

As to the explanation of the transformation the author was in entire agreement with previous observers, believing that it was induced in the connective tissue cells of the host by the parenchyma of the epithelial tumor.

The change in the stroma, which was generally unicentric, began toward the middle of the growth in an area containing a relatively enormous number of mitoses, and of such small size as to suggest very recent origin. In point of time the transformation usually occurred between the fifty-fifth and sixtieth days, and among one hundred tumors examined only four or five showed no change before the sixtieth day,

[1] *Centralbl. f. allg. Path.*, etc., 1909, xx, 1039.
Wien. klin. Woch., 1909, xxii, 1740.
[2] *Jour. Path. and Bact.*, 1910, xiv, 344.

while one had advanced to pure sarcoma at the end of this period. The length of time required, however, was not uniform, for while in some series the onset of the sarcomatous alteration could not be detected until after seventy days, in others the change was present even after thirty-eight. That the variation was not due to individual differences in the mice was proved by the fact that the initiation of the transformation was uniform for all those of any given series. A stay of longer than sixty days in one animal was difficult to secure—otherwise each tumor would doubtless have progressed to the stage of pure sarcoma.

A further case of sarcoma development was recorded by Stahr,[1] affecting certain of the tumors in the ninth generation of a fissure-forming carcinoma. Stahr's observations differed somewhat from those of Haaland and Russell as regarded the point at which the change began, in that while these authors had recorded its first appearance in the middle of the tumor, Stahr found it earliest at the periphery. He agreed with Haaland, however, that the transformation was in some way connected with a low power of growth on the part of the carcinoma cells, and suggested that possibly it might be set going through the agency of materials elaborated by their death.

Clunet[2] discovered still another instance of sarcomatous change in a growth belonging to the third generation of a malignant mammary cyst-adenoma of the mouse, where the transition was complete in every tumor of the sixth generation. Since then the growth, which had reached the thirty-eighth generation, had retained its sarcomatous character unaltered. The malignancy of the newly produced tissue was manifest not only from its histology, but from its clinical behavior, proliferation continuing indefinitely and leading to death from cachexia. Furthermore, the tumors recurred after operative removal, infiltrated the surrounding tissues, and produced metastatic deposits in the lungs.

In discussing the cause of the mutation, Clunet eliminated any chance that the primary tumor had been a carcinoma sarcomatodes, and accepted a malignant change in the stroma as the most probable explanation. He agreed with Haaland's observations on the absence

[1] *Centralbl. f. allg. Path.*, etc., 1910, xxi, 108.

[2] *Recherches exp. sur les Tumeurs malignes*, Paris, 1910, 53.

of any connection between tumor virulence and sarcoma development, for in the first six generations of the tumor, while the sarcomatous change was being initiated, the percentage of takes had been low and negative inoculations or spontaneous regressions frequent. As in Russell's experience, the power of growth increased rapidly after the epithelial components had disappeared.

CULTIVATION OF CELLS IN VITRO

One of the disadvantages of the ordinary method of investigating cancer was the irregularity inherent in the soil where the tumor cells were grown and in which, therefore, they had to be studied. Following the lead of Harrison,[1] who showed that the nerve tissues of the frog embryo could be grown in lymph outside the body, other observers have applied the method, with certain modifications, to the cultivation of the cells of various normal tissues as well as those of tumors.

The use of lymph, however, proved inconvenient for several reasons, and Burrows[2] substituted plasma, which he obtained from blood that had been centrifugalized at a low temperature in paraffined tubes. In the opinion of Carrel and Burrows[3] the pure plasma thus obtained was much superior to oxalated plasma, which could, nevertheless, be used in cases of necessity. The authors described two types of culture — the small hanging drop, and the large plate culture. In making the former, one or two fragments of the tissue which it was desired to cultivate were transferred to a cover-glass and quickly immersed in a drop of plasma, which was spread out in a thin layer before the occurrence of coagulation. The cover-glass was then inverted over a hollow slide to which it was sealed with paraffin to prevent evaporation, and the preparation immediately transferred to the incubator. Large plate cultures were made by spreading fragments of tissue in a thin layer over the surface of a large, black glass plate, and covering

[1] *Proc. Soc. Exp. Biol. and Med.* 1906–1907, iv, 140.
Anat. Record, 1908, ii, 385.
Jour. Exp. Zoöl., 1910, ix, 787.
[2] *Jour. American Med. Assoc.*, 1910, lv, 2057.
[3] *Jour. Exp. Med.*, 1911, xiii, 387.

them quickly with plasma. As soon as coagulation appeared the plates were put into glass boxes containing moist cotton to preserve the proper humidity, and the boxes were sealed with paraffin and kept in the incubator in such a position as to allow the fluid products of the culture to drain to the bottom. The most careful asepsis was, of course, necessary throughout the procedure.

For the study of the cultures, a warm stage was employed on the microscope. Growing cells at the edge appeared as fusiform or polygonal bodies, the cytoplasm of which was filled with refractile granules. Often the movements of the living cells, their modification in shape, and the division of their nuclei could be readily observed. Cultures could be fixed and stained by removing the cover-glass with its adherent tissue to appropriate solutions or, in case the plasmatic medium was thick and the cells had grown in many planes, serial sections of the hardened cultures could be made.

Carrel [1] reported in a subsequent contribution that cell division had been observed in cultivated tissues washed and placed in fresh media, as long as thirty-one days after their removal from the body, and that cultures had survived even nine transfers to fresh plasma.

Lambert and Hanes,[2] discussing the growth of rat and mouse tumors *in vitro*, wrote that the cells of sarcomata possessed the power of wandering out separately into the plasma, probably in search of nourishment, while those of carcinomata, on the other hand, were denied that function and moved outward in a continuous layer. The nuclei in both types frequently contained division figures, and the cells in the two cases were actively phagocytic. The same authors [3] found that rat sarcoma would grow in the plasma of immune rats quite as vigorously as in that from normal or tumor-bearing animals, an observation affording further proof, they believed, of the absence of specific cytolytic substances in the body fluids of animals immune toward transplantable tumors.

They [4] furthermore established the fact that rat and mouse tumors would grow in plasma from alien species, among which the order of

[1] *Jour. American Med. Assoc.*, 1911, lvii, 1611.
[2] *Jour. Exp. Med.*, 1911, xiii, 495.
[3] *Jour. Exp. Med.*, 1911, xiii, 505.
[4] *Proc. Soc. Exp. Biol. and Med.*, 1910–1911, viii, 123.

suitability was: Guinea-pig, rabbit, pigeon, man, dog, goat (no growth).

Those whose interest prompts them to inquire more deeply into the cultivation of cells outside the body should consult the papers of Burrows,[1] Carrel,[2] Carrel and Burrows,[3] and Lambert and Hanes.[4]

Although all these investigators believed that they had observed cell growth taking place outside of the body, some doubt has been expressed as to the correctness of this view. Jolly [5] did not think that Carrel and Burrows had succeeded in demonstrating the survival of cells, and expressed the view that certain of their descriptions were reminiscent of the phenomena of necrobiosis. And although, as was already known, cellular proliferation appeared to be possible for a time in certain tissues, between such a condition and a culture with continuous and progressive development there was a hiatus which might, perhaps, be conquered at some future day. At the time of writing, however, the author considered it an abuse of language to apply the term "culture" to the results so far obtained. In a later article he [6] said that if the spread of cells in cultures were a true growth and not a mere dissemination, one should be able to find evidences of intense cellular multiplication. These he had not been able to verify in the photographs that he had seen.

Ewing [7] was of the opinion that the cells of preparations *in vitro* were elements which had survived and retained a momentum respon-

[1] *Compt. rend. Soc. Biol.*, 1910, lxix, 291.
Jour. Exp. Zoölogy, 1910, x, 63.
[2] *Jour. Exp. Med.*, 1910, xii, 460.
Berl. klin. Woch., 1911, xlviii, 1364.
[3] *Compt. rend. Soc. Biol.*, 1910, lxix, 293, 298, 299, 328, 332, 365, 367.
Compt. rend. Soc. Biol., 1911, lxx, 3.
Jour. Exp. Med., 1911, xiii, 416, 562.
Jour. American Med. Assoc., 1910, lv, 1379, 1554, 1732.
Jour. American Med. Assoc., 1911, lvi, 32.
[4] *Jour. American Med. Assoc.*, 1911, lvi, 33, 587, 791.
Proc. Soc. Exp. Biol. and Med., 1910–1911, viii, 59.
Zeitschrift f. Krebsforsch., 1911–1912, xi, 134.
Jour. Exp. Med., 1911, xiv, 129, 453.
[5] *Compt. rend. Soc. Biol.*, 1910, lxix, 470.
[6] *Compt. rend. Soc. Biol.*, 1911, lxx, 4.
[7] *Zeitschrift f. Krebsforsch.*, 1911–1912, xi, 136.

sible for certain phenomena, but he was doubtful whether there was any real physiological growth. Dying cells did undergo mitosis and, in fact, this process seemed to be increased in dying tissues. All the phenomena so far recorded might be those of necrobiosis, and while the experiments were of interest, the growth of cells outside the body had not yet been observed.

CHAPTER V

RESISTANCE

BASHFORD, Murray, and Haaland[1] have pointed out that the term *immunity*, so often used synonymously with *resistance*, is an unfortunate one, implying as it does a diminished liability to the development of spontaneous cancer. It does not necessarily follow that animals resistant to the inoculation of a tumor are protected against the origin of spontaneous cancer, for Bashford, Murray, and Cramer[2] had a mouse which, having been repeatedly inoculated in vain with Jensen's tumor, nevertheless developed a spontaneous growth of different histological type seven months later. Nor is this an isolated instance. Thorel[3] later recorded the occurrence of spontaneous tumors in twelve mice that had been inoculated one or more times without success from ten days to nine months previously. The possibility that these growths might have resulted from a postponed development of the grafts was eliminated by their location at a site far removed from that regularly chosen for implantation. Moreover, the tumors all occurred in females. Clunet[4] also has reported two spontaneous tumors which arose in mice that had been once unsuccessfully inoculated several months previously.

Resistance is usually divided into *natural* and *acquired*, and the latter, in its turn, into *active* and *passive*.

NATURAL RESISTANCE

Natural resistance is said to be present in consequence of age, race, or ill health and, as it is hardly necessary to state, in species other

[1] *Third Sci. Report, Imperial Cancer Research Fund*, London, 1908, 359.

[2] *Proc. Roy. Soc.*, Series B, 1907, lxxix, 171.
Third Sci. Report, Imperial Cancer Research Fund, London, 1908, 322, 396.

[3] *Verhandl. d. deutschen path. Gesellsch.*, 1908, 12te Tagung, 60.

[4] *Recherches exp. sur les Tumeurs malignes*, Paris, 1910, 24.

than that in which the tumor arose, or, as has been said by Ehrlich[1] and others, outside the limits of bastardy. It is known that sex has no influence in its determination, but the effects of pregnancy have not yet been so clearly defined.

Influence of Age

Although when experimental transplantations were first undertaken it seemed natural to suppose that as cancer originated most frequently in old animals these would offer the best soil for transplantation, it was soon found that the assumption was unwarranted. Thus Loeb[2] noticed that transfer of a rat sarcoma succeeded in both old and young rats, and Bashford and Murray[3] wrote: "Age seems to have no influence on the proportion of successful transplantations, in contrast to its cardinal importance in determining the initiation of the cancer-cycle." The case against the old animal was destined to become even stronger, for in the following year Bashford[4] said: " . . . transplanted growths flourish as well, or better, in young and vigorous mice," and this view had been consistently expressed since that time in communications from his laboratory.[5] In fact, in the paper read before the Royal Society there were described inoculations into two series of mice aged four and five days respectively, with an issue of ten tumors in thirteen mice, or 77 % of successful inoculations. The conditions for tumor growth being thus more favorable in young animals than in old, Bashford and his colleagues were led to indicate and to emphasize that there must be, in consequence, a difference between the conditions necessary for the origin of a tumor and those required for its continued growth.

The experiment was repeated later by Buschke[6] with an entirely

[1] *Arb. a. d. Königl. Inst. f. Exp. Therap.*, Heft i, 1906, 82.
[2] *Jour. Med. Research*, 1901, N.S., i, 36.
[3] *Sci. Reports, Cancer Research Fund*, London, 1904, No. 1, 14.
[4] *Lancet*, 1905, ii, 104.
[5] *British Med. Jour.*, 1906, ii, 208, 1554.
Proc. Roy. Soc., Series B, 1906, lxxviii, 196.
Zeitschrift f. Krebsforsch., 1907, v, 419.
Third Sci. Report, Imperial Cancer Research Fund, London, 1908, 285.
Lancet, 1909, ii, 700.
[6] *Berl. klin. Woch.*, 1911, xlviii, 215.

confirmatory outcome, rats and mice two, three, and four days old offering a soil favorable for the growth of transplanted cancer.

Ehrlich and Apolant [1] arrived at similar conclusions and expressed themselves very much as Bashford had done, namely, that, as transplanted cancer grew as well in young animals as in old, a difference must exist between the conditions necessary for the inception of a tumor and those requisite for its growth in another organism.

Haaland [2] had found it a matter of daily experience that young mice were, in general, suitable for tumor implantation, and Lewin's findings [3] in rats coincided with the conditions already defined in mice. Animals from five to eight weeks of age he found susceptible in the highest degree as compared with old ones. Gierke [4] likewise considered that age did not render animals more suitable for transplantation but that, on the contrary, young mice from eight to fourteen weeks old (before puberty had supervened) were as suitable, and in many cases even more suitable, than older animals.

The experiments of Albrecht and Hecht [5] proved that fully grown mice were fairly resistant to inoculation and that neither these, nor very young animals, were so well adapted as half-grown ones. They finally settled upon healthy animals over four weeks of age as affording the best medium for transplantation.

Influence of Race

Beside the immunity due to age there has been distinguished a state of resistance determined by racial differences, fully described for the first time by Jensen.[6] In addition to inoculating his tumor into white mice he tried to cultivate it in the common house mouse (*Mus musculus*), meeting, however, with such indifferent success that in one series only one tumor was obtained in the ten animals that had been inoculated. Once the growth had been transferred from white

[1] *Berl. klin. Woch.*, 1905, xlii, 872.
[2] *Berl. klin. Woch.*, 1907, xliv, 718.
[3] *Berl. klin. Woch.*, 1907, xliv, 1605.
Zeitschrift f. Krebsforsch., 1907–1908, vi, 303.
[4] *Beitr. zur path. Anat.*, etc., (Ziegler), 1908, xliii, 343.
Third Sci. Report, Imperial Cancer Research Fund, London, 1908, 132.
[5] *Centralbl. f. allg. Path.*, etc., 1909, xx, 1039.
Wien. klin. Woch., 1909, xxii, 1738.
[6] *Centralbl. f. Bakt.*, etc., erste Abt., Orig., 1903, xxxiv, 126.

mice to gray, however, its transplantation from gray to gray became easier, and successful implantations could finally be performed in twenty-seven animals out of eighty-four, although growth was frequently somewhat slower than in white mice. A transfer from the gray variety back to white was readily accomplished. Two attempts to transmit the neoplasm to the long-tailed field mouse (*Mus sylvaticus*) were unsuccessful, nor was the tumor transplantable into other varieties of white mice. The same author,[1] in a later description of two propagable sarcomata of the tame rat, said that they would not grow at all in wild rats and could be inoculated only occasionally into variegated rats from Hamburg, Berlin, and London. On the contrary in white and variegated rats of the laboratory strain and in Copenhagen rats (undoubtedly closely related to the laboratory stock) they could be grown very successfully, the tumor from one rat yielding 87.5 % of daughter tumors and that from the second 57.7 %. The transplantation of both tumors being continued, there occurred during cultivation such an increase in the percentage of successful results that in the fourth, fifth, and sixth generations the yield with the tumor from the first rat had risen to 100 % and with that from the second to 85 %, both calculated in Danish rats. This rise was also evident, however, in foreign rats, for when the first tumor in its fifth generation was inoculated from a Danish rat into five Berlin, three Hamburg, and ten London rats, four, two, and seven, respectively, of these rats, eleven[2] in all, or 61.1 %, developed tumors, while in the first three generations tumors had followed in only 10.5 % of the animals inoculated.

The question of the effect of race upon the suitability of the soil occurred also to Loeb,[3] but he left its final solution to be determined by future investigations. In a later article he[4] described the successful transfer of a sarcoma of the white rat to a cross between the gray and the white rat, and the failure to transplant a tumor of the Japanese waltzing mouse to ordinary white mice.

[1] *Zeitschrift. f. Krebsforsch.*, 1908–1909, vii, 49.

[2] There is a discrepancy in the numbers here: 11 should read 13 and the successful results would, therefore, number 72.2 %. Professor Jensen was kind enough to reply to a letter of inquiry on this point, that 5 Berlin, 5 Hamburg, and 10 London rats were inoculated, and that of these 20 animals, 14, or 70 %, developed tumors. Growth had, however, been so slow, that when his paper was published the results had not been final.

[3] *Jour. Med. Research*, 1901, N.S., i, 36.

[4] *American Medicine*, 1905, x, 265.

Bashford and Murray,[1] having received Jensen's tumor with the information that in Denmark it had grown best in white mice, tested its growth in English white mice, which they found yielded less favorable results than other races of tame English mice, as yellow, black and white, yellow and white, and black; in what were known as "blue mice" they had not been able to get the tumor to grow at all. Bashford,[2] with Murray and Cramer,[3] recording later and more elaborate experiments, described attempts to transplant spontaneous or propagable tumors of Danish, French, and German mice into English breeds. The results, which were entirely analogous to those first obtained, demonstrated that the transfer of malignant new growths to mice of slightly different race might present considerable difficulties. The apparent unsuitability of blue mice for Jensen's tumor, as published in the earlier communication, proved, however, not to be absolute, and in later experiments this breed developed growths which in several cases attained a huge size. The same phenomenon became apparent in other strains of English mice as concerned the inoculation both of Jensen's tumor and one of German origin, and the percentage of successful inoculations increased gradually to a point where the results eventually equaled, or promised to equal, those obtained in Danish and German mice. After Jensen's tumor had been growing in English mice for three years it was inoculated back into Danish mice by Bashford, Murray, Haaland, and Bowen,[4] who recorded that its power to grow in the original soil had not been lost.

According to Michaelis,[5] mice sent him from Denmark provided a better soil for the growth of Jensen's carcinoma than mice obtained in Berlin; and Clowes[6] found difficulty at first in transplanting Jensen's tumor into gray mice of American stock.

Hertwig and Poll[7] did not believe that racial resistance actually deserved the importance that had been ascribed to it. The white mouse, they wrote, has been for years an article of commerce, and

[1] *Sci. Reports, Cancer Research Fund*, London, 1904, No. 1, 13.

[2] *Lancet*, 1905, ii, 1673.

[3] *Sci. Reports, Imperial Cancer Research Fund*, London, 1905, No. 2, Part ii, 22.

[4] *Third. Sci. Report, Imperial Cancer Research Fund*, London, 1908, 273.

[5] *Verhandl. d. Komitees f. Krebsforsch.*, 1903–1904, iii, 38. See *Deut. med. Woch.*, 1904, xxx, 1264.

[6] *Johns Hopkins Hosp. Bull.*, 1905, xvi, 130.

[7] *Abhandl. d. Königl. Preuss. Akad. der Wissensch.*, 1907, 11.

from Berlin, for example, fifty to sixty thousand were exported yearly by dealers who collected them from various small breeding establishments. Some years ago, in fact, many of the so-called "Berlin" mice had been imported from Italy. Thus the investigator could never be sure of the origin of his stock, and it might very well be that what had been described by this or that worker as Copenhagen or Frankfort mice were descended, directly or indirectly, from Berlin mice. On a subsequent page [1] the authors summed up their experience with three tumors that had been transplanted from white to gray mice and conversely. The results were, in the main, so favorable that the authors felt justified in opposing the view that mouse cancer was transferable only between very closely related individuals, and that differences in breed or in diet constituted an obstacle against successful inoculation.

Haaland [2] found difficulty in getting Jensen's mouse tumor to grow in French and Russian mice, and [3] in transferring Ehrlich's mouse sarcoma to Norwegian mice — so much difficulty, in the latter case, that he was barely able to keep the tumor alive. When, a few months later, he was again in a position to inoculate the latter growth into the strain of mice used in Ehrlich's laboratory, he was surprised to see that it flourished with its original vigor. A Berlin stock was susceptible to this tumor, while one from Hamburg was much less so, and in the latter case the tumor had not been able to adapt itself even after five *passages*. Eighteen mice of Danish origin inoculated with this sarcoma did not produce a permanent tumor in one single instance, but a strain of mice that had probably originated in Germany, although it had been several years in Norway, was fairly susceptible. Now it might be imagined, said Haaland, that the susceptibility of certain breeds depended upon general conditions or, in other words, that in such strains cell transplantation would always be more successful. Were this true, one would expect these strains to be susceptible to other tumors as well. But that the case was not so simple Haaland showed by inoculating a mixture of Ehrlich's sarcoma and Jensen's carcinoma. In Berlin mice the sarcomatous element of the mixture

[1] *Abhandl. d. Königl. Preuss. Akad. der Wissensch.*, 1907, 54.
[2] *Ann. de l'Inst. Past.*, 1905, xix, 187.
[3] *Berl. klin. Woch.*, 1907, xliv, 714.

was alone able to proliferate, while in Danish mice, on the contrary, the one tumor that did grow was a pure Jensen carcinoma. Thus it was clear that there were involved, instead of a general condition where certain breeds of mice were susceptible to all growths, special and very specific factors which behaved differently toward different tumors. The Copenhagen mice were at once susceptible to Jensen's carcinoma and resistant to Ehrlich's sarcoma, while conversely, those from Berlin were highly susceptible to the sarcoma and yet resistant toward the carcinoma.

When Haaland left Frankfort he took some of the laboratory strain of mice to Norway, and three months later inoculated six of them with sarcoma fresh from Ehrlich's institute. To this growth, however, they proved themselves highly resistant, although previously mice of the same stock had been susceptible; furthermore, their descendants were also resistant to the tumor. The only possible explanation was that residence in Norway had made the Frankfort mice an unfavorable medium, probably because they had received there a diet different from that to which they were accustomed in Germany.

Jensen [1] also could see no other solution, and suggested that the condition was of practical as well as of theoretical interest because of the chance of influencing metastasis and recurrence in human beings. One could with perfect justice compare tumors obtained by inoculation with those arising by metastasis, and if it should be proved that a radical change of diet was capable of affecting the susceptibility of the mouse to transplantable tumors, the possibility could not be excluded that some influence upon metastasis and recurrence might be achieved in man by the same process.

Gierke [2] did not believe that the conclusion of Hertwig and Poll could be extended to include all mouse tumors, for although there might be some which were insensitive to finer differences among mice, the authors had not proved that this was so of all growths. Gierke transplanted three English tumors into both English and German mice, with the result that the percentage of success was much higher in the former—38, 57, and 75%, as compared with 9, 14, and 21% in the German animals. The rate of growth in English mice was considerably faster, also, than in the

[1] *Zeitschrift f. Krebsforsch.*, 1908–1909, vii, 283.
[2] *Zeitschrift f. Krebsforsch.*, 1908–1909, vii, 331.

others. When, however, there was inoculated into English and German mice a strain of the Jensen tumor that had been growing for years in English breeds, no disparity was found either in the percentage of takes or in the rate of growth. These differences in suitability for inoculation Gierke was inclined to ascribe rather to food and surroundings than to actual racial peculiarities, for the reason that they had been most marked between the stocks of different countries and usually lacking among those native to the same country. Experiments not controlled by simultaneous inoculation into native animals were always open to the criticism that an unfavorable result might have occurred because the tumor happened at the time to be in the negative phase of growth described by Bashford and Murray.

Lewin,[1] on the contrary, expressed his entire agreement with Hertwig and Poll, basing his statement upon experiments conducted in both rats and mice. His rat tumor grew in white, variegated, and black rats from many different localities, and he was able to transplant Jensen's rat sarcoma into animals from Berlin and Düsseldorf while, furthermore, a gray mouse tumor had been inoculated with success into white mice and *vice versa*.

In the experiments of Stahr[2] there were differences in susceptibility between Düsseldorf mice and those from Berlin. The Düsseldorf mice, representing the excess stock of an animal fancier, had been kept for more than half a year in large airy cages and had been fed on hemp seed and milk, but since their arrival at the laboratory they had been given bread soaked in water, food which the laboratory strain of Berlin mice (obtained through an ordinary dealer) had regularly received. Two tumor strains were employed — one from Berlin and one from Nuremberg.

The Berlin tumor grew better at first in the Berlin mice of the laboratory strain than it did in those from Düsseldorf, which had just entered the laboratory. But after the latter strain had been for some months on the same diet and in the same surroundings as the Berlin stock they proved quite as sensitive to the Berlin tumor, or perhaps even more susceptible.

[1] *Berl. klin. Woch.*, 1907, xliv, 1605.
Zeitschrift f. Krebsforsch., 1907–1908, vi, 302.
[2] *Centralbl. f. allg. Path.*, etc., 1909, xx, 628.

The Nuremberg tumor was inoculated into Berlin mice accustomed to the laboratory food and surroundings, as well as into a fresh supply of Düsseldorf mice from the fancier previously mentioned. In each of the two experiments undertaken, the newly introduced mice were less suitable than those of the laboratory strain. The author concluded, although advising caution on account of the small figures at his command, that the Düsseldorf mice reacted differently toward tumor inoculation because they had been kept on a diet different from that of the laboratory strain, and because their health had been impaired by the much less favorable hygienic conditions in the laboratory.

Cuénot and Mercier [1] found, between Parisian mice and those of Nancy, as concerned their susceptibility toward Borrel's tumor "B," a disparity which, exhibiting itself in a smaller yield and a slower growth, rapidly disappeared. The authors, having inoculated many mice of various colors without finding any relation between race and resistance, concluded that immunity must rest upon invisible differences.

Uhlenhuth and Weidanz,[2] in an inquiry regarding the importance of racial differences, determined that English mice were more suitable for the inoculation of an English tumor than Berlin mice, for not only was there a lower percentage of takes in the latter, but spontaneous cure occurred more frequently.

Albrecht and Hecht [3] had difficulty in inoculating spontaneous carcinomata from mice of other countries into Vienna mice. Five foreign tumors were ingrafted unsuccessfully or with difficulty, only two such strains being readily transmissible.

Tyzzer [4] noted a difference in the susceptibility of certain races of mice to the Jensen tumor and, furthermore,[5] that ordinary tame mice were quite insensitive to a growth originating in a Japanese waltzing mouse. Japanese waltzing mice, in their turn, were less suitable than ordinary breeds for the inoculable tumors of the latter.

Gay [6] observed throughout his experiments an important variation

[1] *Compt. rend. de l'Acad. des Sc.*, 1908, cxlvii, 1003.
[2] *Arb. a. d. Kaiserl. Gesundheitsamte*, 1909, xxx, 438.
[3] *Centralbl. f. allg. Path.*, etc., 1909, xx, 1039.
Wien. klin. Woch., 1909, xxii, 1738.
[4] *Jour. Med. Research*, 1907–1908, N.S., xii, 146.
[5] *Jour. Med. Research*, 1909, N.S., xvi, 519.
[6] *Boston Med. and Surg. Jour.*, 1909, clxi, 210.
Proc. Soc. Exp. Biol. and Med., 1908–1909, vi, 74.

in racial sensitiveness to the Flexner rat tumor, for whereas 100% of the most susceptible strain were inoculable, only 50% of the rats from a second dealer could be successfully ingrafted, while among those from still another source the yield was even smaller.

Influence of Health

Mice in poor condition do not offer so favorable a soil for tumor growth as do healthy ones, according to Bashford[1] and Haaland.[2] This may serve to explain the results of those who have described the attainment of resistance by treatment with autolyzed tissues, since the possibility of sepsis in the animals of such experiments has not been eliminated.

Influence of Sex

No observer has yet discovered any difference in susceptibility between males and females — at least this has been the experience of Loeb,[3] Ehrlich,[4] Gierke,[5] Lewin,[6] and many others.

Influence of Pregnancy

It has been asserted and denied that the existence of pregnancy rendered animals less susceptible to implantation; and although Morau[7] and Herzog[8] had written that gestation accelerated the evolution of tumors, and Bashford and Murray[9] that "Pregnancy and full sexual activity in the male (as determined by microscopical examination of the testes) constitute no bar to successful transplantation," Haaland,[10] on the contrary, had found that pregnancy often exerted an inhibitory influence upon the proliferation of tumors, the effect of which was to produce a striking retardation of their growth in pregnant animals as compared with animals not bearing young. Uhlenhuth and Weidanz[11]

[1] *British Med. Jour.*, 1907, ii, 28.
Lancet, 1907, ii, 32.
[2] *Berl. klin. Woch.*, 1907, xliv, 718.
[3] *Jour. Med. Research*, 1901, N.S., i, 36.
[4] *Arb. a. d. Königl. Inst. f. Exp. Therap.*, 1906, Heft i, 81.
[5] *Beitr. zur path. Anat.*, etc., (Ziegler), 1908, xliii, 343.
Third Sci. Report, Imperial Cancer Research Fund, London, 1908, 132.
[6] *Zeitschrift f. Krebsforsch.*, 1907–1908, vi, 304.
[7] *Arch. de Méd. exp. et d'Anat. path.*, 1894, vi, 693.
[8] *Jour. Med. Research*, 1902, N.S., iii, 76.
[9] *Sci. Reports, Cancer Research Fund*, London, 1904, No. 1, 14.
[10] *Berl. klin. Woch.*, 1907, xliv, 718.
[11] *Arb. a. d. Kaiserl. Gesundheitsamte*, 1909, xxx, 440.

had also observed this retardation and, furthermore, that spontaneous regression occurred oftener in pregnant mice.

Bridré [1] chose males for inoculation whenever it was possible because of the low percentage of positive inoculations occurring in pregnant females, while Ehrlich [2] had noticed repeatedly that inoculation into animals bearing young was followed with extraordinary frequency by negative results, or was at least attended by the development of tumors in which growth was greatly retarded.

Pregnancy, according to Albrecht and Hecht,[3] whether already present at the time of inoculation, or commencing afterward, influenced the establishment of a tumor or its subsequent growth just as little as the presence of a tumor influenced conception or pregnancy.

Cuénot and Mercier [4] reported that Borrel's tumor "B" (which rarely underwent spontaneous absorption), if inoculated before fecundation, would develop throughout gestation and recede during lactation. But if one mouse only were born and the activity of the mammary gland were in consequence at a minimum, the tumor did not regress, nor did absorption take place, even in the presence of several young, if a tumor were so situated that its vascularization was independent of that of the mammary gland.

Fichera [5] explained the inconsistencies that had been observed in the relations between pregnancy and tumor growth by assuming that when many embryos were present the specific food-stuffs were almost wholly demanded by them, while if there were but few the nutrient material was available for the tumor cells as well.

ACQUIRED RESISTANCE, ACTIVE AND PASSIVE

Active Resistance evolved by Tumor

It was reported first by Clowes,[6] and later by Gaylord, Clowes, and Baeslack,[7] that the Jensen carcinoma sometimes underwent spontane-

[1] *Ann. de l'Inst. Past.*, 1907, xxi, 763.
[2] *Verhandl. d. deutschen path. Gesellsch.*, 1908, 12te Tagung, 29.
[3] *Wien. klin. Woch.*, 1909, xxii, 1738.
[4] *Compt. rend. de l'Acad. des Sc.*, 1909, cxlix, 1012.
[5] Cited by Apolant, *Jour. Exp. Med.*, 1911, xiv, 320.
[6] *Johns Hopkins Hosp. Bull.*, 1905, xvi, 130.
[7] *Med. News*, 1905, lxxxvi, 91.

ous regression, and this phenomenon was later described in detail by Gaylord and Clowes.[1] Clowes and Baeslack [2] then found that animals in which a cure had taken place were refractory to re-inoculation, since among thirty mice in which the Jensen carcinoma had been absorbed not one developed a tumor upon re-inoculation with the same growth, although ten of the group were subjected to a third implantation.

Flexner and Jobling [3] noted that among seventy rats which had been able to rid themselves of the Flexner-Jobling adeno-carcinoma, only 17 % developed growths upon re-inoculation with that tumor, and Lewin [4] reported that he had seen such resistance frequently after the absorption of his own rat carcinoma.

As a corollary to these observations there arose the question whether a mouse once ingrafted without result could be successfully inoculated at a subsequent trial. Although Jensen [5] had observed that in about half his mice the graft failed to grow and that such animals were then resistant, Bashford, Murray, and Cramer [6] were of the opinion that inoculation of mice in which an implantation had been fruitless succeeded in nearly the same percentage as in the first instance. A year later Bashford [7] reiterated this statement, but qualified it with the remark that if the process were repeated, negative mice being discarded each time, animals could be ultimately obtained with a more pronounced power of resistance, affording, for example, 12 % of tumors as compared with 68 % among controls of the same age. A similar result had been substantiated for mice unsuccessfully injected with two spontaneous sarcomata and various spontaneous carcinomata, when the animals were afterward inoculated with Jensen's tumor. After further investigation he was able to confirm even more fully the results of others, and in a paper written in conjunction with Murray and Cramer [8] said that differences in the size

[1] *Surgery, Gynecology, and Obstetrics*, 1906, ii, 633.
[2] *Med. News*, 1905, lxxxvii, 969.
[3] *Proc. Soc. Exp. Biol. and Med.*, 1907–1908, v, 17.
[4] *Zeitschrift f. Krebsforsch.*, 1907–1908, vi, 306.
[5] *Centralbl. f. Bakt.*, etc., erste Abt., Orig., 1903, xxxiv, 126.
[6] *Sci. Reports, Imperial Cancer Research Fund*, London, 1905, No. 2, Part ii, 51.
[7] *British Med. Jour.*, 1906, ii, 209.
[8] *Proc. Roy. Soc.*, Series B, 1907, lxxix, 179.
Third Sci. Report, Imperial Cancer Research Fund, London, 1908, 331.

of dose would probably explain the apparent contradictions between his own earlier work and that of other observers. In later experiments the mass of tumor absorbed in the course of three or four negative inoculations with 0.05 gram had been so great that the animals could not be regarded as comparable with those in earlier experiments, which had received relatively insignificant doses (0.01 to 0.02 gram).

In this connection the experiments of Bridré [1] are of importance. Unable at first to discover evidences of resistance in unsuccessfully inoculated mice, he conceived the idea that his failure might have been due to the employment of an insufficient primary dose. Accordingly, he inoculated tumor emulsion in amounts five or six times as large as the fragment that had been used in his earlier experiments and obtained an immunity so perfect that no tumors developed in the treated animals, although the controls yielded 55%.

Michaelis, Fleischmann, and Pincussohn [2] had positive results in about 60% of normal mice, while in those which had proved refractory to a first attempt nodules developed after re-implantation in only 12%. It happened occasionally that a mouse inoculated three times in vain could be successfully ingrafted at a fourth attempt.

Borrel [3] noted that while 55% of mice contracted a tumor after a first implantation, only 12 to 15% of the negatives developed them after the second, and none after the third.

Ehrlich [4] found that in mice unsuccessfully inoculated with a hemorrhagic growth giving but a small percentage of daughter tumors, subsequent inoculation, even with a more vigorous strain, produced but few tumors. Furthermore, the refractory condition was still more distinct in those mice which had been able to resist a neoplasm that would grow in a large percentage of the mice inoculated.

Flexner and Jobling [5] reported two hundred and one rats unsuccessfully inoculated with their adeno-carcinoma while its virulence was below the maximum, 49% of which developed tumors upon re-implantation with the virulent tumor.

[1] *Ann. de l'Inst. Past.*, 1907, xxi, 764. [2] *Deut. med. Woch.*, 1907, xxxiii, 827.
[3] *Bull. de l'Inst. Past.*, 1907, v, 603.
[4] *Arb. a. d. Königl. Inst. f. Exp. Therap.*, 1906, Heft i, 90.
Zeitschrift f. aerztliche Fortbildung, 1906, iii, 211.
Zeitschrift f. Krebsforsch., 1907, v, 73.
[5] *Proc. Soc. Exp. Biol. and Med.*, 1907–1908. v, 17.

Clowes and Baeslack [1] stated that in a series where the mice were intentionally treated with a strain so weak as to afford but about 50 % of abortive tumors, the animals in which the attempt had been fruitless all developed fair-sized growths upon re-inoculation with a more vigorous strain. And later, Clowes [2] himself recorded the outcome following re-inoculation of mice refractory to the Jensen tumor. From 30 to 35 % of growths were obtained after the primary inoculation; the first re-inoculation gave 10 %, and the second *nil*. The re-inoculation of the survivors of less vigorous tumor strains with one of more active growth did not indicate, especially when large doses were employed, that there had been conferred as high a degree of resistance as Ehrlich had described, although the difference might be due to diversities in the methods of transplantation employed.

Hertwig and Poll [3] thought that mice were not made refractory by an unsuccessful inoculation and expressed the belief that in such cases there was involved merely the artificial selection of animals naturally immune, as had already been suggested by Jensen [4] and by Michaelis.[5]

Pan-immunity: — Not a little attention has been devoted to attempting a definition of the limits within which immunity produced by the absorption of tumors is specific.

Ehrlich [6] believed that, in general, a preliminary unsuccessful inoculation with carcinoma protected not alone against all strains of carcinoma but equally against all sarcomata, and conversely, that preliminary inoculation with sarcoma would protect not only against all sarcomata but against all carcinomata as well. So far as chondroma was concerned he was of the impression, derived from a series of experiments at that time, however, still unfinished, that in some animals which were resistant to sarcoma or carcinoma the chondroma grew more slowly than usual or, perhaps, not at all. Still, any considerable resistance to the chondroma was achieved apparently only after the sarcoma-carcinoma resistance had been raised to a

[1] *Med. News*, 1905, lxxxvii, 969. [2] *British Med. Jour.*, 1906, ii, 1551.
[3] *Abhandl. d. Königl. Preuss. Akad. d. Wissensch.*, 1907, 26.
[4] *Centralbl. f. Bakt.*, etc., erste Abt., Orig., 1903, xxxiv, 126.
[5] *Zeitschrift f. Krebsforsch.*, 1907, v, 191.
[6] *Zeitschrift f. aerztliche Fortbildung*, 1906, iii, 211.
Arb. a. d. Königl. Inst. f. Exp. Therap., 1906, Heft i, 92.
Zeitschrift f. Krebsforsch., 1907, v, 74.

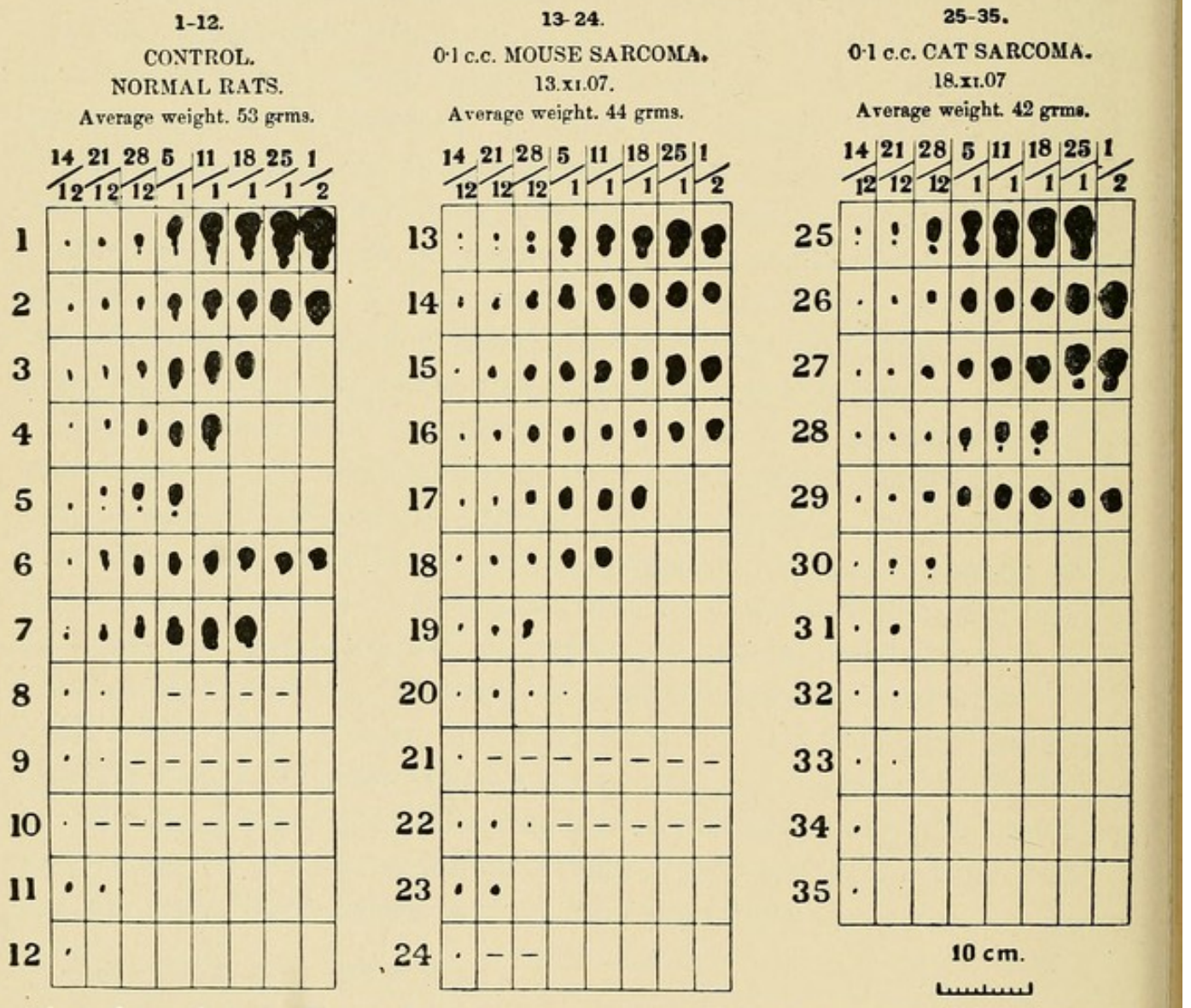
maximum. Hence, as this condition of artificially induced resistance was, to a certain extent at least, not a narrow one, Ehrlich saw no objection to postulating a state of *pluri-immunity* or even *pan-immunity*.

It had been previously recorded by Bashford [1] that mice which had rid themselves of a certain transplantable mammary carcinoma were more resistant to re-inoculation with this tumor than with Jensen's carcinoma. There was, therefore,[2] " . . . a degree of protection which is common, and a certain degree which is specific." But the common protection was, as Bashford, Murray, and Cramer [3] indicated, conferred by the tumor not as tumor, but as mouse tissue, and Bashford, Murray, and Haaland [4] could discover no evidence that carcinoma evolved more resistance to sarcoma than did normal tissue, although sarcoma protected to a high degree against carcinoma. They could not, therefore, subscribe to Ehrlich's belief that protection between carcinoma and sarcoma was mutual and of equal degree.

Haaland,[5] in earlier experiments, had seen that an unsuccessful inoculation with Jensen's tumor did not protect against the subsequent implantation of sarcoma, but Lewin,[6] on the contrary, was convinced that there could exist in the rat conditions of resistance common to sarcoma and carcinoma. Rats unsuccessfully inoculated with sarcoma, or in which a carcinoma had undergone spontaneous absorption, had been, in his experience, refractory to both carcinoma and sarcoma.

Michaelis, Fleischmann, and Pincussohn [7] found that mice unsuccessfully inoculated with Jensen's tumor were not resistant to a Berlin growth, and Gierke,[8] that mice negative to the inoculation of a tumor were protected in an extraordinarily high degree against the same, and to a somewhat lower degree against different tumors.

[1] *British Med. Jour.*, 1906, ii, 209.
Lancet, 1906, ii, 314.
[2] *Science Progress*, 1907, ii, 20.
[3] *Proc. Roy. Soc.*, Series B, 1907, lxxix, 179.
Third Sci. Report, Imperial Cancer Research Fund, London, 1908, 332.
[4] *Jour. Path. and Bact.*, 1908, xii, 436. [5] *Berl. klin. Woch.*, 1907, xliv, 716.
[6] *Berl. klin. Woch.*, 1907, xliv, 1606.
Zeitschrift f. Krebsforsch., 1907–1908, vi, 309.
[7] *Deut. med. Woch.*, 1907, xxxiii, 827.
[8] *Beitr. zur path. Anat.*, etc., (Ziegler), 1908, xliii, 346.
Third Sci. Report, Imperial Cancer Research Fund, London, 1908, 135.



Comparison of growth of the Flexner-Jobling adeno-carcinoma of the rat, in normal control rats and in those treated with mouse and cat sarcoma 19 and 14 days respectively before tumor implantation. All rats inoculated with 0.02 gram of the Flexner-Jobling tumor 2 – xii – 07. There is no protection induced by preliminary treatment with tumors of a strange species.

Albrecht and Hecht [1] noticed that mice in which transplantation had been unsuccessful were, in general, more refractory than normal animals, and in the case of a very virulent carcinoma they had seen this resistance in as many as 50% of the animals, although the phenomenon was in no way constant. Such mice were protected against another strain also, but in a lower degree.

Active Resistance probably evolved only by Intact Tumor Cells of the Same Species: — Having demonstrated that immunity ensued upon unsuccessful inoculation, those engaged in the study of experimental cancer set themselves the task of producing resistance by treatment with something other than living cancer cells of the same species, for when the malignant cell was employed a certain number of animals developed tumors, becoming thereby unavailable for further use.

Michaelis [2] tried repeated inoculations of cancer cells killed by chloroform or heat, but reported that his attempts had been fruitless. Searching further, he inoculated white mice with a gray mouse carcinoma and with a carcinoma from the rat, knowing that by reason of race specificity neither of these tumors would be able to grow in the white mouse; but not the slightest indication of resistance revealed itself in any of the animals. Heated tumor was injected also by Lewin,[3] who could not discover any evidences of resistance after its inoculation.

Although immunity was not evident to Bashford and his colleagues [4] in mice inoculated with growths from other species, Lewin [5] found that he could produce almost complete resistance in mice by two inoculations at short intervals — three to seven days — with rat carcinoma. By preliminary treatment with mouse carcinoma he could protect rats against both carcinoma and sarcoma.

Clowes [6] said that more than two years before the date of writing,

[1] *Centralbl. f. allg. Path.*, etc., 1909, xx, 1039.
Wien. klin. Woch., 1909, xxii, 1740.
[2] *Med. Klin.*, 1905, i, 205.
Zeitschrift f. Krebsforsch., 1907, v, 192.
Berl. klin. Woch., 1907, xliv, 486.
Deut. med. Woch., 1906, xxxii, 1728. See also Michaelis, Fleischmann, and Pincussohn, *Deut. med. Woch.*, 1907, xxxiii, 827.
[3] *Zeitschrift. f. Krebsforsch.*, 1907–1908, vi, 308.
[4] *British Med. Jour.*, 1907, ii, 28.
Third Sci. Report, Imperial Cancer Research Fund, London, 1908, 365, 368.
[5] *Berl. klin. Woch.*, 1907, xliv, 1606. [6] *British Med. Jour.*, 1906, ii, 1550.

experiments had shown him the futility of trying to produce immunity with inanimate materials, and that he and his associates had from the first emphasized the rôle played by the living cell. On this occasion he repeated his belief that actual cell growth must take place in order for immunity to be conferred, and placed in evidence unsuccessful attempts to produce resistance with tumor cells that had been destroyed by heat or treated with certain chemicals, as mercuric chloride, potassium cyanide, iodine, etc. Furthermore, the refractory state could not be elicited by inoculating the nucleoproteids or nucleohistons extracted from tumors.

That intact cells were requisite for the production of resistance was shown by Bridré,[1] who injected the clear fluid obtained by filtering and centrifuging a tumor mush. Growths developed in 40 % of the mice thus treated and in 50 % of the controls, while in mice that had been treated with tumor fragments heated to points exceeding 50° C. there was likewise a barely appreciable immunity to implantation. The general result of his investigations was that the highest resistance followed the inoculation of uninjured cells.

In perfect agreement with this conclusion was the observation of Haaland,[2] that the refractory state did not supervene in mice inoculated with an emulsion of tumor cells devitalized by freezing and grinding.

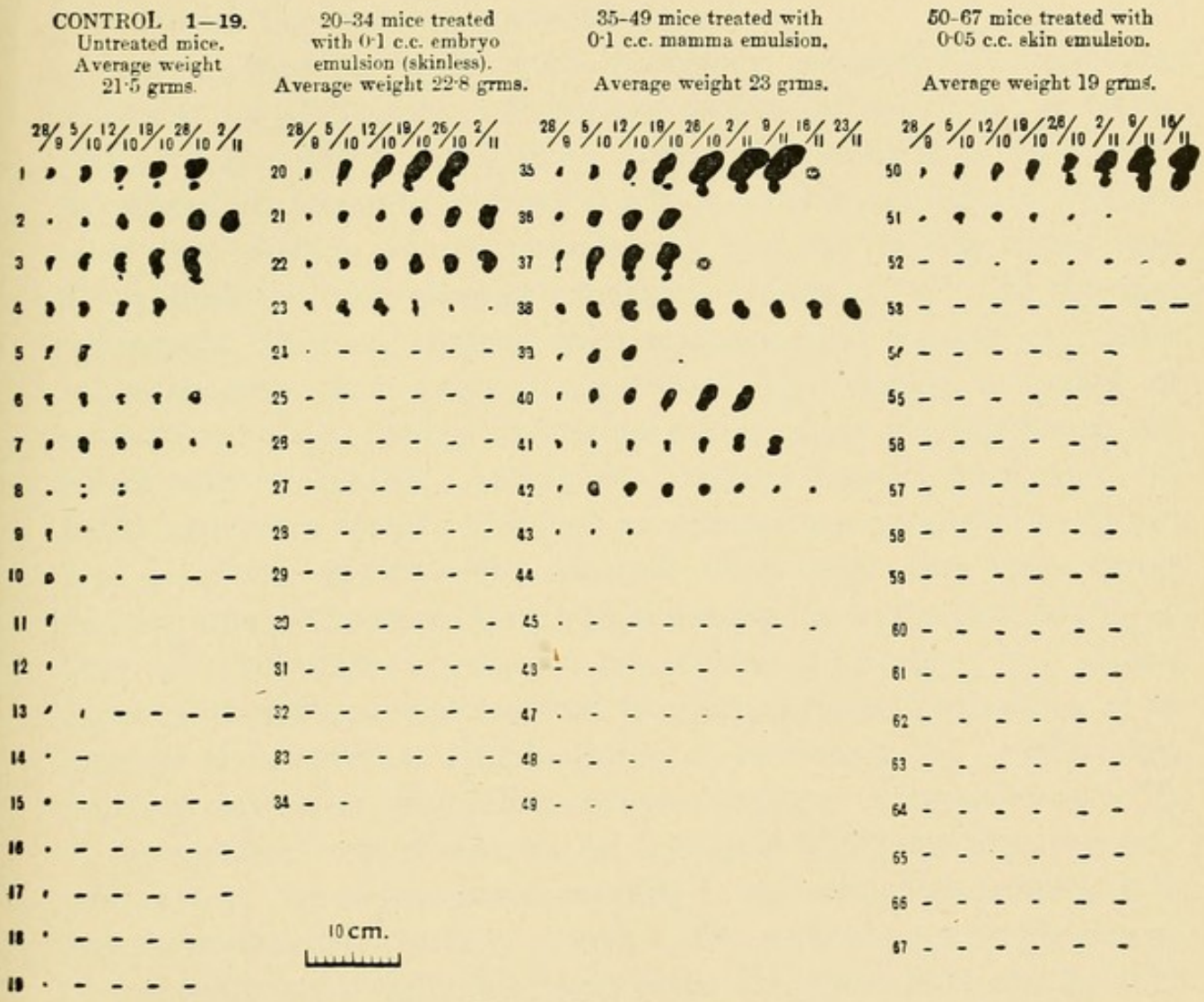
Active Resistance Evolved by Normal Tissue

A useful and extremely interesting method of evoking the refractory condition was described from Bashford's laboratory, where it was found that immunity would follow the inoculation of other material than tumor. Thus he[3] and his co-workers discovered that a preliminary injection of from 0.3 to 0.5 cubic centimeter of normal defibrinated mouse blood induced a definite resistance even in young animals. A repetition of the treatment did not materially increase the refractory condition. Analysis of the phenomenon proved the corpuscles to be the active agent, serum alone being impotent, and it was further found that the effect was best brought out when the

[1] *Ann. de l'Inst. Past.*, 1907, xxi, 768.

[2] *Lancet*, 1910, i, 787.

[3] *British Med. Jour.*, 1906, ii, 209.
Lancet, 1906, ii, 315.
Proc. Roy. Soc., Series B, 1907, lxxix, 180.
Third Sci. Report, Imperial Cancer Research Fund, London, 1908, 333, 369.



* Macroscopic metastases in lungs.

Protection against, or hypersensibility toward, inoculation of a transplantable squamous cell carcinoma induced by treatment with normal mouse tissue 15–16 days before tumor implantation. All mice inoculated in right axilla with 0.01–0.02 of tumor. First charting 10 days later.

minimal tumor-forming dose was employed, a dose lying between one and two centigrams. When larger amounts (five centigrams) were introduced, the percentage of growths in the treated animals was greater, although still not so large as in the controls, while in a series where massive doses were inoculated, nodules developed eventually, although their appearance was somewhat delayed. Here, the authors thought, the larger dose of tumor had so exhausted the protection that growth finally became possible.

Schöne,[1] working in Ehrlich's laboratory, succeeded in producing resistance by preliminary injections of mouse embryo, liver, or testis. Rather large embryos, emulsified without the addition of fluid, were inoculated in amounts of from 0.3 to 0.7 cubic centimeter. White mice were used in all the experiments, and the testing tumor was a vigorously growing alveolar carcinoma known as "5." In the first experiment, thirteen animals were injected eight times — six times subcutaneously and twice intraperitoneally — during a period of seventy-one days, the last treatment taking place one day before the tumor was inoculated. Among the thirteen treated mice, 46.15 % failed to develop tumors, although inoculation was successful in all but 7.14 % of the twenty-eight controls. Not only were there fewer nodules among the treated mice than among the untreated, but those that did occur showed a distinct retardation of growth. In a second experiment eighteen mice received two intraperitoneal injections at an interval of two weeks. Implantation of carcinoma "5," undertaken fourteen days later, was followed by a negative outcome in 66.67 % of the eighteen treated mice but in only 16.67 % among eighteen controls, and of the six tumors developing in the refractory animals only two showed no delay in their growth. The presence of a higher degree of resistance after eight injections than after two could not be substantiated. Liver and testis, although producing immunity, were not nearly so efficacious as embryo. Whether the resistance evoked by normal tissues was identical with that following the inoculation of tumors, Schöne left an open question.

In a later article he[2] said that one single subcutaneous inoculation

[1] *Münch. med. Woch.*, 1906, liii, 2517.

[2] *Verhandl. d. Gesellsch. deutscher Naturforsch. u. Aerzte*, 1907, 79ten Versammlung, erste Teil, 304.

of normal tissue would call forth an efficient immunity and that intraperitoneal inoculations were also active in producing this condition, although here larger doses were preferable (0.5 to 1.0 cubic centimeter). The immunity consequent upon the inoculation of spontaneous tumors was, in general, more marked than that following preliminary treatment with embryo emulsion, although the two types seemed to have a number of characteristics in common.

Investigating the preliminary observations of the authors just cited, Michaelis, Fleischmann, and Pincussohn[1] succeeded in demonstrating a certain amount of resistance after three inoculations of an emulsion of normal mouse liver. Of twenty animals thus treated, only 30% developed tumors, while among eighteen controls there were growths in 72%. As in Schöne's experiments, the nodules that did appear in the treated animals were of retarded growth and attained only a moderate size, afterward remaining stationary. Proliferation in the control mice, on the other hand, was progressive.

Borrel[2] and Bridré[3] inoculated mice with blood and with emulsions of spleen, liver, or testis, in amounts of one cubic centimeter. Two inoculations of blood, of about 0.25 cubic centimeter each, gave but a feeble resistance against the Pasteur Institute tumor "B," 40% of growths developing in treated animals compared with 55% in the controls. Three inoculations of liver emulsion, at intervals of twelve days, produced a higher degree of immunity, while perfect resistance was obtained by the inoculation of spleen. On the other hand, testis was quite powerless to elicit the refractory state, 50% of tumors developing in the treated mice and 55% in the controls.

Lewin[4] extended these studies to rats, and produced resistance against his rat carcinoma and against Jensen's rat sarcoma by a single inoculation with 0.8 to 1.0 cubic centimeter of normal rat blood.

Moreschi,[5] after the inoculation of an emulsion of actively lactating mouse mamma in amounts of 0.15 to 0.20 cubic centimeter, found conditions ranging from hypersusceptibility to resistance. The

[1] *Deut. med. Woch.*, 1907, xxxiii, 827.
[2] *Bull. de l'Inst. Past.*, 1907, v, 605.
[3] *Ann. de l'Inst. Past.*, 1907, xxi, 769.
[4] *Berl. klin. Woch.*, 1907, xliv, 1606.
Zeitschrift f. Krebsforsch., 1907–1908, vi, 310.
[5] *Zeitschrift f. Immunitätsforsch.*, etc., Orig., 1909, ii, 675.

immunity varied toward different tumors, a much more refractory state being present for Ehrlich's carcinoma "11" than for carcinoma "5." Toward a sarcoma the resistance was often evidenced merely by a retardation of growth, although at other times 80 to 87.5% of tumors developed in the resistant animals as compared with 100% in the controls. The large yield was doubtless due to the extraordinary virulence of this growth.

The production of resistance by the inoculation of normal blood and of embryo emulsion was further substantiated by Uhlenhuth and Weidanz,[1] but they were unable to cause its evolution with mouse lens.

Higuchi [2] recorded the achievement of immunity through the introduction into mice of mouse placenta, which was capable of provoking the condition quite independently of its contained blood. It was effectual not only against several carcinomata but against a sarcoma as well.

In experiments with tumors other than Jensen's, Bashford and his associates [3] found that injections of blood protected to a lower degree against their tumor "50" and hardly at all against tumors "32" and "27." In these facts they saw still further evidence of the truth of their oft-repeated statement that the conditions of growth were specific for different tumors, and that factors unfavorable to one particular neoplasm were not necessarily active against all other growths.

Schöne,[5] indeed, had found the limits of specificity so narrow that the embryos of gray mice were not as suitable as those of white for the production in white mice of immunity toward a white mouse tumor, although with extensive treatment a definite result could be achieved. Rat embryos, as a rule, did not educe resistance, although in isolated instances tumor growth seemed to be held somewhat in abeyance.

A lack of specificity in the immune reaction was suggested also by the work of Rous,[6] who showed that resistance toward the implantation

[1] *Arb. a. d. Kaiserl. Gesundheitsamte*, 1909, xxx, 443.

[2] *Sei-I-Kwai Medical Journal*, Tokio, 1911, xxx, 91.
Fifth Sci. Report, Imperial Cancer Research Fund, London, 1912, 83.

[3] *Third Sci. Report, Imperial Cancer Research Fund*, London, 1908, 375.

[5] *Verhandl. d. Gesellsch. deutscher Naturforsch. u. Aerzte*, 1907, 79ten Versammlung, erste Teil, 304.

[6] *Jour. Exp. Med.*, 1910, xii, 344.

of fetal cells could be produced by the inoculation of embryonic tissue, and that it was manifest in an absence of the stroma reaction necessary for a temporary survival of the ingrafted material.

Active Resistance probably evolved only by Intact Normal Cells of the Same Species: — Just as resistance to subsequent inoculation fails to follow preliminary treatment with tumor cells robbed of their vitality by heat or other injurious agents, or with intact tumor cells of different species, so in the case of normal tissue is the injection of uninjured living cells of the same species an essential for the production of the resistant state.

Bashford and his associates [1] were unable to prevent the growth of transplanted cancer in mice by the inoculation of the normal tissues of rats, rabbits, guinea-pigs, or more distantly related species, and Higuchi [2] discovered little if any inhibitory effect after the introduction into mice of placenta, blood, mammary gland, embryo skin, or spleen of rats and guinea-pigs.

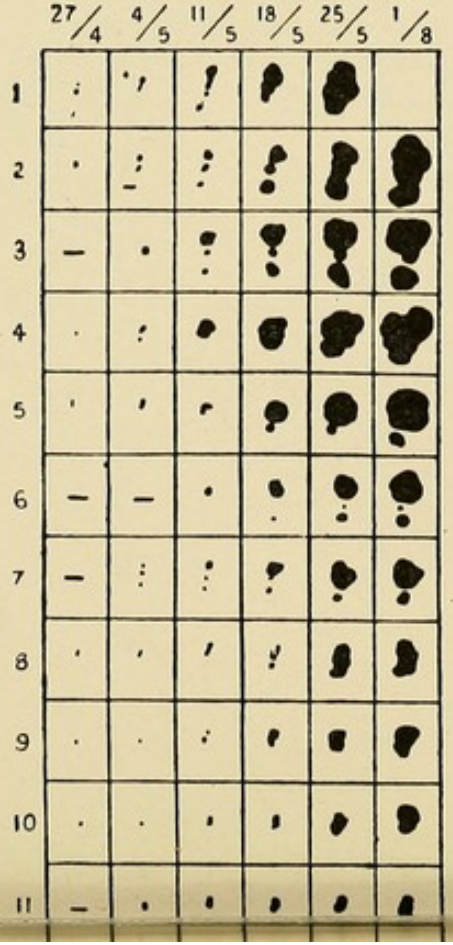
In the experiments of Moreschi,[3] although rat mamma produced not a trace of immunity in mice against one mouse carcinoma, it was efficacious against a second one, and he thought that the presence of the refractory condition depended upon the length of time elapsing between inoculations, no less than upon certain other factors. Proceeding to test the power of rat sarcoma to effect resistance in mice, he discovered evidence that such a condition had been induced. Lactating guinea-pig mamma was also active, but not in so high a degree as mouse mamma. He concluded, therefore, that Bashford and his associates were not justified in denying the possibility of producing resistance with the tissues of strange species.

Similar results were cited by Apolant,[4] who wrote that by treatment with alien blood he had occasionally obtained immunity against mouse carcinoma, and that he was, therefore, unable to agree with Bashford's contention that only tissues of the same species were potent.

[1] *British Med. Jour.*, 1907, ii, 28.
Proc. Roy. Soc., Series B, 1907, lxxix, 182.
Third Sci. Report, Imperial Cancer Research Fund, London, 1908, 334, 365, 376.
[2] *Sei-I-Kwai Medical Journal*, Tokio, 1911, xxx, 91.
Fifth Sci. Report, Imperial Cancer Research Fund, London, 1912, 85.
[3] *Zeitschrift f. Immunitätsforsch.*, etc., Orig., 1909, ii, 681.
[4] *Zeitschrift f. allg. Physiol.*, 1909, ix, Sammelreferat, 91.

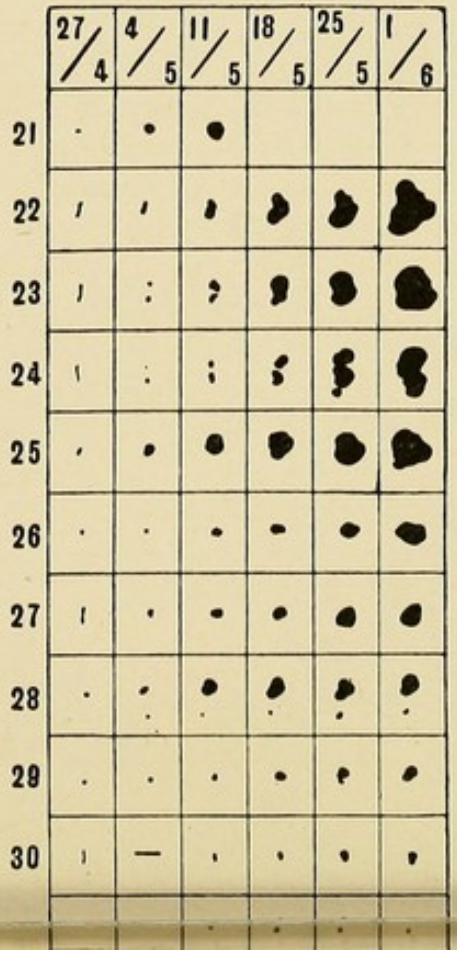
1–20.
CONTROL
NORMAL MICE.

Average weight 16·5 grms.



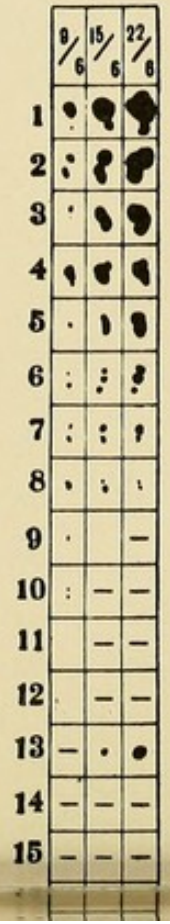
21–39.
0.3 c.c. defibrinated mouse blood subcutaneously.

Average weight 16·75 grms.



1–30.
0.5 c.c. defibrinated mouse blood subcutaneously.

Average weight.
14·2 grms.

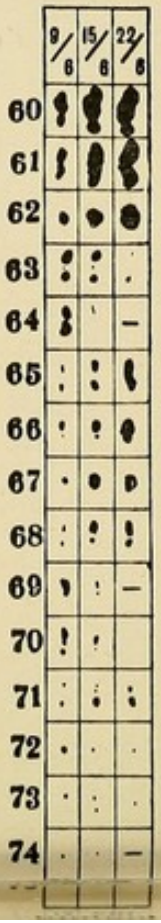


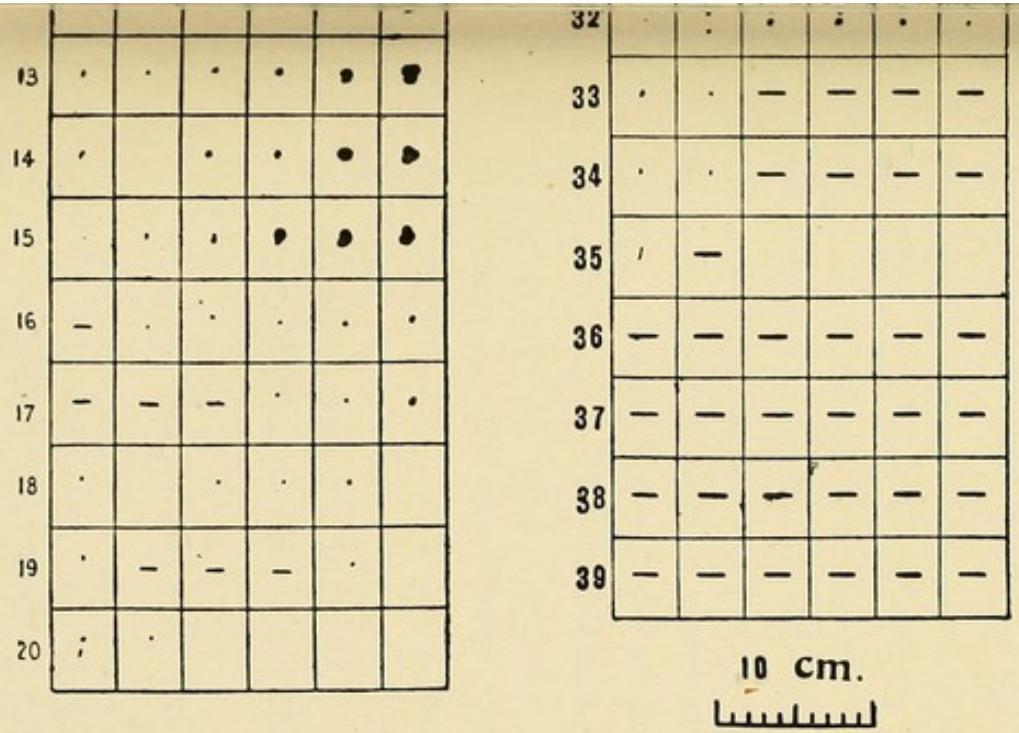
31–59.
Control
Normal
mice.

Average weight.
13·5 grms.

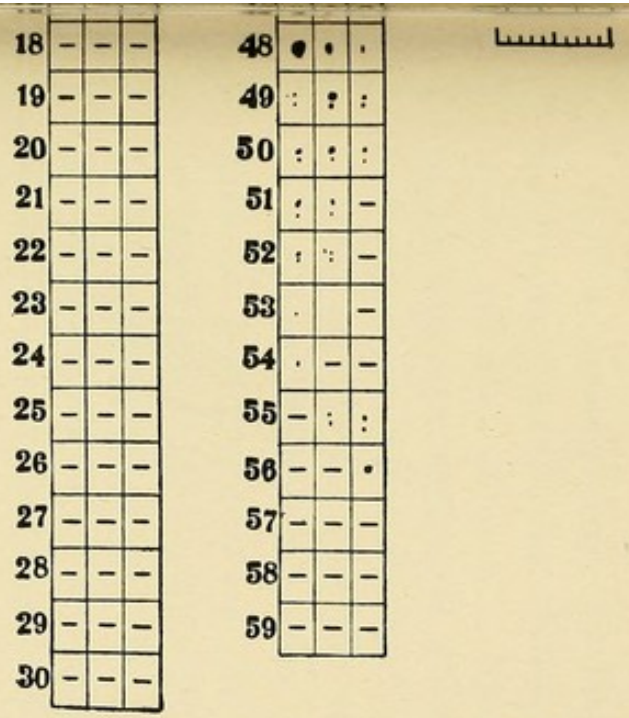
60–75.
0.5 c.c. defibrinated rabbit blood subcutaneously.

Average weight.
15·3 grms.





A slight degree of protection against inoculation of a transplantable mammary alveolar carcinoma of the mouse, by treatment with defibrinated mouse blood 16 days before tumor inoculation. All mice inoculated in right axilla with 0.025 c.c. of tumor emulsion. First charting 11 days later.



Protection against inoculation with Jensen's mouse carcinoma by treatment with defibrinated mouse blood 11 days before tumor inoculation, but not with defibrinated rabbit blood the same length of time before tumor implantation. All mice inoculated in right axilla with 0.025 c.c. of tumor emulsion. First charting 10 days later.

the inoculation of fluids expressed from the organs of normal or tumor-bearing mice; nor was the injection of alien albumin followed by the occurrence of immunity.

Since the majority of the vital functions of the cell were elaborated by its endocellular enzymes, which were best liberated by autolysis, Levin[1] tried to produce the refractory state in rats by previous treatment with autolyzed rat liver. As a certain amount of immunity became evident, he assumed that there may have been present some endocellular enzyme-like substances which autolysis had been powerless to injure.

No Active Resistance with Autologous Tissue: — It was at first thought by Woglom[2] that resistance toward transplanted cancer could be provoked in a mouse by the inoculation of its own spleen, but more extended observation convinced him[3] that neither the spleen nor any other of the various organs or combinations of organs with which he worked could, when inoculated into the mice from which they had been removed, render the animals refractory to the subsequent transplantation of a tumor.

In the meantime the subject had been approached by Apolant and Marks,[4] who concluded that the spleen did not contain enough tissue to yield an amount sufficient for the induction of the resistant state; but whether or not an animal could be made refractory by the inoculation of its own tissues, provided a large enough dose were available, they preferred to leave an open question.

Lambert[5] was unable to detect any evidence of immunity in mice injected with their own blood corpuscles.

Can Tumor-bearing Animals be made Actively Resistant?

It was suggested by Schöne[6] that it might be impossible to provoke a condition of resistance to transplantable tumors in a mouse already bearing a propagable growth. Mice in which a transplantable

[1] *Proc. Soc. Exp. Biol. and Med.*, 1909–1910, vii, 64.

[2] *Jour. Exp. Med.*, 1910, xii, 29.

[3] *Zeitschrift f. Immunitätsforsch.*, etc., Orig., 1911, xi, 683.

[4] *Zeitschrift f. Immunitätsforsch.*, etc., Orig., 1911, x, 159. See also Apolant, *Zeitschrift f. Immunitätsforsch.*, etc., Orig., 1912, xii, 472.

[5] *Proc. Soc. Exp. Biol. and Med.*, 1911–1912, ix, 18.

[6] *Verhandl. d. deutschen Gesellsch. f. Chir.*, 1907, xxxvi, 214. *Deut. med. Woch.*, 1907, xxxiii, 866.

tumor was growing rapidly were inoculated with spontaneous hemorrhagic tumors and at the end of a certain period, within which normal animals would have developed resistance, the growing tumors were removed. After the resulting wounds had healed, secondary inoculations were undertaken which, in the great majority of the animals, were successful. Nevertheless, the procedure had been so complicated, and the number of mice so small, that Schöne did not wish to draw any definite conclusion, preferring to await the outcome of further experiments.

Bashford, Murray, and Haaland[1] encountered similar results, but because of the cardinal importance of dosage and time interval and the complex nature of the experiments, they found it difficult to interpret the outcome, referring to the fact that investigation was still in progress. They had occasionally met with the anomalous finding that treatment which in normal animals would induce a powerful resistance to inoculation, might provoke a relative hypersensibility in those bearing transplanted growths.

This question has been definitely settled by Russell,[2] at least in so far as it concerns the more slowly growing tumors. Among sixty-five mice bearing propagable growths, 63% were found to be receptive to a second inoculation. This figure, however, was reduced in seventy mice to 27%, when the secondary transplantation was preceded some fourteen to sixteen days by an immunizing injection of tumor or mouse embryo emulsion.

Whether or not an animal can be made refractory to the re-inoculation of a tumor which has arisen in it spontaneously, is of supreme importance, since it concerns the search for a means of preventing the growth of metastatic deposits. Haaland[3] found that methods which would render normal mice resistant would prevent neither local recurrence after operation nor the development of new tumors, nor could he influence by the same means either dissemination, with the consequent appearance of metastatic nodules in the lungs, or successful grafting of the mouse with its own tumor cells (artificial metas-

[1] *Third Sci. Report, Imperial Cancer Research Fund*, London, 1908, 392.
[2] *Fifth Sci. Report, Imperial Cancer Research Fund, London*, 1912, 29.
[3] *Jour. Path. and Bact.*, 1910, xiv, 407.
Proc. Roy. Soc., Series B, 1910–1911, lxxxiii, 540.
Fourth Sci. Report, Imperial Cancer Research Fund, London, 1911, 79.

tasis). There seemed, therefore, to be an insensibility of the cell in its own animal to reactions which were effective against strange cells in the same surroundings. The difficulty was, as Haaland indicated, one which was encountered in all other forms of cellular immunity — that of immunizing against cells belonging to the same organism. He investigated, in this connection, the nearest approach to autoplastic transplantation occurring under natural conditions *i.e.* the embedding of the ovum on the uterine mucosa, and found this process as little influenced by methods effective against the transplantation of cancer as was the inoculation of a tumor into the animal to which it was native.

Still another phase of the question has been approached by the same author. If there was difficulty in producing resistance in an animal bearing a transplantable tumor, what conditions obtained when one tried to produce resistance against a transplantable tumor in a mouse already the subject of a spontaneous growth? Haaland [1] found that in animals suffering from spontaneous tumors the absorption of carcinoma "206" went on exactly as it did in normal mice, whence it was plain that in mice spontaneously affected with cancer there existed no condition capable of preventing or of nullifying resistance toward a transplantable growth.

Premetastatic Stage of Active Resistance

In animals bearing transplanted tumors, resistance to re-inoculation has been said by some observers to be present for a certain period after the primary implantation. This period corresponded to the time which elapsed before the appearance of metastases, and was described first by Sticker [2] in the dog as the *premetastatic stage.* After about thirty days the resistance was said to disappear, so that the taking of a second graft or the establishment of metastases became possible.

The presence of this stage in mice Bridré [3] was unable to confirm, for in his experiments animals bearing tumors of different ages were all equally sensitive to re-inoculation.

[1] *Fourth Sci. Report, Imperial Cancer Research Fund*, London, 1911, 83.

[2] *Münch. med. Woch.*, 1906, liii, 1904.
Deut. med. Woch., 1907, xxxiii, 867.
Zeitschrift f. Krebsforsch., 1908–1909, vii, 64.

[3] *Ann. de l'Inst. Past.*, 1907, xxi, 773.

Flexner and Jobling [1] were of the same opinion, and wrote that inoculation of their rat tumor succeeded as well in rats where no visible secondary growths had occurred as in those where metastasis had already taken place.

Gay,[2] in observations upon the Flexner-Jobling rat tumor, found that if a growth were removed during the premetastatic stage a second graft would seldom grow, although after the expiration of this period proliferation would occur in the tumor secondarily inoculated. If the primary nodule were left and a second graft implanted during the premetastatic period, not only did the second tumor fail to grow, but in many instances the first one disappeared. The length of the premetastatic period Gay put at about thirty days.

Jobling,[3] however, working with the same tumor, achieved exactly opposite results. Re-inoculation was possible during the first thirty days in all those rats in which the first growth was not receding, while in some rats with growing tumors inoculation undertaken at a later date was not successful.

Distribution of Active Resistance

Acquired resistance has been studied in relation to its distribution through the organism, and Bashford [4] reported that mice injected with blood were immune to tumor implantation in locations remote from that in which the immunizing treatment had been undertaken. Moreover, it was found by Bashford, Murray, and Cramer,[5] that mice immune after recovery from experimental cancer had undergone some change which, instead of being confined to the tissues in the immediate neighborhood of the spontaneously absorbed tumor, had become generalized, probably by means of the body fluids. In the opinion of Da Fano,[6] the lymphocyte might be the agent which distributed immunity throughout the organism.

[1] *Jour. American Med. Assoc.*, 1907, xlviii, 420.

[2] *Proc. Soc. Exp. Biol. and Med.*, 1908–1909, vi, 75.
Boston Med. and Surg. Jour., 1909, clxi, 207.

[3] *Monographs on Medical and Allied Subjects*, Rockefeller Institute, New York, 1910, No. 1, 57.

[4] *British Med. Jour.*, 1906, ii, 209.
Lancet, 1906, ii, 315.

[5] *Proc. Roy. Soc.*, Series B, 1907, lxxix, 177.
Third Sci. Report, Imperial Cancer Research Fund, London, 1908, 330.

[6] *Zeitschrift f. Immunitätsforsch.*, etc., Orig., 1910, v, 68.

Kraus, Ranzi, and H. Ehrlich [1] suggested, on the other hand, that in animals made refractory by subcutaneous inoculation the immunity was, perhaps, a local condition confined to the subcutis, rather than one of wide distribution throughout the body.

The observation of Bashford, Murray, and Cramer was confirmed by Uhlenhuth, Haendel, and Steffenhagen.[2] These authors found that rats which had become resistant by reason of an unsuccessful subcutaneous implantation could not be inoculated by the peritoneal route, and that resistance in these animals was thus not limited to the subcutis.

Entirely in accord with these observations were those of Woglom,[3] who demonstrated that mice made refractory by the subcutaneous inoculation of embryo skin were as resistant to intrarenal as to subcutaneous grafts of a carcinoma.

According to Levin,[4] also, rats immunized by unsuccessful subcutaneous inoculation of a sarcoma were resistant to inoculation into a parenchymatous organ.

First Appearance and Duration of Active Resistance

Discussing the time of appearance and the duration of the refractory state, Ehrlich [5] wrote that the resistance produced in mice by the unsuccessful inoculation of spontaneous tumors appeared in from seven to fourteen days, and lasted for weeks or even months.

Bashford,[6] in describing the resistance present after the spontaneous absorption of transplantable mouse tumors, said that it persisted for at least six months.

Bridré [7] tested the duration, not only of the resistance consequent upon unsuccessful tumor inoculation, but of that entailed by treatment with normal tissue, and concluded that acquired immunity in the mouse might endure for five months or more.

According to Bashford, Murray, and Cramer,[8] the resistance attend-

[1] *Zeitschrift f. Immunitätsforsch.*, etc., Orig., 1910, vi, 681.
[2] *Arb. a. d. Kaiserl. Gesundheitsamte*, 1911, xxxvi, 484.
[3] *Lancet*, 1911, ii, 92. [4] *Jour. Exp. Med.*, 1911, xiv, 139.
[5] *Zeitschrift f. aerztliche Fortbildung*, 1906, iii, 211.
Arb. a. d. Königl. Inst. f. Exp. Therap., 1906, Heft i, 90, 97.
[6] *British Med. Jour.*, 1906, ii, 209. [7] *Ann. de l'Inst. Past.*, 1907, xxi, 773.
[8] *Proc. Roy. Soc.*, Series B, 1907, lxxix, 180.
Third Sci. Report, Imperial Cancer Research Fund, London, 1908, 333.

ant upon the inoculation of mouse blood appeared after four days, but was more marked after ten. Embryo skin required twenty days to elicit the refractory state in Russell's experiments,[1] or twice the time consumed by blood.

In the rat, Uhlenhuth, Haendel, and Steffenhagen[2] discovered evidence of immunity a month and a half after absorption of a spindle cell rat sarcoma, while Flexner and Jobling[3] found that the resistance left by the retrogression of growing tumors, although not perfect, was of high degree, and that it was most marked immediately after the disappearance of the tumors, becoming reduced subsequently by mere lapse of time. Within a period of ninety days after the retrogression of adeno-carcinomata in sixty-one rats, 16.5% of the animals proved re-inoculable with a tumor which, in the control animals, yielded 86.2% of growths.

Woglom[4] has investigated the onset, extent, and duration of the resistance produced in mice by 0.05 cubic centimeter of embryo skin, normal kidney, or spontaneous carcinoma. The immunity following treatment with embryo skin reached its maximum of 80–100% by the tenth day, remained at a high level until the twenty-fourth, and then declined, to vanish at about the seventy-fifth day. Neither kidney nor spontaneous tumors produced so high a degree of resistance.

Passive Resistance

The presence in the serum of resistant mice of some factor deleterious to the growth of malignant elements was at once suggested when Clowes and Baeslack[5] asserted that exposure of cancer cells to immune serum before inoculation inhibited their subsequent development. The great majority of investigators, however, have been unable to demonstrate inimical substances in the serum either *in vitro* or *in vivo*, although v. Dungern[6] has described the transfer of resistance to rabbits through the serum of others that had spontaneously recovered from a

[1] *Third Sci. Report, Imperial Cancer Research Fund*, London, 1908, 345.

[2] *Centralbl. f. Bakt.*, etc., erste Abt., Ref., 1910, xlvii, Beiheft, 164.

[3] *Monographs on Medical and Allied Subjects*, Rockefeller Institute, New York, 1910, No. 1, 54.

[4] *Jour. Exp. Med.*, 1912, xvi, 629.

[5] *Med. News*, 1905, lxxxvii, 969.

[6] *Zeitschrift f. Immunitätsforsch.*, etc., Orig., 1910, v, 695.

transplantable sarcoma. Twelve to twenty cubic centimeters were injected into the aural vein in six animals simultaneously with the tumor, and in one twenty-four hours previously. In these seven rabbits no tumors arose, although six developed among nine controls.

But in the opinion of Apolant,[1] this growth was hardly analogous to the blastomata hitherto studied, and as it was in all probability of parasitic nature Apolant thought that v. Dungern's results should be accepted with caution.

The production of passive resistance in mice by means of serum drawn from rabbits injected with mouse cancer was attempted by Michaelis.[2] Although this serum was hemolytic for mouse blood, it possessed no immunizing action against cancer.

Ehrlich [3] mixed tumor emulsion with the serum of treated rabbits, but was not able to perceive that the serum exerted any effect upon the cancer cells.

Haaland [4] found that the serum of mice which had been inoculated with Jensen's tumor was powerless to confer resistance against a sarcoma. He furthermore injected the serum of naturally resistant Hamburg mice into young, susceptible mice from Berlin, but discovered no difference between the treated animals and the controls upon subsequent inoculation with a sarcoma. Similarly, Gay [5] wrote that the serum of rats unsuccessfully implanted with the Flexner-Jobling rat tumor, or naturally refractory to it, did not prevent growth if introduced simultaneously with the tumor.

Bridré [6] treated sheep and fowl with fresh cancer, and with preliminary injections of their serum sought to prevent the establishment of tumor grafts in mice. The results were not encouraging, and among the mice inoculated with the specific sheep serum there developed a number of tumors even greater than occurred among the controls.

Bashford, Murray, and Haaland [7] had not been able to demonstrate directly any antibodies in the serum or in the milk of mice naturally

[1] *Zeitschrift f. Krebsforsch.*, 1911–1912, xi, 106.
[2] *Med. Klin.*, 1905, i, 205.
[3] *Zeitschrift f. Krebsforsch.*, 1907, v, 75.
[4] *Berl. klin. Woch.*, 1907, xliv, 717.
[5] *Proc. Soc. Exp. Biol. and Med.*, 1908–1909, vi, 75.
Boston Med. and Surg. Jour., 1909, clxi, 210.
[6] *Ann. de l'Inst. Past.*, 1907, xxi, 774.
[7] *Third Sci. Report, Imperial Cancer Research Fund*, London, 1908, 395, 369.

or artificially refractory to cancer, nor could they, by inoculating the blood of resistant mice, cause the development of a higher degree of immunity than could be obtained with the blood of normal mice.

Uhlenhuth, Haendel, and Steffenhagen[1] made frequent re-inoculations of immune rats to increase resistance to the highest point, so that the presence of any protective bodies existing in the blood might be demonstrated. But when the serum was injected into rats several days antecedent to tumor inoculation, or allowed to act upon a tumor emulsion for half an hour before its injection, no evidence of any protective substance was discovered; on the contrary, Uhlenhuth and his associates found that tumor growth was favorably influenced.

Finally, Russell[2] came to the conclusion that there were no features in the immune condition comparable to the antibodies evolved against the infective organisms.

While the experience of nearly all those who have investigated the question is thus arrayed against the possibility of transferring resistance from one animal to another, authorities are still not entirely unanimous upon this point, and Gaylord[3] has but newly reaffirmed his belief that passive immunity to cancer does actually exist, even though it be not easily or always a demonstrable condition.

ARE NATURAL AND ACQUIRED RESISTANCE TRANSMISSIBLE BY HEREDITY?

The hereditary transmission of *natural resistance* to transplanted tumors was tested by Tyzzer,[4] who found that the offspring of naturally immune mice were more refractory than controls of the same age.[5]

The opposite condition, susceptibility, which had been vouched for by Morau,[6] was also investigated to see whether it was regularly transmitted from generation to generation, or inherited as a Mendelian character. Most of Tyzzer's experiments were performed with the

[1] *Arb. a. d. Kaiserl. Gesundheitsamte*, 1911, xxxvi, 490.

[2] *Third Sci. Report, Imperial Cancer Research Fund*, London, 1908, 357.

[3] *Travaux de la deuxième Conférence internat. pour l'Étude du Cancer*, Paris, 1911, 594.

[4] *Jour. Med. Research*, 1909, N.S., xvi, 519.

[5] Clowes (*Johns Hopkins Hosp. Bull.*, 1905, xvi, 130), however, had recorded the occurrence of ten rapidly growing tumors among sixteen young mice, descendants of parents not susceptible to the Jensen tumor.

[6] *Arch. de Méd. exp. et d'Anat. path.*, 1894, vi, 692.

spontaneous growth of a Japanese waltzing mouse, although the Jensen carcinoma and a tumor from Ehrlich's laboratory were also used. The waltzing mouse tumor grew from the first in practically all Japanese waltzing mice, whereas repeated implantations into ordinary mice were unsuccessful. The cross-breeding of Japanese waltzing mice and common mice was undertaken, therefore, for the purpose of testing the susceptibility of the offspring so produced. The hybrids of the first generation were susceptible, both the progeny of common and of Japanese waltzing mothers; susceptibility, accordingly, might be transmitted by either the male or the female parent. But the members of the next generation, obtained by breeding the hybrids among themselves, were all, like common mice, insusceptible. The appearance of susceptibility in the first generation and its total disappearance in subsequent generations Tyzzer was unable to harmonize with Mendel's law, or with any other principle of heredity yet known. Although a certain number of the mice of the second and third generations manifested the waltzing character, they were nevertheless insusceptible to the waltzing mouse tumor, while the hybrids of the first generation, although none of them exhibited the waltzing character, were susceptible.

Cuénot and Mercier [1] also have asserted that a condition of natural suitability for the inoculation of tumors could be transmitted to the offspring. Every mouse, they concluded, was able to transmit to its descendants a certain sensitiveness to the implantation of a tumor graft. These observers succeeded in breeding two strains of mice — a susceptible and a non-susceptible strain. The non-susceptible group comprised one hundred and three mice, of which seventeen (16.5 %) were successfully inoculated with Borrel's tumor "B." Of the susceptible strain there were eighty-nine, which were inoculated at the same time as the non-susceptible mice and with fragments of the same tumor. Of these, seventy-six (85.3 %) developed growths.

The question of the transmission of *acquired resistance* from a refractory mother to her young has been answered in the negative by Bashford, Murray, and Haaland,[2] who inoculated the offspring of mice that had been rendered highly resistant by repeated treatment with

[1] *Compt. rend. de l'Acad. des Sc.*, 1910, cl, 1443.
Compt. rend. Soc. Biol., 1910, lxix, 645.

[2] *Third Sci. Report, Imperial Cancer Research Fund*, London, 1908, 395.

large doses of Jensen's tumor. Twenty-one of these young mice, when implanted with Jensen's tumor, did not show themselves any more refractory to it than other mice of the same age.

These results correspond to those of Cuénot and Mercier,[1] who were unable to demonstrate resistance in the young born of a mother in which the tumor had been absorbed during lactation.

NATURE OF THE RESISTANT STATE

Although resistance to the implantation of cancer is such a well-recognized condition, no attempt to explain its character has so far been accepted on all sides as complete and satisfactory.

The most widely criticized and skilfully defended hypothesis among those essaying to explain the nature of resistance has been Ehrlich's hypothesis of *athrepsia* which, advanced by its distinguished sponsor[2] as the result of the following chain of reasoning, has been re-stated and defended in a series of subsequent articles.[3]

A mouse carcinoma is able to proliferate to a certain extent in the rat, retrogressing, however, in about a week. If, at the height of its activity, such a tumor were to be inoculated into a second rat, no further growth would take place, whereas if it were inoculated back into a mouse continuation of growth would become possible. One could practise such *zigzag* inoculation, mouse — rat — mouse — rat, etc., as long as desired, without influencing in the least the proliferative power of the tumor. At first it might be thought that the inability of the cells to grow progressively in the rat was the result of natural resistance, that is, of the presence of antibodies able to exert a direct lethal action on the mouse tumor cells. This hypothesis might be dismissed, however, without further consideration, for the initial active growth of the cells after their introduction into the rat would still be inexplicable. A second possibility was conceivable: That antibodies were, indeed, present, but that they were not elabo-

[1] *Compt. rend. de l'Acad. des Sc.*, 1909, cxlix, 1013.

[2] *Zeitschrift f. aerztliche Fortbildung*, 1906, iii, 208.

[3] *Arb. a. d. Königl. Inst. f. Exp. Therap.*, 1906, Heft i, 83.
Zeitschrift f. Krebsforsch., 1907, v, 76.
Harben Lecture, London, June, 1907; *Jour. Royal Inst. Pub. Health*, 1907, xv, 321, 385, 449.
Beitr. zur experimentellen Pathologie u. Chemotherapie, Leipzig, 1909, 77, 134.

rated until after the absorption of the tumor cells, and that then only was the rat made actively refractory. But even this did not suffice to explain the phenomenon, for it would still be asked how re-inoculation into the mouse was possible, not the slightest damage having been done to the proliferative power of the cells during their sojourn in the rat.

Much more satisfactory was a third hypothesis, illustrated by an example from the domain of bacteriology. When the bacillus of influenza was cultivated from the sputum, the first culture was usually successful; and while one subculture could be made occasionally from the original, further transplants were never possible unless blood-agar were used. For the growth of the influenza bacillus there was necessary, therefore, a specific incitant over and above the ordinary nutrient material as it existed in agar. While at the time of the first subculture this condition was fulfilled through the presence of hemorrhagic sputum, growth of the bacillus in subsequent cultures became impossible by reason of exhaustion of the supply of specific material. The situation was similar when a mouse tumor had been transferred to the rat, although here the difficulty could not be lack of nourishment in a banal sense, for the vigorous growth during the first week was opposed to such a conception. It was clear, therefore, that not only was there absent from the rat's organism an antistuff against the cells of the mouse carcinoma, but that this host actually offered nourishment in abundant amount, making active proliferation possible. Apparently, however, the presence of an ordinary food-stuff did not suffice for the continued growth of the foreign cell, and just as the influenza bacillus needed in addition to the agar a small amount of hemoglobin, so did the mouse carcinoma require a certain specific *X-stuff* which was present only in the organism of the mouse, and of which a little had been carried over into the rat at the first inoculation. The tumor grew in proportion to the amount of this material inoculated with it; but once the supply became exhausted, further growth was possible only if the graft were returned to an organism containing the necessary X-stuff, *i.e.* the mouse, and the natural resistance of the rat might thus be referred to lack of a substance indispensable for proliferation of mouse cancer cells. The X-stuff, however, need not be actually wanting in refractory animals, for it might

be present, although not available, nor was athreptic immunity, even when extant, always able entirely to prevent growth. Proliferation might, indeed, occur in spite of it, although in such a case the tumors would be of insignificant size.

Ehrlich was impressed by the relative infrequency with which macroscopic metastases were encountered in the lungs of mice with tumors, and by the observation that if the formation of metastases were imitated in animals already bearing rapidly growing nodules by re-inoculating them with the same or with another tumor, the second implantation would be unsuccessful except in a few instances. This outcome was most readily explained by the existence of the first tumor which, during its rapid growth, devoured with a thousand mouths the nourishment that it required, leaving none for cells secondarily inoculated, or deposited as emboli. These elements found themselves, therefore, under much more adverse nutritive conditions than the cells already established, and were unable, in consequence, to compete with them. He thus saw in the rarity of natural metastases in the mouse, no less than in the failure of secondary inoculations, an indication of the existence of a type of athrepsia different from that naturally occurring in the rat. Entirely in accord with the hypothesis thus outlined could be arranged his observation that with very slowly growing tumors like chondromata, a second inoculation was occasionally successful, since the more slowly the first tumor grew the less nourishment need it assimilate, and the more favorable would be the conditions for cells subsequently introduced.

The absence of macroscopic metastases was accordingly not an indication against the malignant character of a tumor, but rather the expression of a special type of malignancy resulting from an enormous energy of growth.

A partial confirmation of Ehrlich's hypothesis seemed to have been afforded by some experiments of Schöne,[1] who argued that if it were tenable every obstacle to the growth of a second tumor should be removed by the extirpation of the first. This assumption was put to the test in a fairly large number of experiments, with the result that a secondary inoculation done after the expiration of from eight days

[1] *Verhandl. d. deutschen Gesellsch. f. Chir.*, 1907, xxxvi, 213.
Deut. med. Woch., 1907, xxxiii, 866.

to three weeks, when the operation wound had healed, was successful in as large a percentage as in normal animals.

Gierke [1] thought, however, that the experiment did not necessarily prove the mere disappearance of the influence of the tumor to have effected this revolution in the organism of the mouse. If, as he himself thought, the negative result of secondary inoculation in tumor-bearing animals was due not to athrepsia, but to acquired resistance elicited by the introduction and partial absorption of a large dose of tumor at the time of the first inoculation, it would be possible to explain Schöne's facts by the assumption that resistance could be abrogated by drastic operation — an explanation which Schöne himself had not failed to consider.

A result exactly opposite to Schöne's was the outcome of experiments by Uhlenhuth, Haendel, and Steffenhagen,[2] who demonstrated that rats suddenly freed by operation of large tumors averaging about three weeks in age, were resistant to immediate or later re-inoculation, if the growth had been so thoroughly removed that no recurrence took place. In animals with recurrent tumors (and Uhlenhuth and his colleagues noted in passing that such tumors were distinguished by particularly energetic growth) re-inoculation, on the contrary, was always successful. This observation they could not accord with Ehrlich's hypothesis of athrepsia, nor could they coördinate with that hypothesis the particularly favorable results following simultaneous inoculation of rats at several sites. Moreover, they found that tumors secondarily inoculated usually grew in those animals in which the first tumor was flourishing, while, on the other hand, in rats where the first tumor was backward in growth and showed an inclination toward necrosis and regression, re-inoculation was unsuccessful.

The results of their experiments the authors explained as follows : —

After the implantation of a tumor there followed a conflict between its cells and the animal's body. This struggle did not stop, even though the growth established itself, but became, on the contrary, more intense, and there still remained the chance that the organism might

[1] *Beitr. zur path. Anat.*, etc., (Ziegler), 1908, xliii, 352.
Third Sci. Report, Imperial Cancer Research Fund, London, 1908, 143.

[2] *Centralbl. f. Bakt.*, etc., erste Abt., Ref., 1910, xlvii, Beiheft, 158.
Zeitschrift f. Immunitätsforsch., etc., Orig., 1910, vi, 657.
Arb. a. d. Kaiserl. Gesundheitsamte, 1911, xxxvi, 498.

finally conquer. When the host was victorious there ensued cessation of growth on the part of the tumor, necrosis, and finally complete disappearance, and the protective stuffs remaining after destruction or complete ablation of a neoplasm were able to prevent the development of malignant cells subsequently inoculated; the animals were immune. But the tumor cells themselves had time, during the strife, to attain some degree of resistance toward the antistuffs of the organism, so that elements left behind at operation were *serum proof*, and were, moreover, under more favorable conditions for obtaining nourishment than they had been formerly in the presence of a large tumor. Hence, the cells of recurrences were significant for their very powerful growth energy; and as they were able not only to make their way against antistuffs, but even to paralyze these bodies, there was no bar to re-inoculation in an animal with a recurrent tumor. That, in the absence of recurrence, re-inoculation even with the original serum proof tumor was negative, could be explained on the supposition that the action of the protective bodies was concentrated on the new graft, which had been so disturbed in its growth by removal to another part of the body that it succumbed to their attack.

The work of these authors has been criticized by Russell [1] on the ground that incomplete excision was not the factor which determined whether re-inoculation would or would not be successful. This was decided solely by the reaction taking place between the animal and the growth first implanted. Furthermore, the assumption of an alteration in the tumor parenchyma, by virtue of which it became resistant to immunity, accorded but ill with Russell's experience, for attempts to obtain such refractory neoplasms by cultivating those tumors which had grown as an exception in mice immunized with embryo skin had not produced a growth resistant to the forces of immunity called into play by embryo skin.

The deductions drawn by Uhlenhuth and his colleagues were refuted also by Apolant,[2] who saw in the experiments a substantiation rather than a contradiction of Ehrlich's views. In the first place, the rapid growth of recurrences could not be explained by assuming that their cells were serum proof, for the fact that re-inoculation with the same

[1] *Fifth Sci. Report, Imperial Cancer Research Fund*, London, 1912, 16.

[2] *Zeitschrift f. Immunitätsforsch.*, etc., Orig., 1911, x, 103.

tumor was fruitless stood in direct contradiction to such an assumption. The authors' explanation of this discrepancy Apolant could not admit, for if the cells were serum proof they would be able to resist the attacks of antistuffs. As for the dislocation of tumor cells from their original position, while it was no doubt true that they suffered injury during the process, still it was the very method by which successful tumor transplantation had been effected over a period of ten years. Apolant could conceive of no more striking proof of Ehrlich's views than the rapid growth of recurrences, for the same quantity of specific nutrient material which, before the operation, had to suffice for the whole tumor, was now at the disposal of a relatively small number of cells, and their more rapid proliferation was, therefore, readily comprehensible.

When Uhlenhuth and his associates asserted that the result of double and triple inoculations was not in accord with the hypothesis of athrepsia, they had overlooked Ehrlich's condition that the presence of a rapidly growing tumor was essential for the supervention of athreptic immunity.

Although the authors under discussion had found that the yield from re-inoculation of operated rats depended entirely upon whether the first growth had or had not been radically removed, Apolant could find no evidence that the outcome of the second inoculation was dependent upon the appearance or non-appearance of recurrences, for in about 20% of the rats and 15% of the mice there were striking exceptions to Uhlenhuth's results. It was a question whether all the cases that remained free from recurrence had really been subjected to complete operation. During the radical excision of large growths extensive defects ensued, to heal only with difficulty and under great tension, and the nourishment of tiny fragments remaining would therefore be seriously impaired by the tightly stretched skin, their cells undergoing necrosis and absorption, although larger pieces with better circulatory connections might be able to continue growth. In the former case active resistance would supervene from the absorption of tumor remnants, while in the latter there would be no absorption and consequently no resistance.

Clowes[1] found that if mice already developing growths were inocu-

[1] *British Med. Jour.*, 1906, ii, 1551.

lated a second time from five to ten days after the first implantation, tumors were rarely produced, or, if produced, grew far more slowly than in normal controls. Less than 6% of tumors resulted from such secondary inoculations, while in the normal controls the yield was 95%. While thus agreeing with Ehrlich so far as observation went, Clowes could not subscribe to his hypothesis, and explained the failure of the second graft by the assumption of immune forces induced in the serum as the result of a reaction against the developing tumor.

Investigating athreptic immunity, Hertwig and Poll[1] determined that a growth already present, whether small or large, extended no protection against the development of new tumors when, at the expiration of varying periods, second, or even third inoculations were performed; nor could they discover any restrictive influence upon the younger through the deprivation of nourishment. In many cases, indeed, the nodules were of similar proliferative energy, and those secondarily inoculated not seldom outstripped neoplasms already established. The tumor with which they were working, even though it were not of such vigorous growth as that used by Ehrlich, might still be fairly classed among the rapidly growing, for after two or three months it reached, in many of their mice, a weight equal to that of the animal bearing it.

Lewin,[2] who worked with both rat and mouse tumors of rapid growth, expressed his entire agreement with the findings of Hertwig and Poll, for he had never failed successfully to re-inoculate tumor-bearing animals.

Borrel,[3] repeating Ehrlich's experiments, achieved the same result at which Ehrlich had arrived, when tumor emulsion was inoculated after the manner practised at the Frankfort laboratory, although when intact fragments were ingrafted a contradictory outcome was obtained. When the site of the inoculation of an emulsion was examined microscopically, it could be shown that only a part of the introduced material had proliferated. The remainder, having been absorbed, might have given

[1] *Abhandl. d. Königl. Preuss. Akad. d. Wissensch.*, 1907, 31.
[2] *Berl. klin. Woch.*, 1907, xliv, 1606.
Zeitschrift. f. Krebsforsch., 1907–1908, vi, 307.
[3] *Bull. de l'Inst. Past.*, 1907, v, 594.

rise to cytolytic antibodies able to embarrass the development of cells deposited at a second inoculation. The cells first introduced were not affected by these hypothetical antibodies because time was required for their elaboration and they were, therefore, unable to exert injury upon a tumor in which growth had been already inaugurated. But such antibodies might prevent the development of cells subsequently inoculated, by being present at a time when vascular connection had not yet been established.

Contrasted with this first condition was the second where, an entire fragment having been inoculated, no absorption took place in the majority of cases. In such an event, re-inoculation performed after an interval of from eight to twenty-one days, at a time when the first tumor had already advanced to a large size, was entirely successful.

Bridré[1] also was unable to substantiate Ehrlich's observations, finding, on the contrary, that mice already bearing a tumor offered a favorable soil for a second, whether this were of the same or of a different type. Indeed, in certain experiments, mice in which tumor "B" was already established could be re-inoculated with this growth in 100% of cases, while the controls did not furnish more than 80%. Although the proportion of successful inoculations was not always thus increased, tumor-bearing mice were at least as susceptible to re-inoculation as normal animals. The results of Bridré's inoculations were not due to the low virulence of the tumor first implanted, and he explained the discrepancy between his results and Ehrlich's by the difference in the amounts of tumor inoculated.

The experiments of Borrel having been repeated in Ehrlich's laboratory, Apolant[2] contended that athrepsia was readily demonstrable after the inoculation of tumor fragments, its presence being indicated in such a case by the much smaller weight of the secondary tumors as contrasted with those in the controls.

Michaelis[3] could not see that the presence of one growth prevented re-inoculation of a second, but suggested that his failure might have been due to absence in his own tumor of the extreme proliferative power possessed by that of Ehrlich.

[1] *Ann. de l'Inst. Past.*, 1907, xxi, 771.
[2] *Zeitschrift f. Immunitätsforsch.*, etc., Orig., 1911, x, 104.
[3] *Zeitschrift f. Krebsforsch.*, 1907, v, 195.

Bashford and Murray[1] had written two years before the publication of Ehrlich's hypothesis ". . . that transplantation can be successfully performed in animals in which tumours have already developed fourteen days to ten weeks after the first effective transplantation, *i.e.* both when the primary tumour is small, and when it has attained a large size." Subsequent experiments gave them no reason to retract this statement, and after four years of further experience, in a paper published with Haaland, they[2] stated that a series of inoculations into tumor-bearing animals confirmed what had previously been said. While in experiments with rapidly growing tumors they did encounter the phenomenon described by Ehrlich, they believed that the result of a secondary inoculation was determined, not by the growth rate of the tumor already established, but by the size of the dose primarily inoculated. Mice in which the first tumor had developed from a small quantity were re-inoculated with large or small amounts, and conversely, the same manner of re-inoculation was practised on mice whose primary tumor had developed from a large dose. The results in both series were entirely in accord and led the authors to express the conviction that concomitant immunization, evolved by the absorption of tumor at the time of the first inoculation, was the most natural explanation of the facts, and that the athreptic explanation was inadequate. A negative result on re-inoculation was thus ". . . due to a secondary change in the positive animal following on the absorption of tumour material. The quantitative relations obtaining between the tumour tissue introduced and the degree of protection resulting, demonstrate, just as effectually in the case of animals bearing tumours as in the case of normal animals, that we have here to deal with an active immunity." When a growth had reached a very large size, re-inoculation into the animal bearing it was either negative or was followed by small stationary tumors only. This occurred, however, because the limit of nutritive capacity in the animal had been reached, and the authors compared the issue with the poor results obtained after inoculation of mice in ill health.

[1] *Sci. Reports, Cancer Research Fund*, London, 1904, No. 1, 15.

[2] *Third Sci. Report, Imperial Cancer Research Fund*, London, 1908, 386. *Zeitschrift f. Immunitätsforsch.*, etc., Orig., 1909, i, 519.

Gierke,[1] working in the same laboratory, concluded from extensive experiments that ". . . a mouse with a tumour offers conditions far more favourable to subsequent inoculations than those obtaining in normal mice." There were two possible explanations for this fact. "Either these animals have been picked out by the primary inoculation, which are naturally suitable for tumour implantation, and, therefore, they are susceptible also to a second inoculation, or, the suitability for implantation is increased in certain cases by the primary growing tumour." He himself inclined to choose the second explanation, although realizing that the experiments did not afford an absolute demonstration of its validity. The contrast between these results and Ehrlich's, as it was probably not due to variations in material, Gierke ascribed to dissimilarities in technic, and particularly to differences in dose. Such large amounts as had been employed in Ehrlich's laboratory were in great part absorbed, only a comparatively small number of the cells taking part in the formation of the new growth, and the absorption of such a large amount of tumor would increase resistance toward a second graft.

Jobling,[2] without wishing to discuss Ehrlich's views, offered the results of secondary and tertiary inoculations with the slowly growing Flexner-Jobling rat adeno-carcinoma. His experiments showed, in brief, that a proliferating tumor did not prevent the successful implantation of another of the same kind, although the secondary inoculations were likely to yield a smaller number of growing nodules than the first. In rats with stationary or receding growths, however, re-inoculation usually failed.

The athreptic hypothesis has been subjected by Bashford and Russell[3] to a criticism even more searching than any so far described, and based upon the microscopic examination of the re-inoculation site at successive intervals.

Study of young grafts had already demonstrated that the tumor cells continued to proliferate, that the stroma died and was replaced by one furnished from the new host, and that in mice resistant to

[1] *Beitr. zur path. Anat.*, etc., (Ziegler), 1908, xliii, 347.
Third Sci. Report, Imperial Cancer Research Fund, London, 1908, 137, 139.

[2] *Monographs on Medical and Allied Subjects*, Rockefeller Institute, New York, 1910, No. 1, 57.

[3] *Proc. Roy. Soc.*, Series B, 1909–1910, lxxxii, 298.
Lancet, 1910, i, 782.

carcinoma there was a failure to supply this specific scaffolding, whether immunity had been induced by the previous absorption of tumor or of normal tissue.

It was found that re-inoculation failed except in those mice in which the first tumor had continued to grow rapidly. When growth had slackened, or when absorption had begun, no tumor developed from secondary inoculation; or, in other words, the better the growth of the first tumor, the more favorable were the chances that a second implantation would be successful.

At the site of the re-inoculation there was an inhibition of the stroma reaction in those mice in which the primary tumor had become slower of growth or had begun to decrease in size, and it was thus demonstrated beyond reasonable doubt that the actual mechanism in play was the induction of a resistance identical with that already described in other refractory animals. The successful re-inoculation of mice bearing the most rapidly growing tumors was readily understandable on this basis, for the absorption of tumor tissue was here almost *nil*, and resistance had, therefore, not been established. The authors concluded that to assume the presence of a special, or athreptic, immunity in tumor-bearing animals would be superfluous.

Ehrlich's[1] reply to his earlier critics was an emphasis of his former statements, that in order for the phenomenon of athreptic immunity to be brought out the preliminary inoculation must have been made with a tumor strain of maximal virulence, and that retardation of growth was just as much an evidence of athrepsia as it was, in active resistance, an evidence of partial immunization. The conception *tumor virulence* embraced two qualities not always separable from one another, namely, *proliferative energy*, measured by the rapidity of growth, and *transplantability*, evaluated by the percentage of successful inoculations. While these two qualities generally went hand in hand, exceptions occurred now and again, the most important being a chondroma which, in spite of its feeble growth energy, could be successfully transferred in 100% of cases. As regarded transmissibility, a limit was naturally imposed upon the increase of virulence at 100%, while for the augmentation of growth energy the limit varied with the different strains. Lowest with chondroma, higher — although

[1] *Verhandl. d. deutschen path. Gesellsch.*, 1908, 12te Tagung, 14.

very variable — with carcinoma, it reached its maximum in the sarcomata, among which tumors could be obtained that in three or four weeks might equal the size of the animal itself.

Ehrlich expressed the conviction that over and above characteristics so obvious as inoculation percentage and rapidity of growth, there were other and more latent qualities to be interpolated in the conception of virulence. Thus he had observed that two strains of carcinoma, although they were about equal in inoculation percentage and growth energy, varied in the power to prevent successful re-inoculation, one entirely excluding it, the other limiting it only to a slight extent. The factor distinguishing the former he provisionally described as *exhaustive* or *ereptive*, but left open the question whether or not it rested upon a maximum avidity of the tumor cells or upon some specific relationship toward the materials necessary for tumor growth. The expression *maximal virulence* he preferred to restrict to such tumors as had all three qualities developed in the highest degree, and in which they persisted without variation.

Criticizing Borrel's comparison of the results following the inoculation of emulsion and intact grafts respectively, Ehrlich replied that in his own experience tumors had always exhibited a more rapid growth after having been introduced in the form of an emulsion — a fact that harmonized but poorly with any supposition implicating antibodies. Accepting for the moment, with Borrel, the assumption that antibodies were always elaborated in great quantity after the inoculation of an emulsion, it was difficult to understand why they did not hinder the growth of the first tumor. It was certain that a nodule already established and, therefore, well vascularized, would be subjected to a much greater chance of contact with antibodies circulating in the blood than would one newly introduced under the skin, although it could, of course, be objected that the proliferating tumor had become proof against the new-formed antibodies. However, all the re-inoculations at the Pasteur Institute had been made with growths which, having survived many generations of emulsion inoculation, were serum proof to the same degree as the tumor already present; and as there was no ground for ascribing to either neoplasm differences in the grade of resistance, Borrel's explanation was untenable.

Nevertheless, his experiments had been repeated, with the result

that athrepsia had been demonstrable even after the insertion of tumor fragments, although, to be sure, the same grade had not been reached as might have been attained after introduction of an emulsion. The explanation, however, was quite opposite to Borrel's. As growth in tumors resulting from the implantation of intact grafts was appreciably slower than in those following the inoculation of an emulsion, there was absent in the former case that most important element in the production of athreptic immunity — rapid proliferation.

Direct proof of the existence of athreptic immunity had been afforded further by the transplantation of artificial tumor mixtures, already described in detail by Apolant.[1] Several years before the publication of Ehrlich's present article, the prompt development of a mixed tumor would follow the inoculation of a mixture of one of their sarcomata with a carcinoma. But repeated at the time of writing, when the virulence relationship of the two strains had altered in favor of the former, the same experiment would yield a pure sarcoma. However, by the action of extreme cold the virulence of the sarcoma could be diminished to a point where it was again equal to that of the carcinoma, so that a mixed tumor would ensue upon injection of a mixed emulsion. When the virulence of the sarcoma was still more reduced, a pure carcinoma could be obtained from the mixture. These results demonstrated that Apolant had been dealing with an overgrowth by the stronger component or, in other words, by the one which more strongly attracted the nutriment to itself. The action of antibodies did not in any way enter into the discussion.

If, now, it were imagined that a highly virulent sarcoma and a carcinoma of lower virulence, instead of being mixed, had been inoculated separately at two different sites, no essential change in the athreptic influences would have been brought about, and the explanation of the development of the more vigorous tumor only would offer no difficulty.

With these reflections as a basis, Ehrlich sought to define clearly the presence of athreptic influences by making primary and secondary inoculations, not with the same tumor, but with tumors differing in histology as well as in virulence. In the first two experiments the preliminary inoculation was done with a sarcoma, re-inoculation with

[1] *Zeitschrift f. Krebsforsch.*, 1907–1908, vi, 251.

a carcinoma of weaker virulence following nine or fourteen days afterward. It was found that in the series re-inoculated after fourteen days not a single carcinoma had grown, while in the nine-day series only two insignificant tumors occurred among eighteen inoculations. Controls in both cases showed a large number of growths. When the experiment was reversed and the tumor of lower virulence was inoculated first, to be followed nine days later by the more virulent growth, there was found but slight difference in size between the sarcomata in normal mice and those that had been inoculated into animals already bearing carcinomata, although there was considerable disparity between these carcinomata and those in normal mice. Thus it was evident that a tumor of low virulence hindered only to a slight degree the growth of a more virulent one inoculated afterward and, moreover, that it was considerably impeded in growth, even in spite of its greater age, by the more virulent neoplasm subsequently ingrafted. The experiment demonstrated in a salient way the significance of athreptic influences, and was entirely analogous to Apolant's inoculations of tumor mixtures, for in both cases the development of different growths in the same animal was dependent upon the respective virulences of these grafts or, in other words, upon the avidity of their cells for nutriment.

In order to prove the principle of athrepsia it was sufficient to show by a series of trustworthy experiments that one growing tumor was able to exert an adverse influence upon the growth of another, for a retardation of proliferation was the expression of a partial immunity.

Ehrlich concluded this article with the implication that the negative result attendant upon the inoculation of pregnant animals, or the slow growth of such tumors as did occur, was added evidence in favor of the hypothesis of athrepsia.

v. Dungern and Coca[1] considered that immunity to the transplantation of tumors was a condition of allergie. In studying a hare sarcoma that was transmissible to rabbits, they found that while there developed only a slight circumscribed thickening about a first graft in the rabbit, a second one educed a much stronger reaction, in the shape of a more or less severe inflammatory swelling. The response was well marked by the end of twenty-four hours, still clear after two days, and receded on the third day. Thus there appeared phenomena around

[1] *Zeitschrift f. Immunitätsforsch.*, etc., Orig., 1909, ii, 391.

the second implantation of foreign sarcomatous tissue which might be described as a local reaction of hypersusceptibility.

Histological investigation of the inoculation site in immune animals on the second day revealed, beside necrosis, the presence of a great number of macrophages in the tissues, capillaries, and small veins, while in normal rabbits, on the contrary, such cells were of rare occurrence. A few days later the inoculation site in immune animals was crowded with these elements, and surrounding the blood vessels could be found great collections of lymphoid and plasma cells. The most striking lesion of all, however, was a thrombosis (by masses of macrophages) of nearly all the smaller and larger veins in the neighborhood of the central necrosis. In several cases the authors observed after the second inoculation a softening of the remains of the graft first introduced, small nodules that had been firm for weeks or months becoming soft in a few days, and here there was present the same macrophage reaction. The condition corresponded entirely with that found in tuberculous tissue, composed as it was of a central necrotic zone surrounded by endothelium-like cells, large typical giant cells with peripheral nuclei, and beyond these, lymphoid and plasma cells. Exactly the same appearances were to be seen in transplanted tumors undergoing spontaneous absorption, as well as in the lymph nodes in cases where a tumor of particularly vigorous growth had reached a considerable size. The lesion was distinct, also, in hares.

This reaction, which v. Dungern and Coca considered significant for the elucidation of tumor immunity, they correlated with hypersusceptibility. Upon the first inoculation foreign protoplasmic substances, in the form of tumor cells, entered the circulation and were taken up by the macrophages or their mother cells, producing a condition of sensitiveness in these elements which, therefore, reacted more quickly and more strongly when, at a second inoculation, the identical foreign protoplasm was again encountered. The specific stimulus might act either by chemotaxis or by stimulating the development of macrophages from other cells. Observation spoke more strongly for the second possibility, for when the reaction was pronounced there was always an unmistakable growth of the vascular endothelium coupled with the appearance of many karyokinetic figures, while in contradistinction to this there was no evidence of endothelial proliferation

in the absence of the macrophage reaction. The lesion, while generally very marked in the case of transplanted tumors, was not so distinct where primary growths were concerned.

To see if it were possible to find any constant differences between fragments of tumor implanted into normal mice and those which had been introduced into resistant animals, Russell[1] subjected the grafts to a systematic examination. The material was obtained by the removal of small implantations six, twelve, twenty-four, etc., hours after their inoculation. The phenomena during the first two days were the same in both series of mice, but after this period it was, as a rule, easily possible to distinguish between grafts from resistant and those from normal animals. In a refractory mouse the processes occurring on and after the third day were as follows: —

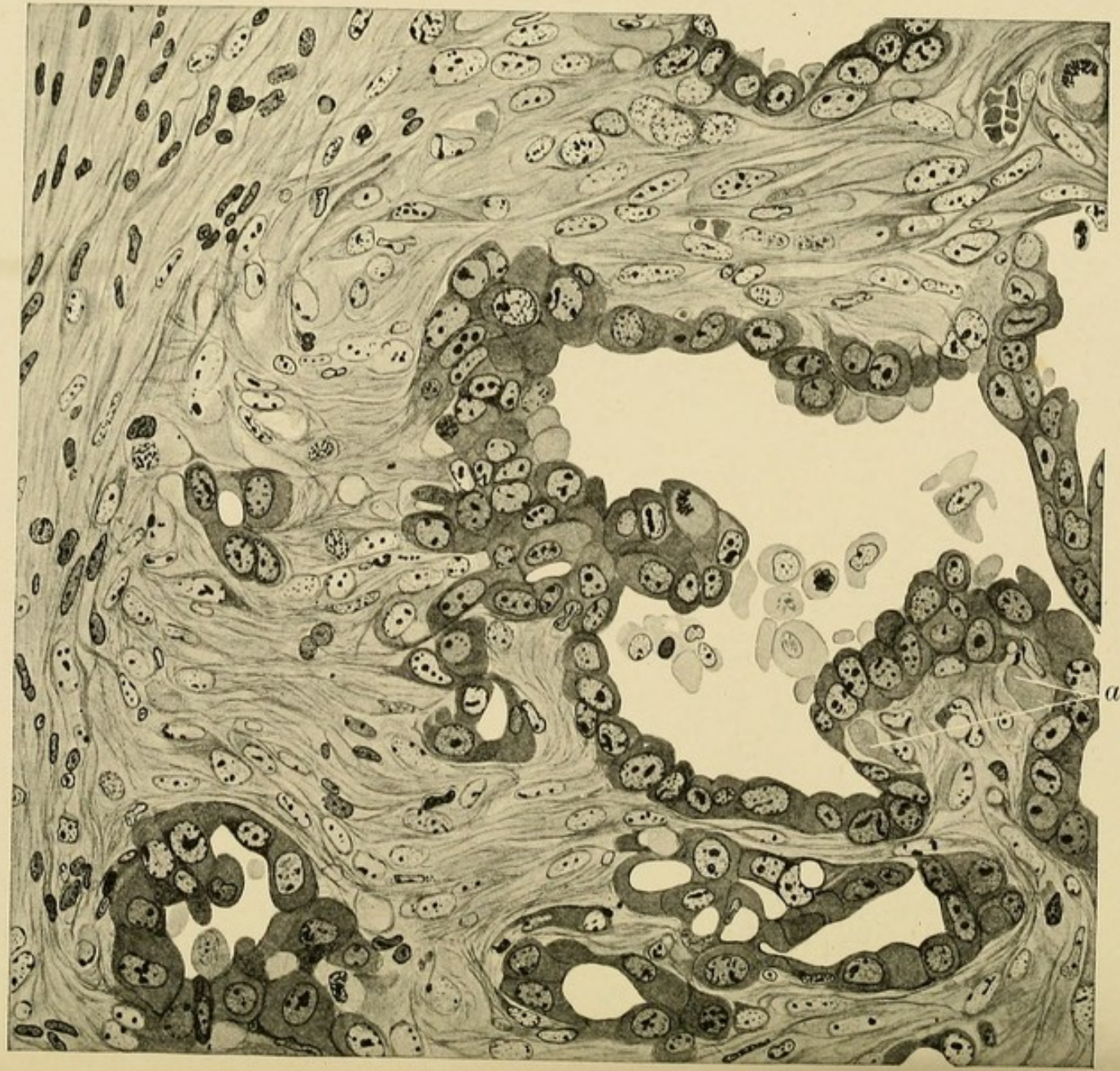
"The necrosis in the center of the graft extends, until all the graft has degenerated with the exception of the epithelial cells in the immediate neighborhood of the host tissues; the fibrinous exudate becomes absorbed and the number of polymorphonuclear leucocytes decreases. The acinous arrangement is destroyed, and the tumour cells lie as a single layer of cells between the necrotic center of the graft and the host tissues. There is not the active proliferation of the host fibroblasts seen normally, nor is there any development of new capillaries. The epithelial cells still continue to divide mitotically.

"As shrinkage of the necrotic center of the graft occurs, there is produced a cleft between the graft and the host tissues, and the epithelial cells spread themselves out over this free surface, producing a cystic cavity. . . .

"The line of contact between the host cells and the pedicle of this dead material does not show anything comparable to the rich development of new tissues seen in . . . a normal graft at four days. When one examines the outer wall of this space with a high power, the lining membrane is seen to be composed of a layer of columnar epithelium, one or more cells deep and sharply demarcated, as if by a basement membrane, from the host tissues. The cells still exhibit mitoses, but not in the number customary in a growing graft.

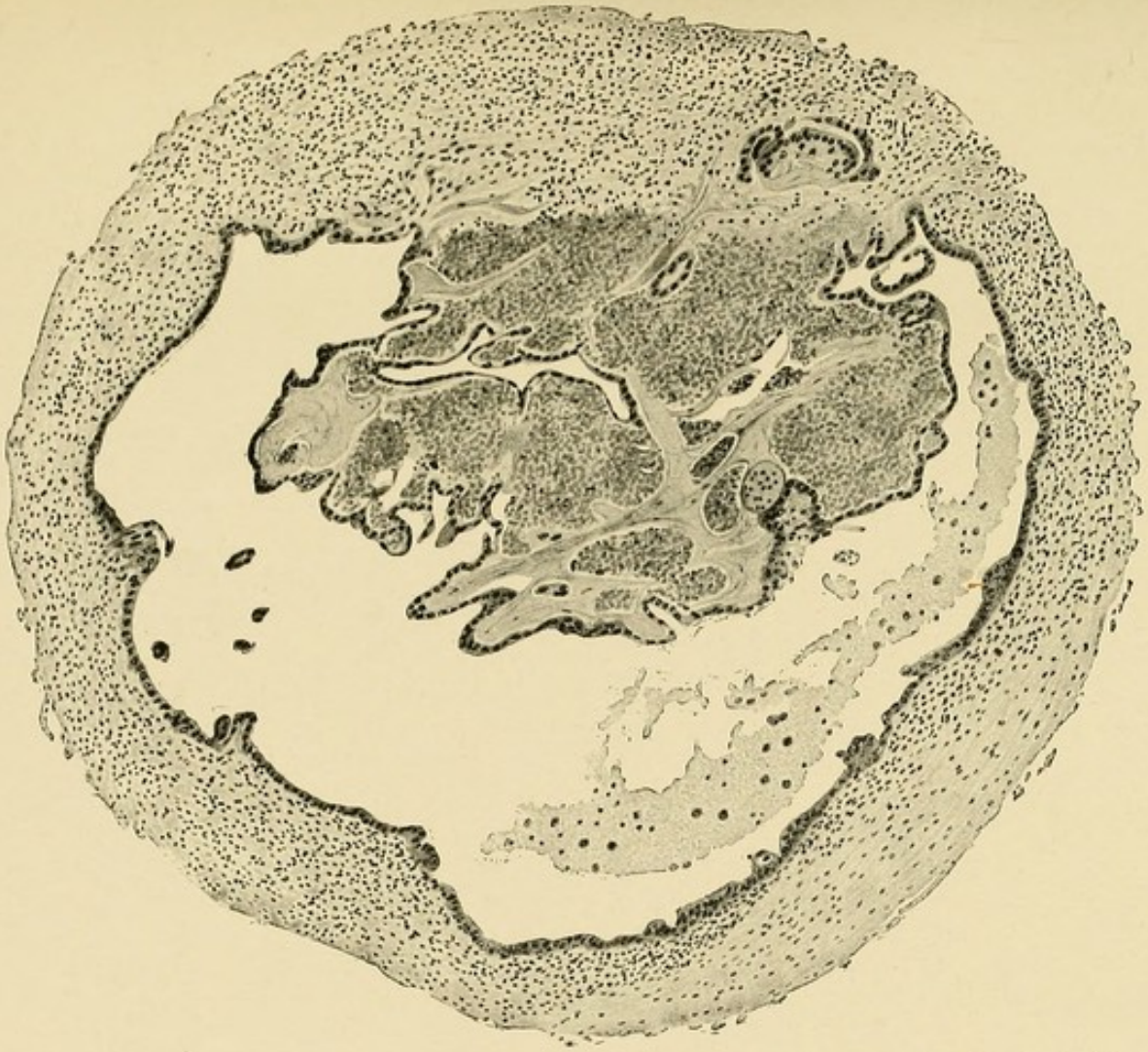
"The host tissues themselves are more cellular than the normal areolar tissue of the mouse, but this is mainly due to the presence of

[1] *Third Sci. Report, Imperial Cancer Research Fund*, London, 1908, 341.



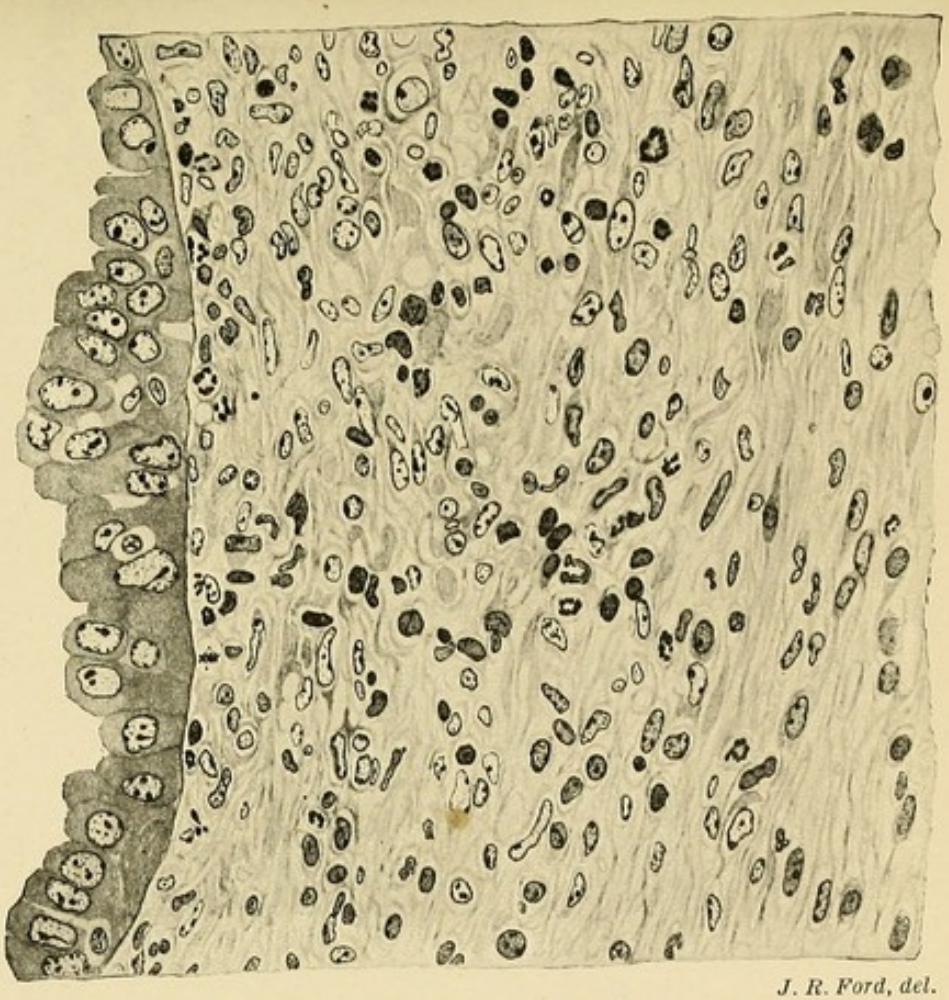
J. R. Ford del

matoxylin. $\times \frac{330}{1}$.



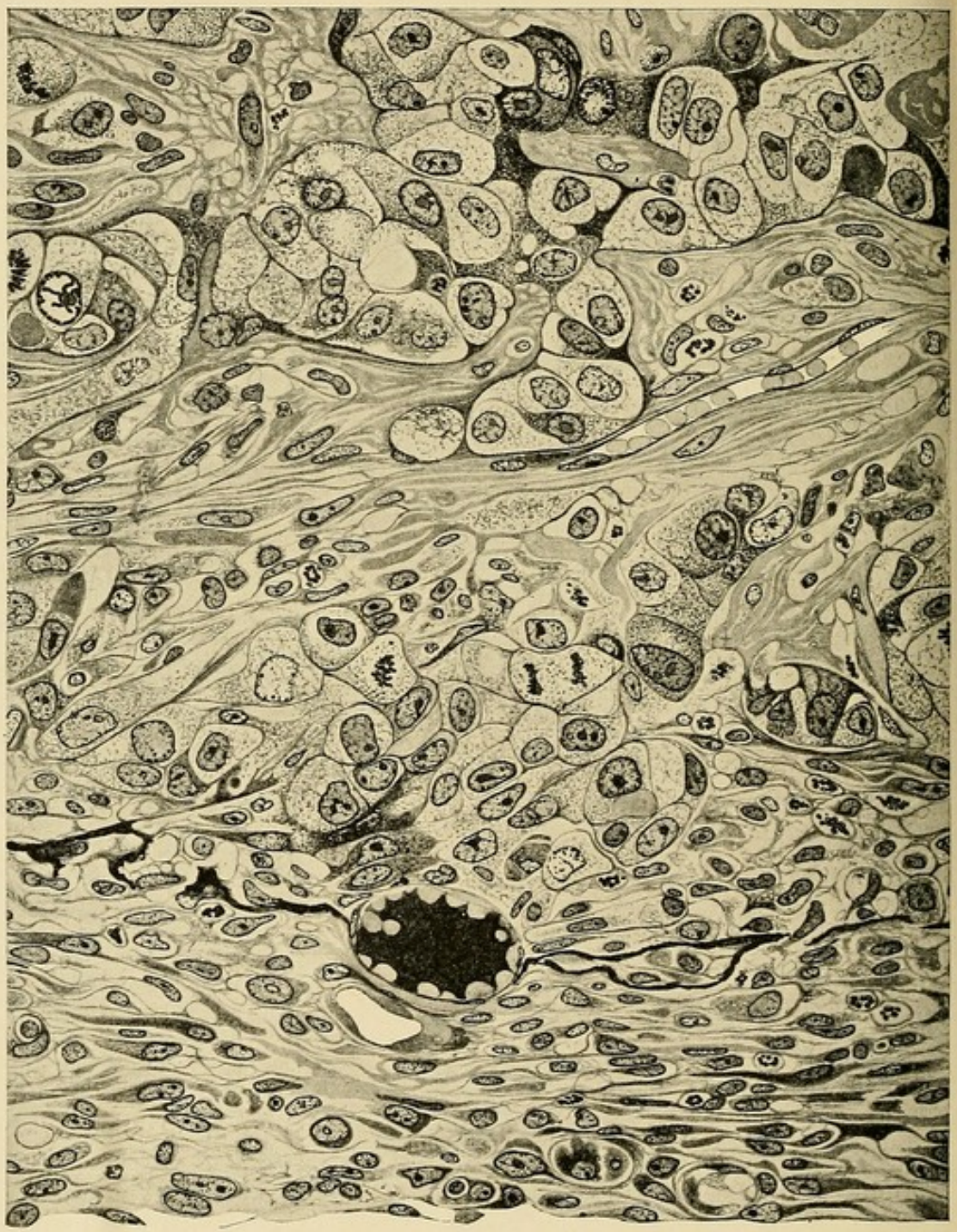
J. R. Ford, del.

Graft of the same tumor strain in an immune mouse, removed 5 days after inoculation. Osmic acid, hematoxylin. $\times \frac{60}{1}$.



J. R. Ford, del.

Part of left-hand figure under higher magnification. Right side of cyst wall adjoining host tissues, showing the sharp demarcation between the tumor cells and the surrounding connective tissue. No new formation of large fibroblasts or young capillaries. Osmic acid, hematoxylin. $\times \frac{330}{1}$.



J. R. Ford, del.

Graft of the same tumor removed from an immune rat 72 hours after implantation. Osmic acid, hematoxylin. $\times \frac{410}{1}$.

polyblast cells and polymorphonuclear leucoctyes. There is no evident increase in the vascularity of the tissues. The absorption of the necrotic mass takes place very slowly, only after about seven days do the fibroblasts and polyblasts penetrate into it in any number, and about 12 to 14 days is necessary for the whole to be cleared up."

The outstanding feature in refractory animals was thus the failure to supply a vascular stroma.

The absence of the stroma reaction in resistant animals having been demonstrated, Russell sought to explain the reason for it, and suggested that it might be ascribed to one of two factors.

". . . either the tissues of the animal have been altered in such a way by the process of immunization that they no longer react to the stimulus of the cancer cell, or else the cancer cell itself becomes robbed of its power of inciting a specific reaction."

The author was inclined to believe that the immunity was directed against the chemotactic influences exerted by the cancer cell on the connective tissues of the host.

"There must be present in the resistant animals, either in the circulating fluids or in the tissues, something which inhibits this chemotaxis," although all attempts to demonstrate *in vitro* the presence of an active cell poison had as yet been inconclusive.

"That the induced resistance to inoculation is not due to a very active cell poison may be seen from the power which the cancer cells retain, of continuing their proliferation for 7–10 days in this unfavourable medium, provided that they can obtain sufficient nourishment. Further, the cells at the periphery of the graft are those which go on growing, and these are the very cells which are most exposed to the influence of any supposed poison, whereas the cells towards the centre of the graft, which are not so exposed to a free supply of this inimical substance, are the ones which die rapidly because of the interference with their food supply."

Russell's observations were extended by Woglom[1] to include an investigation of the Flexner-Jobling adeno-carcinoma of the rat, and it was shown that here, as in the mouse, the failure of grafts to establish themselves in immune animals could be referred to an absence of the specific stroma reaction.

[1] *Fifth Sci. Report, Imperial Cancer Research Fund*, London, 1912, 43.

Burgess[1] investigated in the same way the phenomenon of natural resistance in mice, working with an adeno-carcinoma discovered by Tyzzer in a Japanese waltzing mouse. This tumor would grow in hybrid mice of the first generation obtained by cross-breeding Japanese and common mice, and in Japanese waltzing mice, but in all others, including the offspring of hybrids of the first generation, inoculation was invariably unsuccessful.

The study of early stages showed that in susceptible Japanese waltzing mice the tumor received a new fibrous and vascular stroma from the tissues of the host. In non-susceptible hybrids of the second generation stroma and blood vessels were supplied in the same way, but after about a week the graft became surrounded by an inflammatory exudate which impaired its nutrition. Apparently as a part of this reaction there occurred in many of the non-susceptible mice an overproduction of fibrils in the more centrally located portions of the new stroma, but no such inflammatory process was seen in growths of a corresponding age in Japanese mice. In the resistant animals, and probably as a result of impaired nutrition, peripheral extension of the nodule ceased and central necrosis advanced, until ultimately the entire tumor had undergone necrosis and absorption.

In the case, then, of animals belonging to very closely related varieties capable of interbreeding freely, resistance was only developed after several days of vigorous growth on the part of the graft. It was not, therefore, a result of the failure of the specific stroma reaction for, in fact, this eventually surpassed the response in susceptible mice. The active resistance of the non-susceptible animals was apparently due to an inflammatory lesion which interfered with the nutrition of the tumor.

While Russell, Woglom, and Burgess had examined more particularly the phenomena in play in the graft itself, Da Fano[2] investigated the reactions taking place in the normal tissues of the host.

As lymphocytes appeared in great number about inoculations of immunizing material during the evolution of resistance, their relation to this condition could not be denied. Furthermore, their presence

[1] *Jour. Med. Research*, 1909, N.S., xvi, 575.

[2] *Zeitschrift f. Immunitätsforsch.*, etc., Orig., 1910, v, 1.
Fifth Sci. Report, Imperial Cancer Research Fund, London, 1912, 57.

was independent of the amount of material inoculated. They were absent, or appeared only in small number after the inoculation of dead tumor, or in animals already immune. In growing carcinomata they were to be found only in places where local healing was in progress. The carcinoma cells seemed to exert some sort of specific influence on the lymphocytes, and the latter to spread the resistant state throughout the organism.

Many of the statements referring to these elements might be applied to the plasma cells, which Da Fano believed also played an important rôle in the resistant state. As they were not found normally in the subcutaneous tissue of the mouse, their presence in immune animals might be taken to mean that in the mechanism of resistance there were concerned, beside a local reaction, changes in the entire organism, of which the plasma cells were a morphological expression.

CHAPTER VI

HYPERSUSCEPTIBILITY

Not only has it become possible to affect animals in the direction of making them less susceptible to tumor growth; the occurrence of a modification in the opposite direction has been suggested by several writers — a transformation of the organism in the course of which it comes to offer a more favorable soil for the proliferation of malignant new growths.

Flexner and Jobling[1] described a series of experiments in which rats were treated intraperitoneally with an emulsion of adeno-carcinoma that had been heated for half an hour at 56° C. Grafts of the same tumor introduced subcutaneously into these rats twenty-four hours afterward grew at the same rate as the grafts implanted in controls. But when tumor was inoculated from ten to thirty days following the injection of heated emulsion, not only did the number of successful inoculations tend to exceed that in the controls, but the individual growths developed with greater rapidity, reached a size more than double that of the control tumors, and showed a far smaller percentage of retrogressions. The promoting influence was less effective at thirty days than at ten, but indications existed which seemed to show that if the preliminary treatment were repeated once or twice at ten-day intervals, the conditions favoring the growth and persistence of the tumors could be maintained, and, possibly, still further increased.

In a second article the authors[2] recorded an analysis of the phenomenon previously described. One-half of a group of rats with stationary or receding growths was inoculated with heated emulsion and ten days later with tumor, as a result of which 60 % developed growths. The other half was inoculated without an antecedent injection of heated emulsion, and in only 36 % was the outcome successful.

[1] *Proc. Soc. Exp. Biol. and Med.*, 1906-1907, iv, 156.
[2] *Proc. Soc. Exp. Biol. and Med.*, 1907-1908, v, 16.

Normal control rats yielded 100 %. In a similar experiment carried out on a second group of rats in which the growing tumor was later absorbed, only 9 % of those that had not received heated tumor emulsion were successfully inoculated, while tumors developed in 30 % of those that had been subjected to preliminary treatment.

Again, a series of rats injected with heated emulsion and successfully inoculated, the tumors having finally undergone absorption, was divided into two parts. One of these groups received a second injection of heated emulsion, the other was kept for controls. At the expiration of ten days both groups were inoculated with tumor grafts, in consequence of which 30 % of each developed growths, while 100 % occurred in the normal controls. It appeared, therefore, that no promoting effect arose from a second injection of immune rats with heated tumor emulsion. What seemed equally surprising was the high percentage of successful secondary inoculations in this group, as contrasted with the low percentage among the rats in the preceding group which had not received the emulsion — that is, 30 % against 9 %. If, however, this group were compared with the one in which, after spontaneous recovery, heated emulsion was injected for the first time and followed by new grafts, the percentage of successful re-inoculations was found to be identical in both, namely, 30 %.

The authors thought it premature to attempt a discussion of these results, which seemed to imply that by the injection of heated tumor emulsion a state of susceptibility to implantation could be preserved, while at the same time the tumor originally implanted had undergone absorption.

No promotion of growth followed previous treatment with heated and unheated emulsions of various organs, or with the sera of normal rats, of naturally resistant rats, of rats that had acquired resistance through the absorption of their tumors, or of rats with growing tumors.

Gaylord [1] repeated these experiments with a sarcoma of the rat, but was unable to demonstrate hypersensitiveness, or any definite condition, following the injection of heated tumor material.

[1] *Jour. American Med. Assoc.*, 1908, li, 252.

Bashford, Murray, and Haaland[1] had found that if a dose of spontaneous tumor not exceeding 0.1 cubic centimeter were used in an attempt to produce resistance the effect was usually not very pronounced, and that when inoculation was performed after a long interval a condition of hypersusceptibility might be shown to exist.

They had seen evidence supporting the occurrence of the same condition after the inoculation of mouse mamma[2] and skinless mouse embryo. The treatment of mice with rat mamma, six weeks before tumor implantation, had also evolved a state of hypersensibility, although where such treatment preceded the inoculation of the tumor by only sixteen days, none was apparent.

It seemed to the authors that both the time interval and the dose of normal tissue employed were of importance in determining whether protection or hypersusceptibility would supervene. Hypersensibility seemed to be a less specific phenomenon than resistance, and one that could be obtained by preliminary treatment with various substances, particularly, however, with the tissues of strange species.

Moreschi[3] observed, after two injections of lactating mouse mamma, a distinct stimulation of growth when tumor inoculation was performed from ten to twelve days after the last treatment. Preliminary injection of mice with functionating rat mamma favored proliferation in two different strains of carcinoma, when the interval between treatment and the introduction of the graft was from nine to fifteen days. But if the tumor were inoculated after from thirty to thirty-seven days the growth stimulus was repressed, to be supplanted by a distinct resistance. Lactating guinea-pig mamma did not produce hypersusceptibility.

Gay[4] found that the blood of normally insusceptible rats or of rats unsuccessfully inoculated with the Flexner-Jobling adeno-carcinoma, injected into normally insusceptible rats before tumor inoculation, or concomitantly with it, led to a larger percentage of growths.

[1] *Third Sci. Report, Imperial Cancer Research Fund*, London, 1908, 368, 376, 381.

[2] See plate facing page 144.

[3] *Zeitschrift f. Immunitätsforsch.*, etc., Orig., 1909, ii, 675.

[4] *Proc. Soc. Exp. Biol. and Med.*, 1908–1909, vi, 75.
Jour. Med. Research, 1909, N.S., xv, 186.
Boston Med. and Surg. Jour., 1909, clxi, 211.

According to Haaland,[1] an obvious hypersensibility followed the inoculation of cancerous or normal cells that had been mechanically disintegrated by freezing and grinding. That this condition was not a phase antecedent to the establishment of resistance was demonstrated by varying the time interval between preliminary treatment and tumor implantation, from which it became apparent that there was as little immunity after thirty days as after ten or twenty. A dose of 0.1 cubic centimeter of disintegrated tissue appeared to produce the optimum of hypersusceptibility. Animals could also be hypersensitized with 0.5 cubic centimeter of the expressed fluid of cancer or normal tissue, or through treatment with cells devitalized by autolysis, heat, or exposure to radium.

Leitch[2] observed hypersensibility in mice that had been treated with the supernatant fluid from an emulsion of ground mouse cancer cells in physiological saline solution. The fluid was injected into the peritoneum on three occasions at ten-day intervals, one-half a cubic centimeter at the first, and one centimeter at the last two treatments, and tumor inoculation was undertaken twenty-four days after the last injection. The amount introduced represented, in the opinion of the author, an exceedingly minute dose of epithelium.

Uhlenhuth, Haendel, and Steffenhagen[3] were of the impression that in rats previously treated with the serum of resistant rats, tumor development and growth were favorably influenced. Hypersensibility was noted, also, when tumor emulsion had been mixed before inoculation with the serum of resistant rats.

Another type of sensibility to tumor cells was described by Yamanouchi,[4] wherein mice bearing tumors were said to react immediately, and with very characteristic symptoms, to the intraperitoneal inoculation of an emulsion of the same tumor. The pathognomonic symptoms, a staring coat and a condition of immobility followed after twenty-four hours by death, occurred neither in normal mice, nor in

[1] *Proc. Roy. Soc.*, Series B, 1909–1910, lxxxii, 293.
Lancet, 1910, i, 787.
[2] *Lancet*, 1910, i, 991.
[3] *Centralbl. f. Bakt.*, etc., erste Abt., Ref., 1910, xlvii, Beiheft, 164.
Arb. a.d. Kaiserl. Gesundheitsamte, 1911, xxxvi, 490.
[4] *Compt. rend. Soc. Biol.*, 1909, lxvi, 754.

those which had been unsuccessfully inoculated. That mice in the latter category were not affected seemed to demonstrate that the condition was not an anaphylaxis (in the usual sense of the word) toward the tissue of the tumor.

Apolant [1] was unable to reproduce this phenomenon, and offered the tentative explanation that Yamanouchi's growth had been infected, and that the hypersensibility had been directed toward bacteria rather than toward tumor cells.

Similarly, the experiments of Rous[2] did not support the findings reported by Yamanouchi.

ANAPHYLAXIS

Pfeiffer and Finsterer [3] found that guinea-pigs which had received forty-eight hours previously an intraperitoneal inoculation of the serum of a carcinomatous individual, showed anaphylactic symptoms (among them a drop of several degrees in the body temperature) upon injection of the expressed juice of the patient's tumor. A similar reaction could be produced, also, in an animal sensitized with the serum of a person other than the one furnishing the tumor, so that a patient with carcinoma seemed to produce anaphylactic antibodies, not only against his own tumor, but against carcinomata in general.

Ranzi,[4] who said that he had not found the reaction specific, was criticized by Pfeiffer,[5] chiefly upon technical grounds.

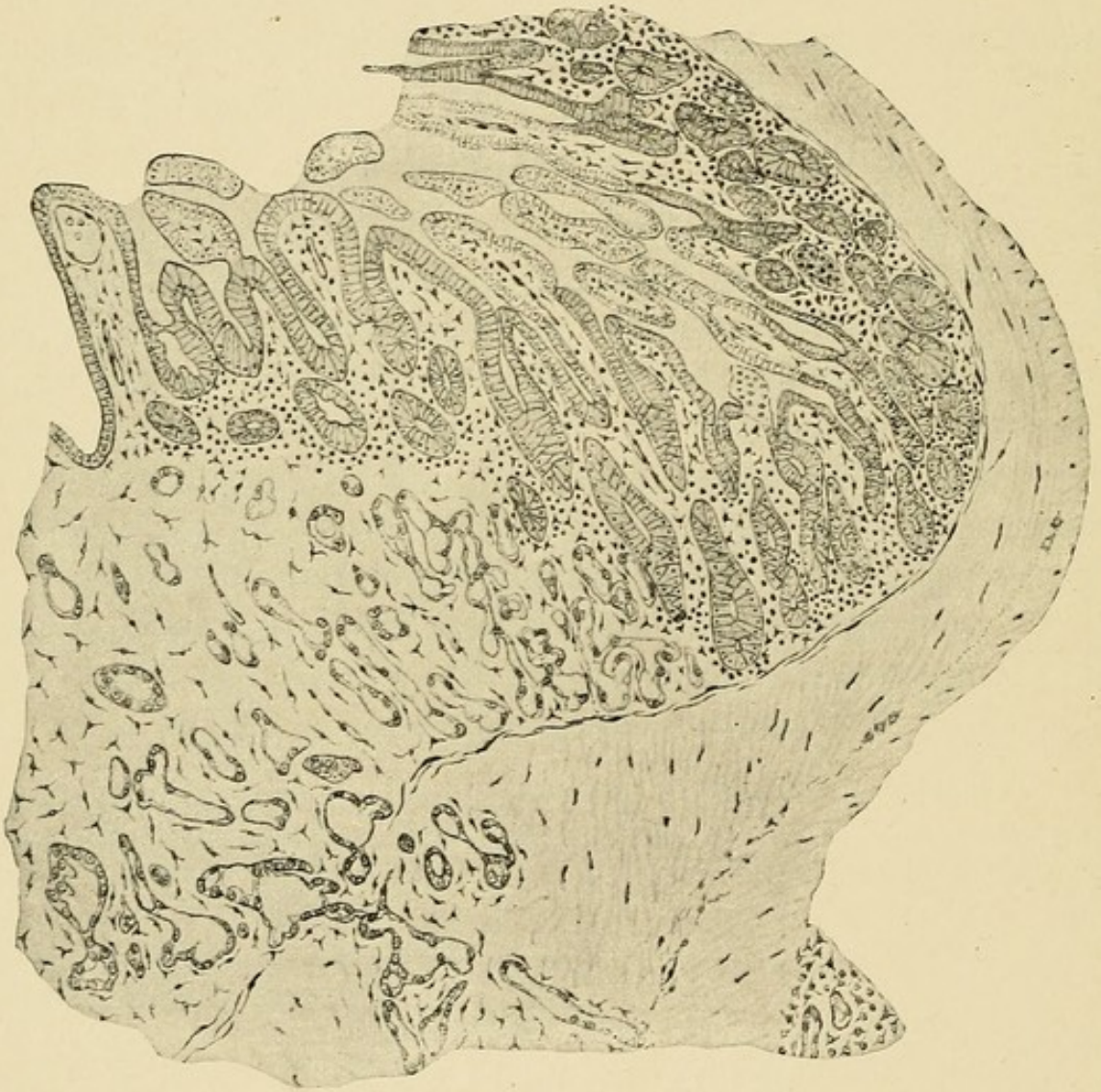
[1] *Zeitschrift f. Immunitätsforsch.*, etc., Orig., 1909, iii, 108.

[2] *Zeitschrift f. Immunitätsforsch.*, etc., Orig., 1910, iv, 238.

[3] *Wien. klin. Woch.*, 1909, xxii, 989. See also Pfeiffer, *Wien. klin. Woch.*, 1909, xxii, 1227.

[4] *Wien. klin. Woch.*, 1909, xxii, 1372.

[5] *Wien. klin. Woch.*, 1909, xxii, 1375.



Adeno-carcinoma from small intestine of the mouse. Margin of growth, showing mode of extension laterally and through muscular wall. $\times \frac{60}{1}$.

CHAPTER VII

THE SPONTANEOUS TUMOR

FREQUENCY OF TUMORS AMONG THE LOWER ANIMALS

To review fully the occurrence of spontaneous tumors among the lower animals is not within the scope of this volume. It is sufficient for the present purpose merely to state that both benign and malignant tumors are being discovered constantly in mammals, birds, am-

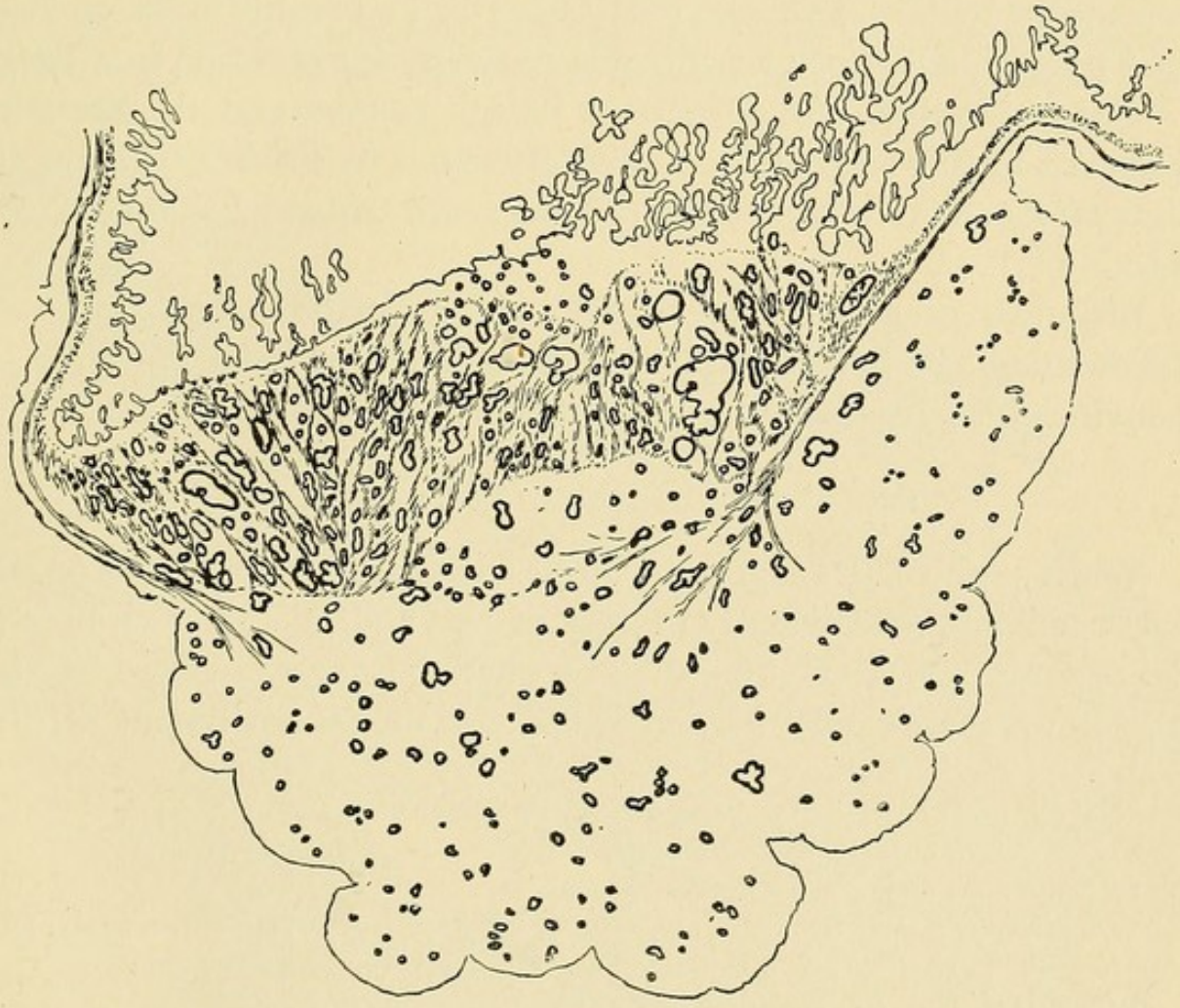


FIG. 8. — Grouse: Primary adeno-carcinoma of small intestine. Infiltrative growth in all the coats, great thickening of circular muscular layer, the surface of which at one part forms the base of the ulcer. $\times \frac{1.5}{1}$.

phibians, and fish, and that the close degree of relationship with man into which many of the subjects are thrown has not been proved in the slightest degree responsible for the development of the disorder. If malignant disease be more common among the domestic animals it is only because, as Bashford and Murray[1] have pointed out, enjoying a longer span of life by reason of man's care more of them reach the cancer-bearing age, and because they are, moreover, under more constant observation than are animals living in a state of nature.

The zoölogical distribution of cancer has been discussed by these two authors together, and by Murray alone,[2] and they have recorded, among tumors observed by themselves or others, malignant growths in the cow, dog, horse, sheep, pig, cat, hen, Indian parakeet, macaw, grouse, canary, giant salamander, frog, triton, codfish, gurnard, and trout. According to Murray, no case of malignant tumor has yet been found among the reptiles, although Pettit described a fibroma of the stomach in a python, and Koch a papilloma of the occipital region in a lizard.

The occurrence of malignant or benign tumors and the literature germane to this subject have been discussed by Sticker,[3] Ehrenreich and Michaelis,[4] Plehn,[5] Casper,[6] Tyzzer and Ordway,[7] Fiebiger,[8] and McCoy.[9]

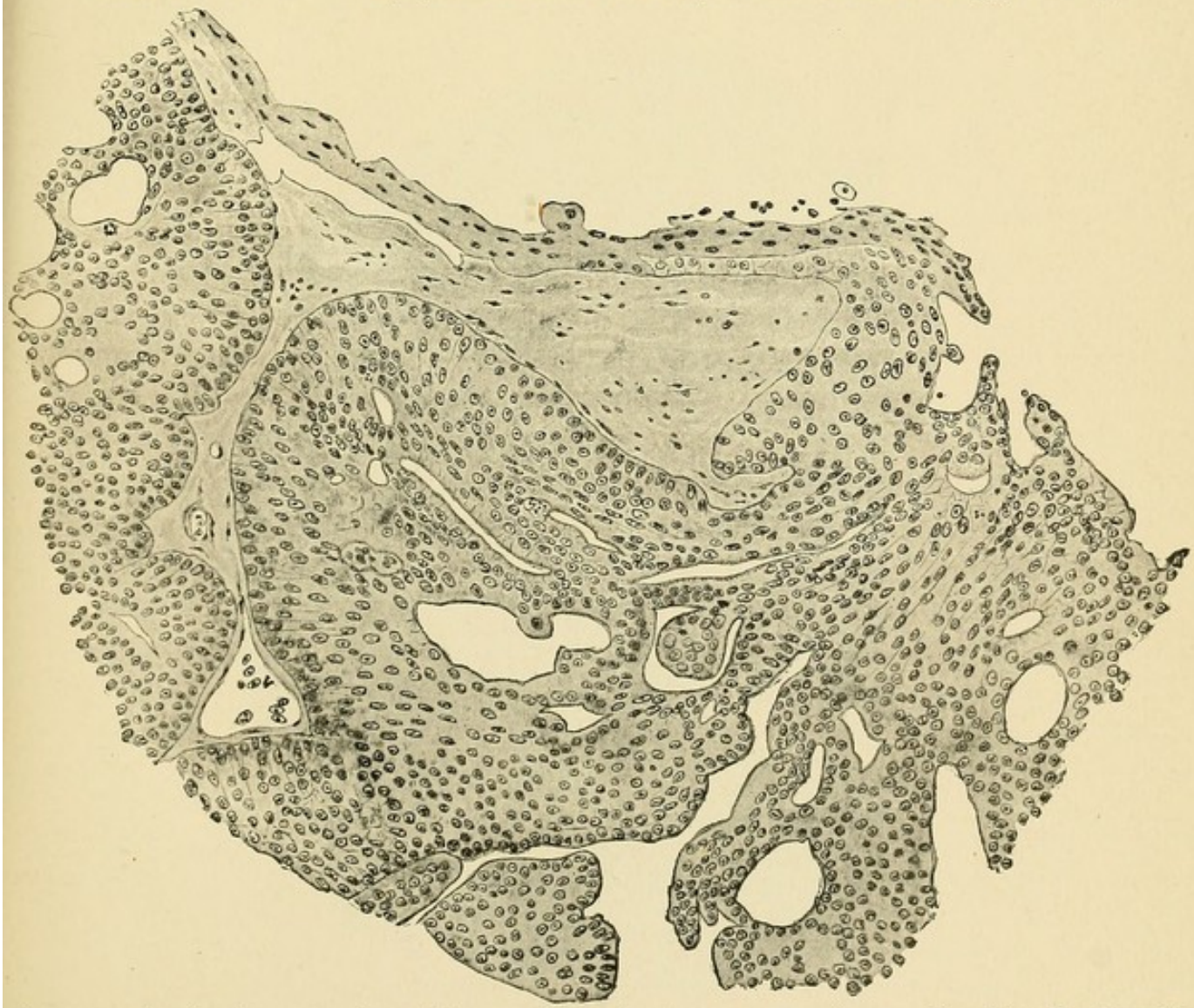
Instances of growths apparently malignant have been reported, furthermore, in the vegetable kingdom, none of them, however, so convincing as Jensen's[10] transplantable tumor of the beet.

STRUCTURE OF ORIGIN IN THE MOUSE

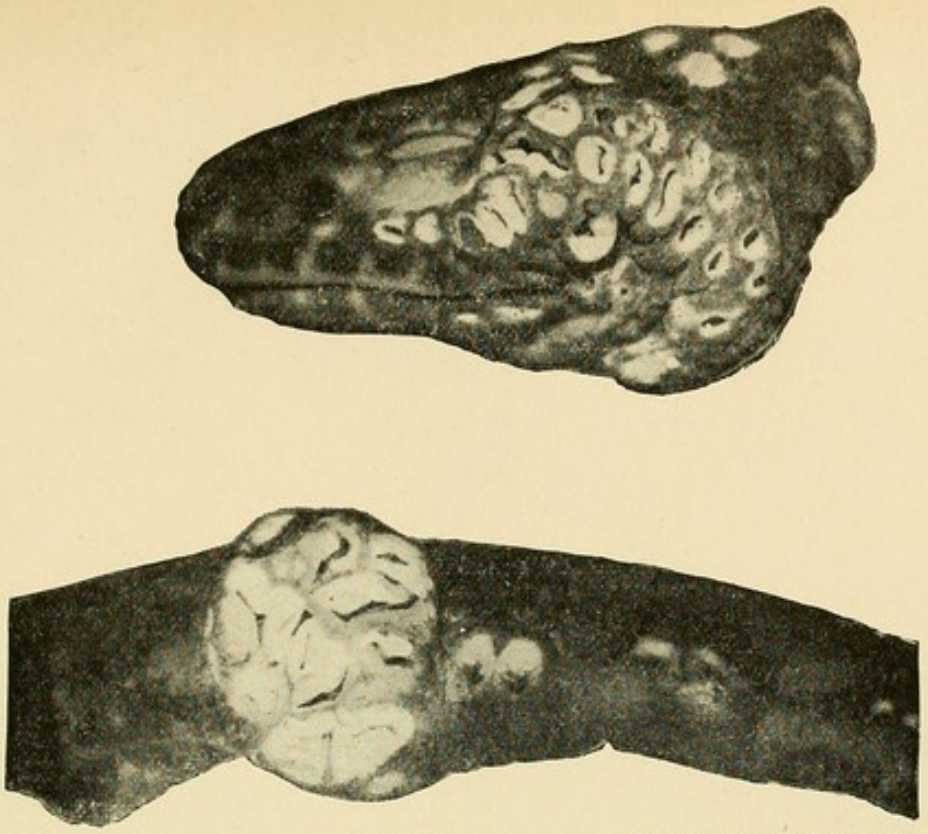
When pathologists began to interest themselves in tumors of the lower animals, preëminently in those of rats and mice, and to attempt transplantation, the first question that had to be answered was whether these growths were actually malignant. The gravest doubts of their

[1] *Sci. Reports, Cancer Research Fund*, London, 1904, No. 1, 5.
[2] *Third Sci. Report, Imperial Cancer Research Fund, London*, 1908, 41.
[3] *Arch. f. klin. Chir.*, (v. Langenbeck), 1902, lxv, 616, 1023.
[4] *Zeitschrift f. Krebsforsch.*, 1906, iv, 586. [5] *Zeitschrift f. Krebsforsch*, 1906, iv, 525.
[6] *Ergebn. d. allg. Path.*, etc., (Lubarsch and Ostertag), 1907, xi, Abt. 2, 1068.
[7] *Jour. Med. Research*, 1909, N.S., xvi, 459.
[8] *Zeitschrift f. Krebsforsch.*, 1908–1909, vii, 165, 371, 382.
[9] *Jour. Med. Research*, 1909, N.S., xvi, 285.
[10] *Travaux de la deuxième Conférence internat. pour l'Étude du Cancer*, Paris, 1911, 243.

Malignant adeno-carcinoma of skin glands from frog. The growth has penetrated beneath the dense lamellar layer of the dermis, *a*, and infiltrates the subjacent muscles of the thigh, *b*. $\times \frac{35}{1}$.

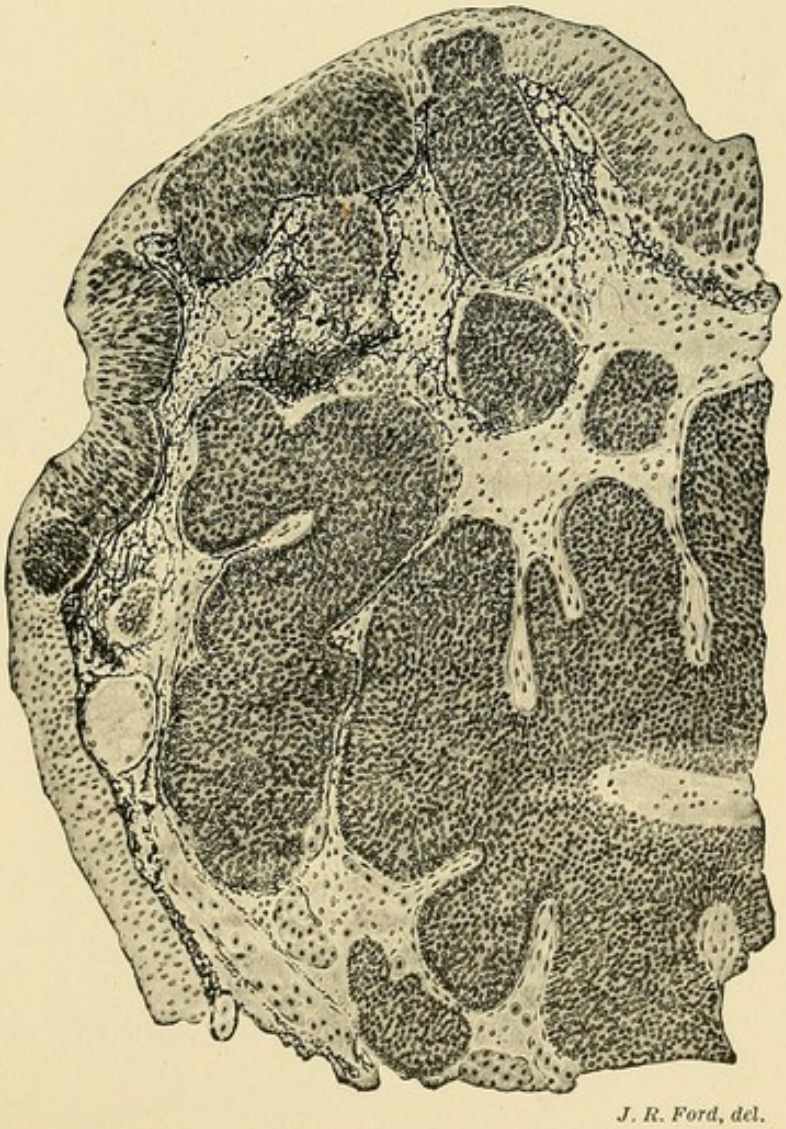


Same growth, showing connection with normal skin, the tumor apparently spreading laterally along its deep surface.



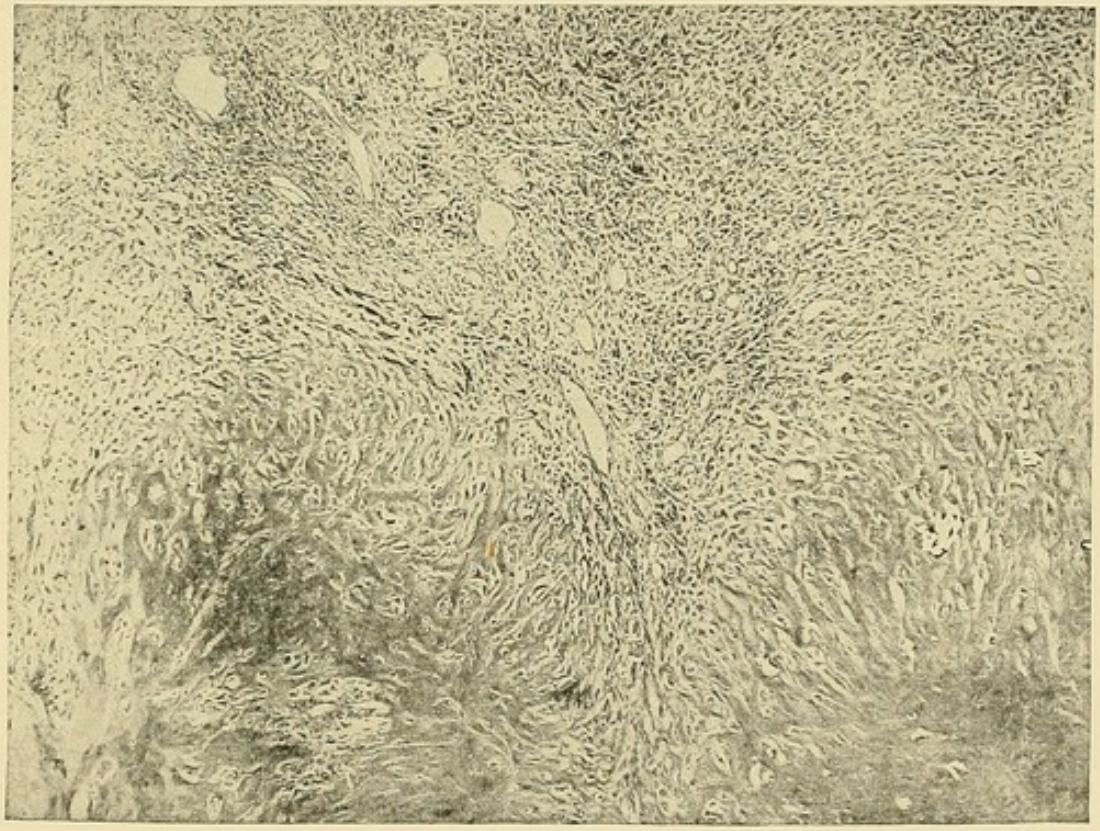
Photograph by Dr. Zarnik.

Carcinoma of skin glands from triton. Macroscopic appearance of growth at angle of mouth and scattered nodules on tail. × 5.



J. R. Ford, del.

High power view of above growth. Alveolar part of tumor and commencement of a columnar cell portion (right upper). Note how sharply the alveolar plugs are marked off from the surrounding normal skin. × $\frac{65}{1}$.



Microphotograph by R. Muir.

Osteo-sarcoma of operculum from cod. Portions of two osseous nodules are shown, with surrounding vascular spindle cell tissue. $\times \frac{60}{1}$.

true neoplastic nature existed on many sides, and the patient collection of data regarding their life history was necessary before the problem could be settled. In addition, one of the most important tasks was to determine the structure giving origin to the tumors, a question to which the answer at the beginning of the investigation was by no means certain.

Eberth and Spude [1] had described tumors in three mice and had attempted to transplant these growths, but, puzzled by the lack of apparent connection between the neoplasms and any epithelial structure, concluded that they were endotheliomata, in spite of their morphology. As Apolant [2] said later, however, if these authors had only examined a section of normal mouse mamma, they never would have mistaken ducts and alveoli for altered lymph vessels.

The ostensible want of relationship between epithelial structures and tumors seemingly of epithelial origin continued, nevertheless, to confuse many of Eberth and Spude's successors. Jensen,[3] although not certain of the source of his tumor, was inclined to derive it from the glandular structures of the skin or from the epidermis, while Pick and Poll [4] described an epithelial tumor of the scapular region in a white mouse and suggested that it had originated in the sweat glands. According to both Apolant [5] and Murray,[6] however, the sweat glands of the mouse are restricted to the soles of the feet.

v. Hansemann [7] expressed the opinion that the tumors found in mice did not proceed from the skin, nor did he consider that they were of glandular origin, and held that the hibernating gland should be considered in any attempt to account for their genesis. This gland, a structure of very extensive distribution extending from between the shoulders forward to the thymus, running thence toward the aorta and widening out on either side to reach finally the region of the kidney, was not found, however, in the neighborhood of the inguinal folds, where

[1] *Arch. f. path. Anat.*, etc., (Virchow), 1898, cliii, 60.
[2] *Arb. a. d. Königl. Inst. f. Exp. Therap.*, 1906, Heft i, 32.
[3] *Centralbl. f. Bakt.*, etc., erste Abt., Orig., 1901, xxxiv, 33.
[4] *Berl. klin. Woch.*, 1903, xl, 519.
[5] *Arb. a. d. Königl. Inst. f. Exp. Therap.*, 1906, Heft i, 40.
[6] *Third Sci. Report, Imperial Cancer Research Fund*, London, 1908, 85.
[7] *Verhandl. d. Komitees f. Krebsforsch.*, 1903–1904, iii. 38. See also *Deut. med. Woch.*, 1904, xxx, 1264.

tumors, nevertheless, not infrequently originate. He was inclined, on the whole, to believe that the growths were derived from endothelium, although he confessed his inability to advance proof of this contention.

Michaelis,[1] because of the alveolar structure of Jensen's tumor, raised the question whether it might not represent an adenomatous growth springing from a gland without lumina, and suggested the two glands of this type found in the mouse at the sides of the trachea.

The studies of Bashford and Murray,[2] and of Apolant,[3] were the first to fix definitely the tissue from which originated the great majority of the epithelial tumors of the mouse. Apolant, having noticed that nearly all tumors occurred among females on the ventral aspect of the body, was led to inquire what subcutaneous structure was limited to this surface of the animal, extended from the jaw to the region of the genitals, and was best developed in females. The mammary glands met the requirements so adequately that Apolant could not escape accepting them as the organs giving origin to the tumors. Of these glands the mouse possessed five on each side, the forward pair of which abutted directly upon the submaxillary glands, while that nearest to the caudal end of the animal was in close proximity to the vulva. Microscopic examination, furthermore, demonstrated absolutely a mammary genesis for most of the tumors, and made it extremely probable for the remainder.

Similar observations by Bashford and Murray established, in addition, a mammary origin even for tumors situated upon the back. They found that the five pair of mammary glands occupied the sides and front of the body from the neck to the anus, and, extending at the shoulders around the sides of the thorax both behind and in front of the fore-limbs, met dorsally in the middle line. At the inguinal region there was a similar extension along the crest of the ilium and over the inner aspect of the thigh. Histological proof of the mammary origin of these tumors was not wanting, for it was found that they formed a graduated series, extending from growths built up of small acini indistinguishable from those of the normal resting mamma,

[1] *Verhandl. d. Komitees f. Krebsforsch.*, 1903–1904, iii, 37. See also *Deut. med. Woch.*, 1904, xxx, 1264.

[2] *Sci. Reports, Imperial Cancer Research Fund*, London, 1905, No. 2, Part ii, 15. *Lancet*, 1907, i, 798. *Third Sci. Report, Imperial Cancer Research Fund*, London, 1908, 83.

[3] *Arb. a. d. Königl. Inst. f. Exp. Therap.*, 1906, Heft i, 17.

through others in which the acini were dilated into cystic spaces reproducing in exaggerated form the puerperal condition, to alveolar growths built on the plan of the rapidly growing mammary foundation of the new-born mouse. Tumors of other types were also encountered in the mamma — angiomata, sarcomata, and squamous cell

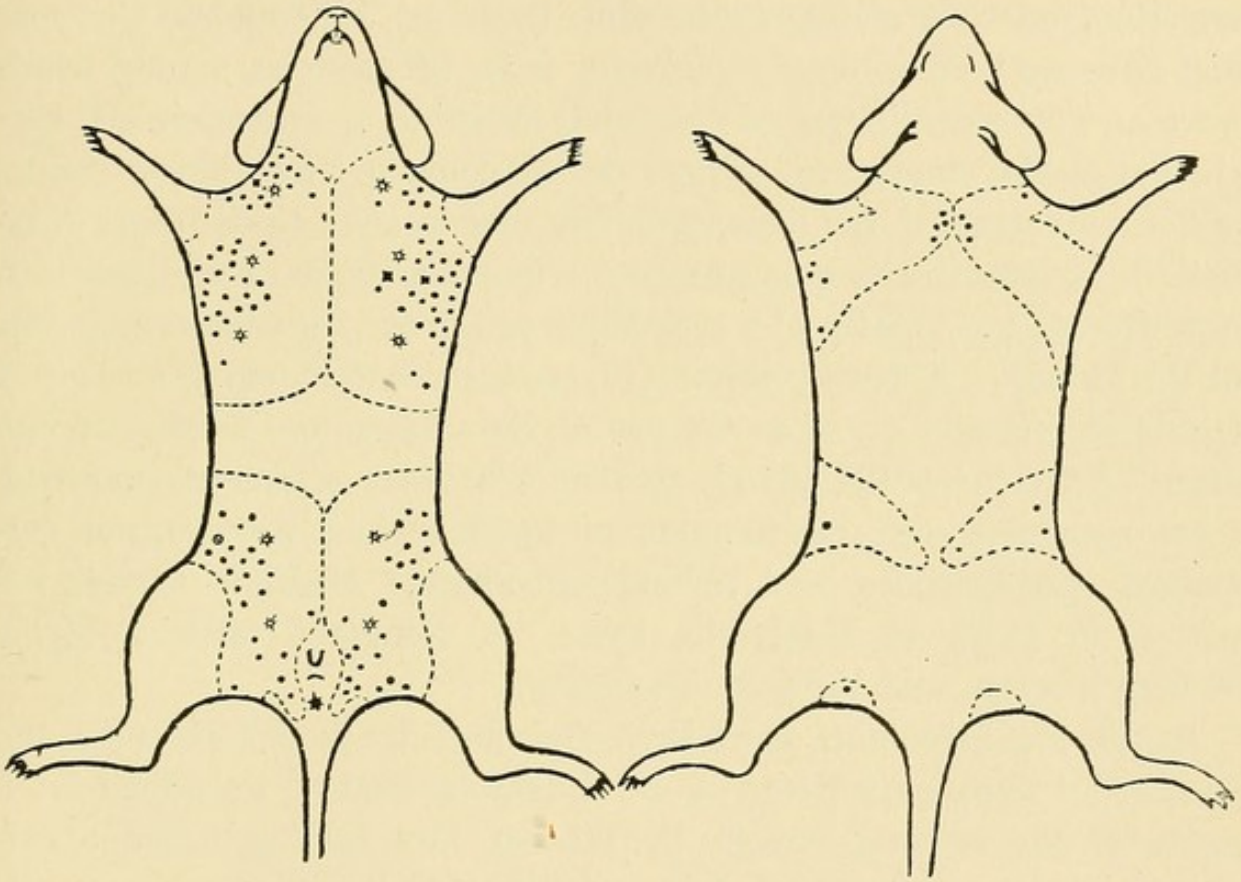


FIG. 9.— Mouse : Sites of 142 spontaneous mammary carcinomata, shown by black dots; nipples by stars.

carcinomata, and in a proportion analogous to that met with in mammary neoplasms of the human subject.

Deton,[1] having reconstructed wax models from serial sections of two spontaneous carcinomata from the mammary region in the mouse, concluded that the tumors had not originated in the mamma. Apolant,[2] however, could not accept this view, because, in his opinion, the method was not sufficiently accurate to demonstrate the relations existing between tumor and mamma.

It has been said frequently that new growths in the mouse occurred solely in the mamma, and that their analogy with malignant new growths in the human subject was materially lessened by this

[1] *Zeitschrift f. Krebsforsch.*, 1909–1910, viii, 459.
[2] *Arch. f. mikroskop. Anat.*, 1911, lxxviii, 156.

observation. That the belief was erroneous, however, will appear from the most cursory review of the literature, and it is only necessary to direct attention to a few of the recorded tumors to show that the types of neoplasia found in the mouse have been as divers as in man. Thus Borrel [1] found a squamous cell carcinoma of the floor of the mouth and Haaland [2] four similar cases, while Bashford, Murray and Cramer,[3] and Murray [4] alone, have reported a series of growths, among which were an adeno-carcinoma of the small intestine, a squamous cell carcinoma of the stomach, a spindle cell sarcoma of the kidney region, and an adenoma of the liver, probably malignant. Ehrlich and Apolant [5] have described a spontaneous carcinoma sarcomatodes, Ehrlich [6] a chondroma, and Haaland [7] a melanoma of the ear, an adeno-carcinoma of the kidney, a hypernephroma (?), an adeno-carcinoma of the ovary (probably primary), a fibro-myoma of the uterus, two adeno-carcinomata of the perputial gland, several sebaceous adeno-carcinomata, three squamous cell carcinomata of the mouth, a spontaneous carcinoma sarcomatodes, and several sarcomata. Multiple tumors occurred in 17 % of Haaland's cases, as compared with 15 % in Murray's series, and 12 % in Apolant's.

In the rat, sarcomata have been described by Loeb,[8] Herzog,[9] and Jensen,[10] a fibro-epithelioma of the tongue by Stahr,[11] an adeno-carcinoma of the seminal vesicle by Flexner and Jobling,[12] and a carcinoma of the mamma by Lewin and Michaelis.[13]

[1] Cited by Haaland, *Ann. de l'Inst. Past.*, 1905, xix, 188.

[2] *Ann. de l'Inst. Past.*, 1905, xix, 188.

[3] *Sci. Reports, Imperial Cancer Research Fund*, London, 1905, No. 2, Part ii, 47.

[4] *Third Sci. Report, Imperial Cancer Research Fund*, London, 1908, 69.

[5] *Berl. klin. Woch.*, 1907, xliv, 1399.

[6] *Arb. a. d. Königl. Inst. f. Exp. Therap.*, 1906, Heft i, 65.

[7] *Fourth Sci. Report, Imperial Cancer Research Fund*, London, 1911, 1.

[8] *Jour. Med. Research*, 1901, N.S., i, 28. [9] *Jour. Med. Research*, 1902, N.S., iii, 74.

[10] *Zeitschrift f. Krebsforsch.*, 1908–1909, vii, 45.

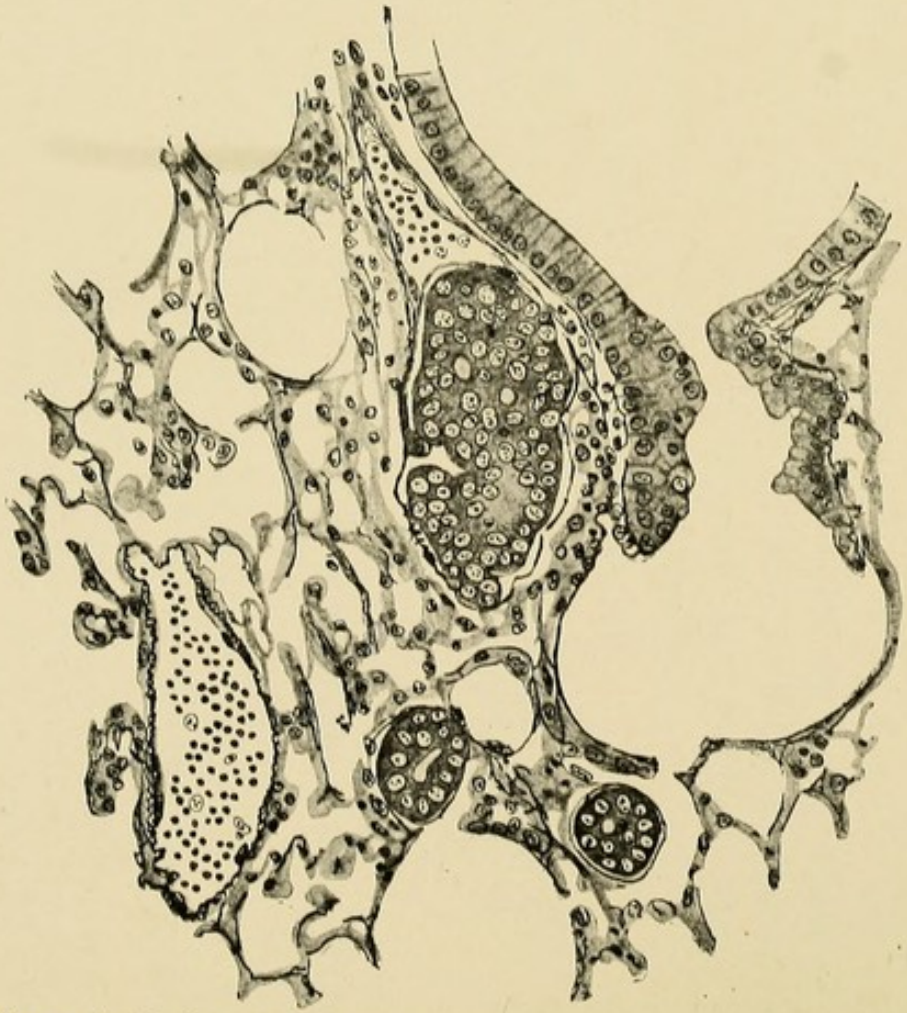
[11] *Centralbl. f. allg. Path.*, etc., 1903, xiv, 1.

[12] *Proc. Soc. Exp. Biol. and Med.*, 1906–1907, iv, 12.

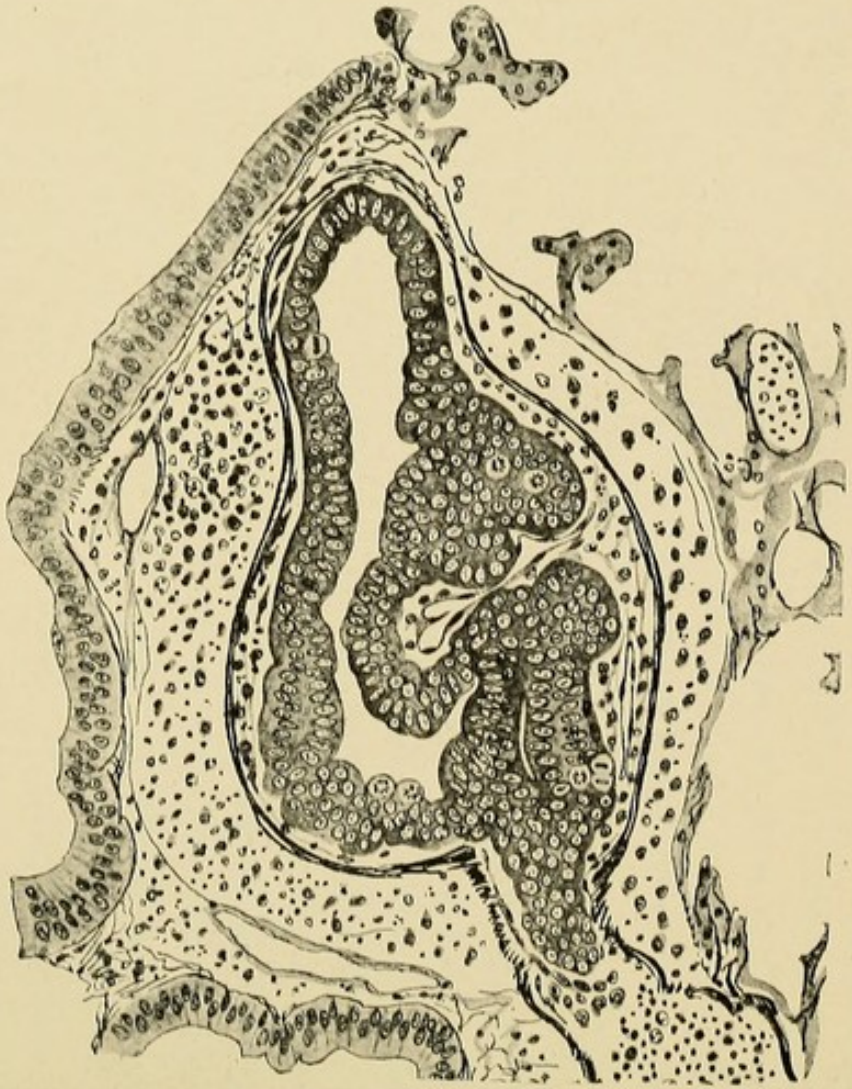
Monographs on Medical and Allied Subjects, Rockefeller Institute, New York, 1910, No. 1, p. 1.

Flexner and Jobling at first reported their tumor as a sarcoma. During continued propagation, however, it altered in character; in later articles (*Proc. Soc. Exp. Biol. and Med.*, 1908, v, 52, 91) the authors described the gradual ascendancy of epithelial elements (discovered upon reëxamination in the spontaneous tumor) and disappearance of structures previously thought to be sarcomatous. The tumor is now an adeno-carcinoma.

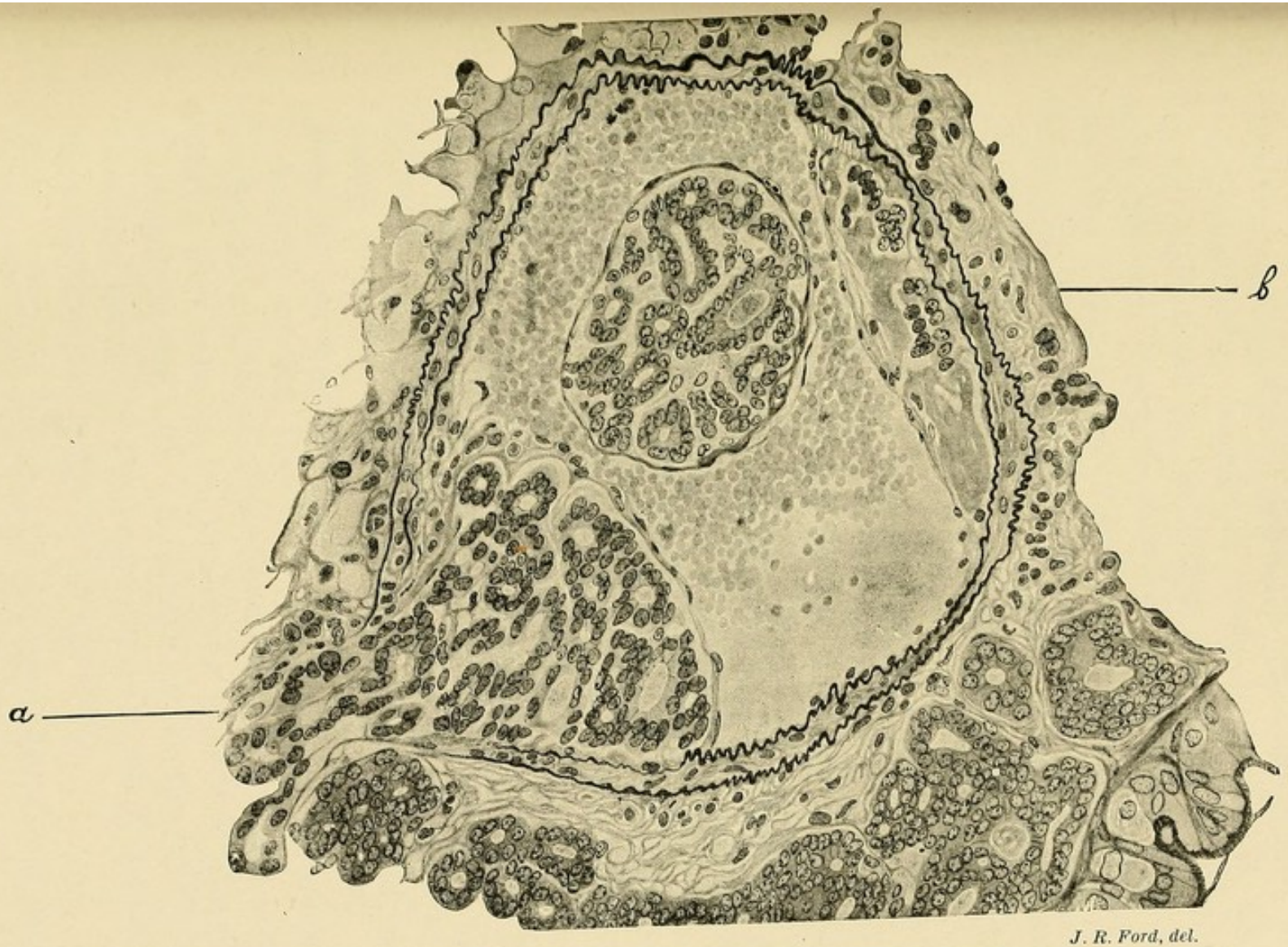
[13] *Deut. med. Woch.*, 1907, xxxiii, 657. See also Lewin, *Berl. klin. Woch.*, 1907, xliv. 1602.



Hemorrhagic alveolar mammary carcinoma of mouse. Capillary emboli distending the vessel wall and projecting into the air cells of the lung. $\times \frac{160}{1}$.



Mammary adeno-carcinoma of mouse. Embolic metastasis in pulmonary artery. The embolus, which is entirely within the internal elastic lamina, has become vascularized by capillaries from the intima of the



J. R. Ford, del.

Pulmonary metastasis of a hemorrhagic adeno-carcinoma. (*a*) healthy embolus breaking through vessel wall; (*b*) healed nodule enclosed by sclerotic hyaline connective tissue. $\times \frac{350}{1}$.

v. Hansemann,[1] while not denying the occurrence of carcinoma in the mouse and, indeed, admitting that Borrel's tumors were true carcinomata, was inclined to place Jensen's among the endotheliomata. Certainly, he[2] contended, Bashford was unwarranted in calling it a carcinoma solely because it produced metastases, for secondary deposits were found in connection with many endotheliomata. In order, however, to be convinced of the endothelial nature of these neoplasms it was necessary to examine the spontaneous tumors, for in transplanted growths the evidence was not so striking.

Lazarus-Barlow[3] concurred in the opinion that the tumors found in mice were more properly classed among the endotheliomata.

THE QUESTION OF MALIGNANCY

Occurrence of Metastases

Jensen and all others who believed in the malignancy of his tumor were very severely arraigned by Williams,[4] who asserted that they had relied too much on histological appearance and not enough on clinical observation, and ascribed to their narrow outlook the "extraordinary concatenation of blunders with which the history of the experimental study of cancer is cumbered."

Such a statement, however, scarcely does justice to the care which the investigators of experimental cancer have bestowed upon the clinical course of the disease in the mouse and rat, for one of the most eagerly prosecuted inquiries has been that relating to the presence or absence of metastasis formation and infiltrative growth.

As early as 1896, Livingood[5] discovered in the shoulder region of a white mouse, an adeno-carcinoma which not only recurred several months after removal, but produced a metastatic deposit in the lung. In another mouse, with a carcinoma on the fore-leg, there were several secondary tumors in the lung and, as in the first case, they were of the same structure as the primary growth.

[1] *Berl. klin. Woch.*, 1905, xlii, 315. [2] *Zeitschrift f. Krebsforsch.*, 1905, iii, 570.
[3] *Proc. Roy. Soc. Med.*, 1908, i, Path. Section, 171.
[4] *Trans. Path. Soc. London*, 1907, lviii, 38.
The Natural History of Cancer, New York, 1908, 187.
[5] *Johns Hopkins Hosp. Bull.*, 1896, vii, 177.

Loeb [1] found metastases in rats with transplanted tumors, and situated in regions which they could have reached only through transportation by the blood or lymph streams. He pointed out that rats bearing propagable neoplasms did not live for more than two or three months, and that it was perhaps necessary, as in man, that the growth be present during a longer period in order that secondary deposits might occur.

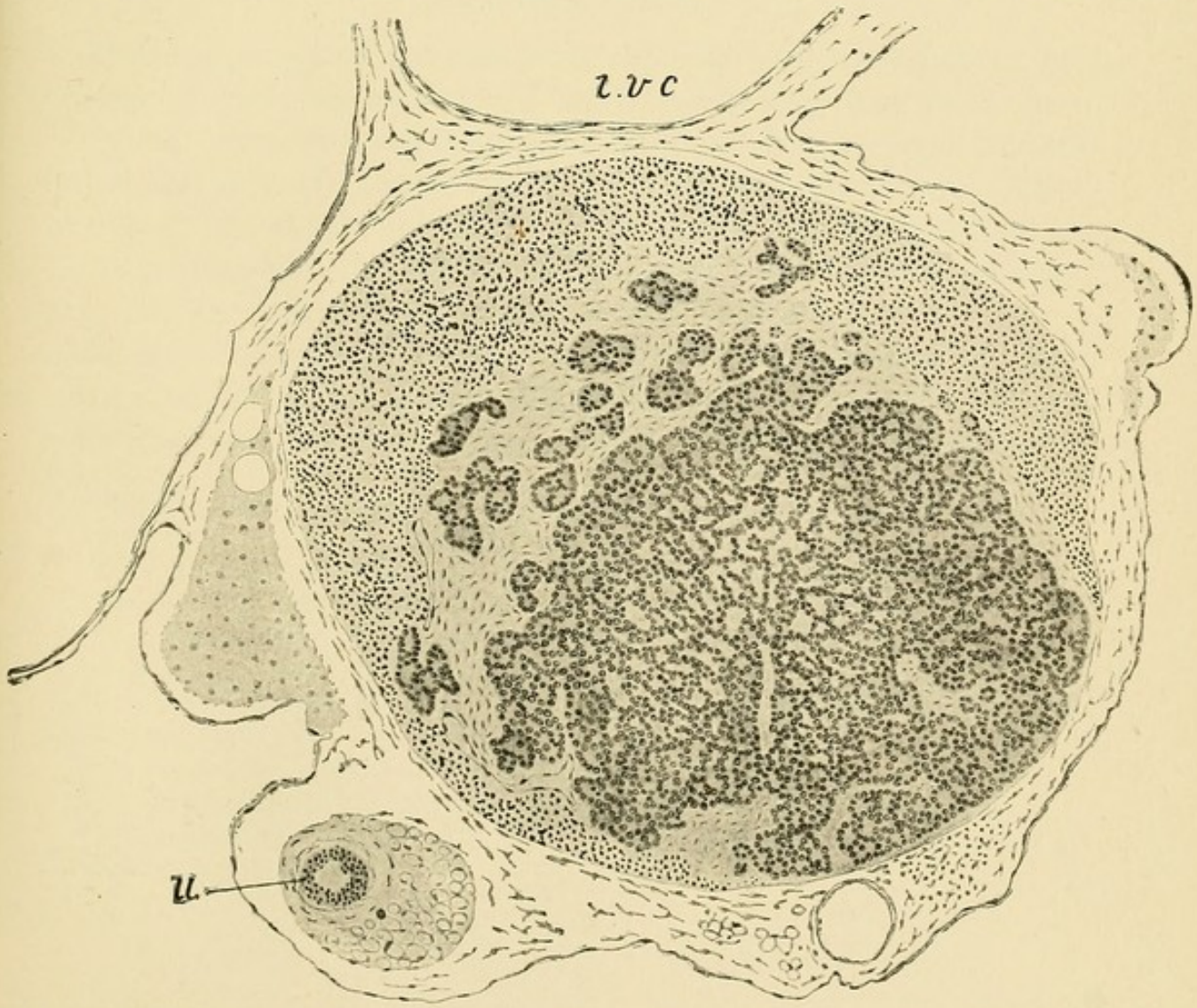
Borrel [2] discovered metastases in the lungs and the lymph nodes of mice bearing tumors, and Haaland [3] concluded, after an examination of serial sections of the organs of mice spontaneously affected with cancer and of those inoculated with the Jensen carcinoma, that metastasis by way of the blood stream was of common occurrence, at least in association with growths which were at all advanced in age. Among six cachectic mice bearing Jensen's tumor he found pulmonary nodules in five, although he was able to confirm Jensen's statement that metastases could not be detected in the lymph nodes, while among spontaneously affected mice lymphatic deposits were difficult to demonstrate, and might be considered rare. Even after a much more extensive experience with spontaneous tumors, Haaland [4] had not found the lymph nodes involved nearly so often as the lungs, in which metastatic nodules clearly visible to the naked eye were discovered in 38 % among two hundred and seventy-three cases. In other locations, however, secondary deposits were much more rare, occurring but four times in the liver, once in the kidney, once possibly in the ovary, thrice on the peritoneum, once in the retroperitoneal tissue at the site of the adrenal, once under the diaphragm, once in the posterior mediastinum, and once in the spleen. In one case the cells of a lung metastasis had grown through the vessels into the heart, where they floated free in the blood stream.

Bashford and Murray,[5] in the earliest days of their experience with the Jensen tumor, failed to discover secondary nodules, but in the following year, in collaboration with Cramer,[6] described them in the lungs of mice inoculated with this growth.

[1] *Jour. Med. Research*, 1902, N.S., iii, 48.
[2] *Ann. de l'Inst. Past.*, 1903, xvii, 114.
[3] *Ann. de l'Inst. Past.*, 1905, xix, 172, 184. *Deut. med. Woch.*, 1905, xxxi, 1239.
[4] *Fourth Sci. Report, Imperial Cancer Research Fund*, London, 1911, 54.
[5] *Sci. Reports, Cancer Research Fund*, London, 1904, No. 1, 13 (footnote).
[6] *Sci. Reports, Imperial Cancer Research Fund*, London, 1905, No. 2, Part ii, 38.

J. R. Ford, del.

Metastasis in cervical lymph node of the mouse, from a spontaneous mammary carcinoma. $\times \frac{20}{1}$.



Metastasis in aortic lymph node of the mouse, from another spontaneous mammary carcinoma. *i.v.c.*, inferior vena cava; *u*, ureter. The growth has expanded the capsule of the gland and the lymphoid tissue is reduced to a crescent. $\times \frac{20}{1}$.

Murray [1] found metastases in the lung in rather less than 50 % of sixty-eight mice with spontaneous tumors, even though these organs had not been completely investigated in all the cases, and Gierke [2] discovered secondary nodules in the same location in eight out of thirty-five cases. Lymphatic metastases were detected by Murray in three cases of his series, and by Gierke in one.

In the rat, Flexner and Jobling [3] reported a tumor which metastasized extensively throughout the bodies of animals into which it was transplanted and which, in the fifth generation, involved even the regional lymph nodes, although no secondary deposits were found in the animal primarily affected. Michaelis [4] discovered in the same species a carcinoma of the mamma which, although it had not given rise to metastases in the rat bearing the spontaneous growth, produced them after inoculation, nevertheless, in the lungs, liver, and glands (*Drüsen*) of other rats.

Infiltrative Growth

The malignancy of the tumors of mice has been impugned on the further ground that evidence of infiltrative growth had never been observed. But as early as 1904 Bashford and Murray [5] had investigated this type of extension, and had made the statement in a footnote that the malignancy of the Jensen tumor was demonstrated by its infiltrative growth, while in the following year Bashford, Murray, and Cramer [6] described the phenomenon in more detail.

Baeslack,[7] also, had discovered infiltration on the part of some tumors of the Jensen series. Michaelis,[8] on the contrary, said that infiltrative growth had never been seen; and although a year later he[9]

[1] *Third Sci. Report, Imperial Cancer Research Fund*, London, 1908, 98.

[2] *Beitr. zur path. Anat.*, etc., (Ziegler), 1908, xliii, 331.
Third Sci. Report, Imperial Cancer Research Fund, London, 1908, 118.

[3] *Proc. Soc. Exp. Biol. and Med.*, 1906–1907, iv, 12.
Jour. American Med. Assoc., 1907, xlviii, 420.
Jour. American Med. Assoc. 1908, l, 66.

[4] *Zeitschrift f. Krebsforsch.*, 1907, v, 190. See also Lewin and Michaelis, *Deut. med. Woch.*, 1907, xxxiii, 657.

[5] *Sci. Reports, Cancer Research Fund*, London, 1904, No. 1, 13.

[6] *Sci. Reports, Imperial Cancer Research Fund*, London, 1905, No. 2, Part ii, 39, 47.

[7] *Deut. med. Woch.*, 1905, xxxi, 957.

[8] *Deut. med. Woch.*, 1904, xxx, 1264.

[9] *Med. Klin.*, 1905, i, 203.

still felt that an important characteristic of malignancy was lacking while this method of extension remained undemonstrated, a brief note [1] published shortly afterward contained the affirmation that he had observed infiltration in certain stages of his tumors, and that the last distinction between human carcinoma and those found in the mouse had thus disappeared. Metastases he had been able to demonstrate, once in the lungs and once in the lymph nodes in two mice with spontaneous tumors, and in the lungs of a mouse bearing a transplantable growth.

The explanation of Apolant [2] that infiltrative growth, although it did occur in the mouse, was not so marked as in human beings because the tumors developed in loose connective tissue, where they had an abundance of room in which to grow without involving skin and musculature, was entirely in harmony with the observation of Bashford, Murray, and Cramer,[3] that the Jensen tumor forsook its expansive growth and assumed the infiltrative type, when dense tissue opposed its progress.

Bashford and Murray,[4] in a detailed account of sporadic mammary carcinoma in the mouse, described metastases in the lymph nodes and lungs, and invasion of the capsule of the tumor by strands and columns of cancer cells. The involvement of structures adjacent to the growth made complete surgical ablation possible only when both capsule and surrounding tissue were removed, the mere shelling out of the tumor from its capsule having been found inadequate. Their observations permitted the conclusion: ". . . that we are dealing with new growths of the mammary region of the mouse, which grow progressively (recur after incomplete removal), infiltrate the surrounding normal tissues, and produce metastases of the same histological type in the lungs and lymphatic glands. They lead to the death of the animal." Murray [5] found, indeed, that over one-half of the tumors recurred following attempts at total excision, after an interval of from two weeks to several months. Furthermore, he had been able to demonstrate by histological examination that the encapsulation was only apparent.

[1] *Med. Klin.*, 1905, i, 496.

[2] *Arb. a. d. Königl. Inst. f. Exp. Therap.*, 1906, Heft i, 60.

[3] *Sci. Reports, Imperial Cancer Research Fund*, London, 1905, No. 2, Part ii, 40.

[4] *Lancet*, 1907, i, 800.

[5] *Jour. Path. and Bact.*, 1908, xii, 437.

The malignancy of mouse tumors was adequately defended by Apolant[1] in a recent reply to v. Hansemann's objections[2] against comparing these growths to those of man. More recently still v. Hansemann[3] has receded from his original position, and now admits metastasis to be an occasional event attending spontaneous growths, although still denying that the tumors so widely regarded as mammary originate in the mamma.

The two main objections urged against the malignancy of mouse and rat tumors, namely, absence of metastases and of infiltrative growth, have thus been overborne by a mass of evidence gradually collected in laboratories throughout the entire world.

Altmann's Granules as a Criterion of Malignancy

The question of the malignancy of these growths was attacked from still another side by Beckton,[4] who found that Altmann's granules, although they were demonstrable in most of the varieties of normal cells and in those of inflammatory lesions and benign tumors, were partly or entirely absent from the cells of malignant growths in the human subject. In a considerable number of reputedly malignant dog tumors which this author had the opportunity to examine, Altmann's granules were found in much greater numbers than in malignant neoplasms of human origin, while, as concerned normal tissues, the granules were usually missing from the cells of stratified squamous epithelium and unstriped muscle. In the embryos of the mouse, the rat, and the chick, granules of medium or small size were discoverable in moderate numbers in the earlier periods of development, but during the later stages these and other granules might be either present, or absent as in some squamous epithelia, unstriped muscle, and the epithelium of the collecting tubules of the kidney.

On the basis of these observations the author suggested that an examination for the presence of Altmann's granules might yield a useful diagnostic criterion regarding the malignancy of a given tumor, exclud-

[1] *Berl. klin. Woch.*, 1912, xlix, 495.
Arch. Middlesex Hosp., 1909, xv, 182.
British Med. Jour., 1909, ii, 859.
British Med. Jour., 1910, ii, 1422.

[2] *Berl. klin. Woch.*, 1912, xlix, 224.

[3] *Berl. klin. Woch.*, 1913, l, 81.

[4] *Jour. Path. and Bact.*, 1909, xiii, 185.
Lancet, 1909, ii, 391.
Jour. Path. and Bact., 1910, xiv, 408.
Arch. Middlesex Hosp., 1910, xix, 103, 111, 115.

ing, of course, such growths as had arisen from tissues that did not normally contain the granules, and having regard to the anomalous position occupied by ovarian and thyroid tumors. Turning his attention to tumors of the mouse, Beckton examined twenty-one unselected examples of propagable sarcomata and carcinomata, two-thirds of which afforded definite evidence of malignancy in the absence of granules from their cells, while in the remaining third the granules were present. The latter group, however, Beckton hesitated to stamp as non-malignant because of the present uncertain state of available knowledge on the subject. While, then, Altmann's granules were found on the whole in decidedly greater numbers than in malignant new growths of human origin, still, in about two-thirds of the cases the mouse tumors were as devoid of granules as were human new growths of undoubted malignancy.

Later papers by Colwell and Beckton,[1] Beckton and Colwell,[2] Beckton and Russ,[3] and Miller,[4] dealt with various technical matters in relation to the granules under discussion.

The experience of Beckton was controverted by Bensley,[5] who believed that the cells of malignant tumors in man did contain Altmann's granules, and even in greater number than they could be detected in the tissues from which the tumors arose.

A disagreement as to the granule content of tumor cells is, however, not a novelty, for while Raum [6] believed that he had demonstrated the presence of Altmann's granules in the cells of both carcinomata and sarcomata, Burkhardt,[7] on the other hand, was convinced that the properties of the cancer cell were altered in such a way as to destroy the granules.

In any case, it is doubtful whether these bodies have any significant relation to the biological qualities of a tumor.

[1] *Arch. Middlesex Hosp.*, 1911, xxiii, 41.
[2] *Arch. Middlesex Hosp.*, 1911, xxiii, 52.
[3] *Arch. Middlesex Hosp.*, 1911, xxiii, 99.
[4] *Arch. Middlesex Hosp.*, 1911, xxiii, 106.
[5] *Trans. Chicago Path. Soc.*, 1910, viii, 78.
[6] *Arch. f. mikroskop. Anat.*, 1892, xxxix, 137.
[7] *Arch. f. klin. Chir.*, (v. Langenbeck), 1902, lxv, 135.

ÆTIOLOGY

Influence of Infectivity

As the investigation of mouse tumors became more widespread, instances of apparent contagion through the medium of "cancer cages" began to appear in the literature. Thus Borrel [1] recorded a breeding establishment in which three cases of cancer had occurred in a single month. Upon making a visit to this establishment he was informed that in two years there had been found, in the same cage, more than twenty tumor mice, an enormous percentage compared with the number of animals kept on hand. The dealer had been in the habit of selling the young mice, and the growths had been observed solely in the older ones retained for breeding purposes. Borrel offered as further evidence of infection the instance of a cage in which five or six cancers had arisen during one year, although in other establishments, which furnished many hundreds of mice yearly, no case had ever been discovered. All these observations pled for the existence of a cancerous virus, a conception which Borrel [2] has consistently upheld in subsequent monographs. He has always insisted that transplanted tumors represented only the second stage in the development of cancer — that of malignant growth. Because in spontaneous neoplasms this secondary period was preceded by a transformation of normal cells into malignant elements, it was to the earlier stage that research should be devoted, and especial attention had accordingly been bestowed upon the youngest tumors in the study of his own material.

Cysts were not uncommonly found among mice in the groin or axilla, both of which were sites of election for the biting insects. In these cysts, or in the connective and glandular tissue surrounding them, Borrel could frequently discover *Cestodes* or *Nematodes*, and these helminths seemed to be without doubt a causative factor in the production of the lesions. In the connective tissue, muscles, or capillaries adjoining young adeno-carcinomata, he had also succeeded in demonstrating nematodes, and in a minute tumor had found the

[1] *Ann. de l'Inst. Past.*, 1903, xvii, 113.

[2] *Bull. de l'Inst. Past.*, 1907, v, 497.
Travaux de la deuxième Conférence internat. pour l'Étude du Cancer, Paris, 1911, 193.

trail of such an organism, with the parasite lying at the end of it. Similar worms had been discovered, moreover, in the lung and the lymph nodes of its hilum, as well as in the circulation of mice suffering from generalized lymphomata, but he had not yet thoroughly examined normal mice for their presence. While the temptation was great to consider the nematodes, introduced by biting insects, as the carriers of a virus, their relation to malignant growth could be no more than suggested.

It was possible, however, to express greater certainty in the case of sarcoma of the liver in rats. Four years ago he had reported a rat cancer propagable through three generations, and had attributed the growth to the cysticercus of *Taenia crassicola*, while in another case there had been discovered a tiny adeno-carcinoma of the kidney developing about a cysticercus. Similar cases have been reported by Regaud,[1] Saul,[2] Bridré,[3] and by Bridré and Conseil.[4]

But not only were endoparasites found in connection with malignant growths. Certain ectoparasites, as the *Acaridae*, might be at fault, and in the mouse, for example, there was recognized a cutaneous affection which was distinguished by warty excrescences upon various parts of the body. In the early stages of this disease, an adenomatous condition of the sebaceous glands, there could be discovered a still unidentified mite. These organisms, furthermore, played an important rôle in the lympho-sarcoma of dogs,[5] and one of them, the *Demodex*, was connected with cancer of the face in man. While an infection with such insects was frequently present in the nipples of cancerous women, Borrel did not wish to draw any conclusions regarding the relation of the parasite to cancer of the breast, and pointed out that the great majority of healthy persons would present the demodex in certain portions of the skin — perhaps hundreds to the square centimeter. Various authors had wrongly ascribed to him

[1] *Compt. rend. Soc. Biol.*, 1907, lxii, 194.
[2] *Centralbl. f. Bakt.*, etc., erste Abt., Orig., 1909, xlix, 80.
Berl. klin. Woch., 1911, xlviii, 341.
Deut. med. Woch., 1911, xxxvii, 233.
[3] *Compt. rend. Soc. Biol.*, 1909, lxvi, 376.
[4] *Bull. de l'Assoc. franç. pour l'Étude du Cancer*, 1909, ii, 171.
Bull de l'Assoc. franç. pour l'Étude du Cancer, 1910, iii, 318.
[5] *Compt. rend. de l'Acad. des Sc.*, 1907, cxliv, 344.

the hypothesis that such organisms as these were the parasites of cancer, although he actually believed that they acted only as carriers of a virus. Because a nail occasionally produced tetanus, one would never think of describing it as the parasite of that disease; it was merely that the bacillus causing tetanus was able to develop after having been carried into the tissues by the foreign body.

Attention was directed by Loeb and Jobson[1] to a ranch in Wyoming, upon which there had been discovered one or two cases of carcinoma of the inner canthus of the eye every year for the past ten years, among two thousand head of cattle. This incidence was fifty times greater than the average, and the similarity of the tumors in respect of character and location was remarkable. The animals on the neighboring ranches were free from carcinoma.

Michaelis[2] mentioned a dealer who had found five tumors arising successively among the mice of a single cage. The last two of these growths were examined, and proved to be malignant adenomata.

Gaylord and Clowes[3] reported that in the spring of 1902 there were brought to the State Cancer Laboratory in Buffalo a number of rats inoculated with a cystic sarcoma of the thyroid, and that for the accommodation of these animals two large cages and a number of smaller ones were constructed. For a certain period of time these cages contained numbers of successfully inoculated rats, but from December, 1902, until the summer of 1903, they were empty. During and after the summer of 1903 the large cages were again put to use, and a year later one of the rats kept in them developed a fibro-sarcoma. Eight others were accordingly placed in this cage, while into the second one there were put six or eight more. Four months later the two surviving males in the first cage had developed tumors, one of which was a cystic spindle cell sarcoma of the thyroid, the other a fibro-sarcoma. No tumors appeared either among the inmates of the control cage or of the other cages in the laboratory although there were no less than one hundred rats on hand, nor did any develop among the animals in the smaller cages, which had been sterilized.

[1] *Jour. Comparative Med. and Vet. Archiv.*, 1900, xxi, 388. See also Loeb, *Arch. f. klin. Chir.*, (v. Langenbeck), 1903, lxx, 845, and *Centralbl. f. Bakt.*, etc., erste Abt., Orig., 1904, xxxvii, 236.

[2] *Zeitschrift f. Krebsforsch.*, 1906, iv, 2. [3] *Jour. American Med. Assoc.*, 1907, xlviii, 15.

A second observation by the same authors related to the endemic occurrence of cancer among mice. There was discovered in the possession of a dealer a cage in which about sixty spontaneous tumors had occurred in the course of three years. The location of this cage had been changed frequently and the stock had been entirely removed on at least one occasion. The dealer was of the impression that most of the affected mice had been females, and added that he had never seen a tumor on the back of a mouse.

Balancing these, and many similar observations, stands the following quotation from Bashford[1]: "... alleged epidemics have often been recorded in ... mice and rats housed together in small cages. Satisfactory proof that these aggregations of cases were due to infection has not been furnished, and the alternative explanation, that they arise as the result of in-breeding cancerous stock, has naturally suggested itself. Our very detailed observations on tens of thousands of mice have not revealed in our laboratory anything which we would call an epidemic. When, however, we take into consideration the manner in which cases of carcinoma mammæ have been sent to us by breeders we find the same kind of evidence as that which has led observers in France, America, and Germany to assert that epidemics of cancer occur in breeding establishments. We may illustrate this kind of evidence by the numbers of tumour-mice sent in by four of the breeders who supply us with mice, under a guarantee that no fresh stock has been introduced. From January 1, 1906, to October 31, 1907, Mr. A sent us ten cases, Mr. B, six cases, Mr. C, thirty-five cases, and Mr. D, eighteen cases of carcinoma of the mamma. These figures, which are more remarkable than any others yet published, are no evidence that there was an endemic or epidemic occurrence of cancer in the breeding-cages of Mr. C or Mr. D. The proportions of mice supplied to us in the same period to cases of cancer were as follows: —

	Mice with Tumour	Total Mice
"Mr. A	10	1,302
Mr. B	6	1,547
Mr. C	35	9,698
Mr. D	18	11,842

[1] *Proc. Roy. Soc. Med.*, 1909, ii, General Reports, 72.

"The numbers of tumours occurring in these stocks of mice have been determined solely by the number of mice of 'cancer age' under observation. This is brought out particularly clearly in the difference between the age constitution of the stock of Mr. C and Mr. D, since the stock of the latter contains constantly a much higher proportion of young animals, and he supplies us with most of our young mice. Further, if we note the dates on which tumours are sent to us and arrange them in columns, we find that the crops of tumours coincide with the ageing of groups of mice. Thus those apparent aggregations of cases, wrongly called epidemics by too enthusiastic advocates of a parasitic origin for cancer, also give no indication of haphazard in-breeding leading to a preponderance of cases of cancer of the mamma. The incidence of the disease for mice continues to obey the laws of age- and sex-distribution, even where in-breeding is proceeding haphazard."

Influence of Age and Sex

Most investigators are agreed that spontaneous malignant tumors are much more common in female than in male mice, that the most frequent site is the mammary gland, that the tumors are nearly always of epithelial origin, and that, as in man, age plays an important part in preparing the animals for their inception.

Malignant growths are found in the mouse with comparative frequency if they are sought, and whereas in 1905 Bashford, Murray, and Cramer[1] had been able to discover but twelve among nearly thirty thousand tame mice of all ages, Murray,[2] six years later, recorded 8.6 % in two hundred and twenty-three females of non-cancerous ancestry, and of all ages from six months upward.

Apolant,[3] among two hundred and twenty-one tumor mice (two hundred and seven white and fourteen gray), found two hundred and seventy-six growths, thirty-eight of the mice having from two to five each, while in confirmation of observations previously published with Ehrlich[4] not a single case had occurred in a male. Among these two

[1] *Sci. Reports, Imperial Cancer Research Fund*, London, 1905, No. 2, Part ii, 15.

[2] *Proc. Roy. Soc.*, Series B, 1911–1912, lxxxiv, 42.
Fourth Sci. Report, Imperial Cancer Research Fund, London, 1911, 129.

[3] *Arb. a. d. Königl. Inst. f. Exp. Therap.*, 1906, Heft i, 15.

[4] *Berl. klin. Woch.*, 1905, xlii, 872.

hundred and seventy-six tumors, two hundred and thirty-three of which were examined and found to be epithelial, two hundred and fifty-nine were on or near the ventral surface of the body.

Among two hundred and eighty-eight tumor mice, according to Haaland,[1] only six were males, and of the three hundred and fifty-three tumors detected in these mice, three hundred and eleven presented the structure of mammary adeno-carcinoma.

Influence of Lactation

Apolant[2] was struck by the fact that tumors in the mouse were found most often in an organ of very vigorous functional energy. That, in contrast to man, a single organ should be so uniformly affected, he thought might be partly explained by the injuries inseparable from physiological activity. This interpretation has been discounted considerably, however, by the later observations of Haaland.[3]

"Of 74 mice which had developed mammary tumours, and which were under observation from birth, only 33 are recorded to have littered previously to the development of the tumour, while no litter is recorded for 41. . . . Of the 33 having littered previously to the development of the tumour: —

" 1 litter	is recorded	in 11	cases
2 litters	are "	7	"
3 "	"	7	"
4 "	"	5	"
5 "	"	2	"
7 "	"	1	case.

"The figures show that it is hardly likely that excessive physiological demands made upon the mamma play the determining rôle in the development of tumours of this organ, but that other factors must be looked for."

Influence of Heredity

One aspect of the investigation of cancer to which the mouse lends itself most readily is that concerning the relation of heredity to the

[1] *Fourth Sci. Report, Imperial Cancer Research Fund*, London, 1911, 9.
[2] *Arb. a. d. Königl. Inst. f. Exp. Therap.*, 1906, Heft i, 40.
[3] *Fourth Sci. Report, Imperial Cancer Research Fund*, London, 1911, 43.

occurrence of spontaneous tumors, and this because mice rarely live to be over two years old. The difficulty of settling the question in man is well illustrated by a paragraph from Newsholme :[1] —

"The fact that several members in successive generations of a given family have died from cancer is commonly accepted as proof that the disease is hereditary. This is far from being the case. Cancer causes a certain average number of deaths among a given number of persons. . . . Given a certain probability of death from cancer, and knowing the number of a family, it is easy to calculate the probability of one, two, or more of them dying of cancer, quite independently of heredity. Even if heredity were proved to be absolutely inoperative, it is certain that there would be families among whom numerous deaths from cancer would occur. It does not prove heredity to show that in one family five deaths, say, occurred from cancer. This might happen from mere chance, and in fact, such cases must occur without heredity at all. De Morgan worked out the probability of 1000 successive heads being thrown in tossing a coin, and he showed that given a sufficient number of people starting to toss coins, it was a certainty that at least one of them would toss 1000 consecutive heads. So, given a sufficient number of families, it is a certainty, even if there be no such thing as heredity, that of at least one family, say ten members will die of cancer. The only absolute proof of heredity would be to show that cancer occurred frequently in certain families, and practically nowhere else ; short of this the probability of heredity of cancer would be increased if it could be shown that cancer was much more common in certain families than in the average for the whole community, due allowance being made for variations in age and sex-distribution."

The suitability of the short-lived domestic animals for the investigation of problems in heredity was indicated by Bashford,[2] who reported later [3] that after three years of failure he had been able to obtain offspring from mice with spontaneous cancer, and expressed the hope that data relating to the existence of hereditary susceptibility might be obtained in time. In the following year [4] breeding experiments were well under way, and by crossing spontaneously affected animals

[1] *The Elements of Vital Statistics*, London, 1899, 248. [2] *British Med. Jour.*, 1903, ii, 128.
[3] *British Med. Jour.*, 1906, ii, 207. [4] *British Med. Jour.*, 1907, ii, 27.

with the offspring of cancerous parents, strains of mice were being obtained in which the cancerous heredity was one-half, three-fourths, fifteen-sixteenths, or even higher.

A preliminary account of these experiments was given by Bashford and Murray,[1] and a completer analysis, founded on more extended observation, by Murray.[2] The following table, abbreviated from Murray's, shows the ratio of deaths from cancer to deaths from all causes, among five hundred and sixty-three[3] mice of a highly inbred stock with a heredity more or less cancerous, the figures referring to females only. The table " . . . shows a rapidly increasing proportion of deaths from cancer commencing after six months is passed, and attaining a maximum in the three-monthly period ending at 18 months. In the succeeding periods the frequency diminishes, till in mice over 24 months old the frequency is barely twice that found in mice under 9 months old. Similar figures for the human female give a corresponding curve."

TABLE I (*after Murray*)

Age (months) . .	0–3	–6	–9	–12	–15	–18	–21	–24	Over 24	Total
Total mice . . .	—	—	100	104	88	94	69	55	53	563
Percentage of mammary cancer	—	—	**5.0**	**10.6**	**18.2**	**28.0**	**14.5**	**14.0**	**9.4**	**14.4**

The five hundred and sixty-three mice were then distributed into two groups. The first contained three hundred and forty mice of recent cancerous ancestry, of which the mother, one or both grandmothers, or all three had been cancerous. To the second were assigned two hundred and twenty-three mice of remote cancerous ancestry — mice, that is, where no cancer had occurred in

[1] *Proc. Roy. Soc.*, Series B, 1909, lxxxi, 310.

[2] *Proc. Roy. Soc.*, Series B, 1911–1912, lxxxiv, 42.
Fourth Sci. Report, Imperial Cancer Research Fund, London, 1911, 114.

[3] Through a clerical error the total was given in Murray's paper as five hundred and sixty-two.

either the mothers or the grandmothers. The majority of the cases of cancer occurred in the members of the first division, as may be seen from tables II and III (abbreviations of Murray's) which show the percentage of cases occurring in the first and second groups respectively.

TABLE II (*after Murray*)

Age (months) . .	0–3	–6	–9	–12	–15	–18	–21	–24	Over 24	Total
Total mice . . .	—	—	62	63	62	56	40	29	28	340
Percentage of mammary cancer	—	—	**6.5**	**11.1**	**24.2**	**32.1**	**25.0**	**17.2**	**10.7**	**18.2**

TABLE III (*after Murray*)

Age (months) . .	0–3	–6	–9	–12	–15	–18	–21	–24	Over 24	Total
Total mice . . .	—	—	38	41	26	38	29	26	25	223
Percentage of mammary cancer	—	—	**2.6**	**9.8**	**3.8**	**21.6**	**0.0**	**11.5**	**8.0**	**8.6**

The figures were submitted, further, to mathematical analysis involving a determination of the standard errors of the differences between the cancerous and non-cancerous groups. Taking the crude data, the actual percentages amongst all the offspring were : —

Ancestry cancerous 18.2%
Ancestry non-cancerous 8.6%

When a correction was made for the varying age-distributions of the two groups by calculating corrected percentages based on the age-distribution of all mice as a standard, and reducing the numbers for the five hundred and sixty-three mice to the corresponding proportions per thousand, the difference was increased only from 9.6 to 9.8%. As the standard error of this difference was 2.96, the difference was 3.3

times the standard error, and the chance of its occurring as a mere fluctuation of random sampling only about one in a thousand.

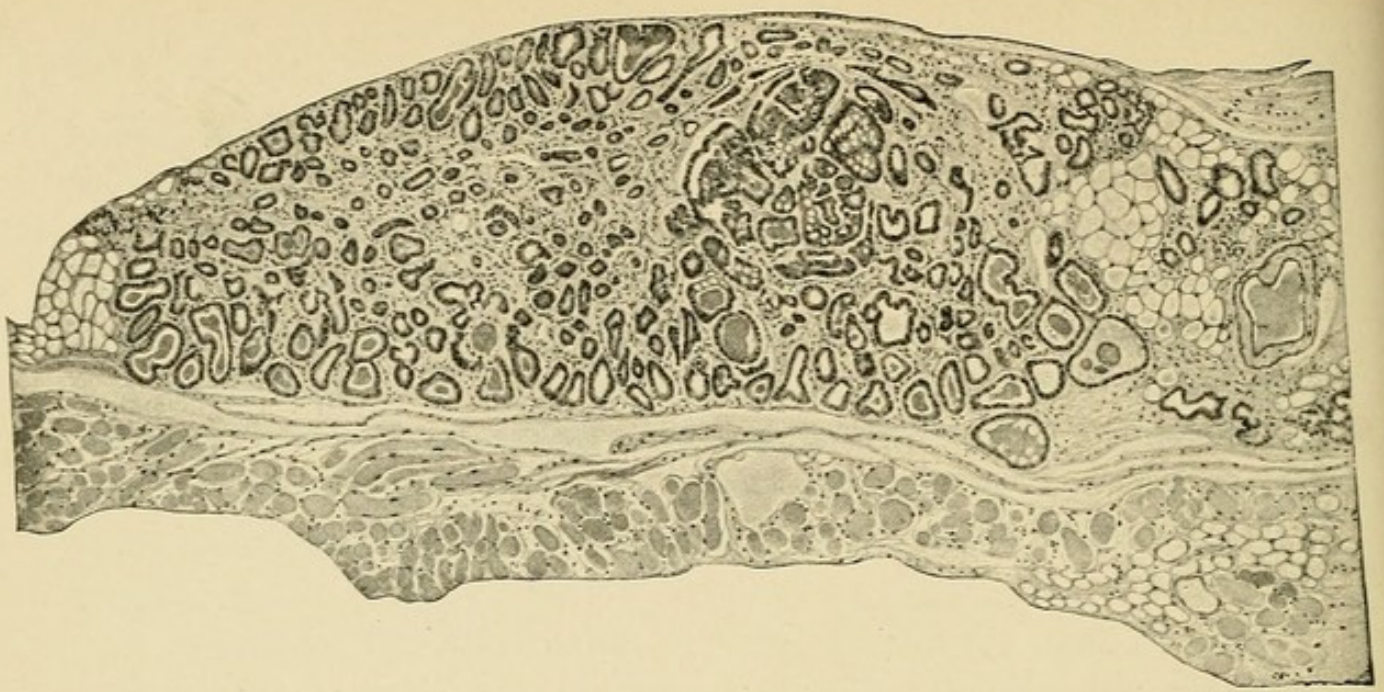
Murray thought, therefore, that the disparity between the two groups was not due to chance but, on the contrary, almost certainly significant, and that the increased liability was probably in the nature of a predisposition of one particular tissue or organ system to undergo cancerous transformation under the wear and tear of life. The difference between the two series was apparent at all ages, and the age of maximum incidence did not appear to have been lowered in the predisposed group.

In discussing the application of these results to man, Bashford [1] extended a warning against too pessimistic conclusions, pointing out that the influence of heredity had been demonstrated only for stocks where this factor had been concentrated by careful mating. Such a concentration could occur in man only by hazard as a coincidence of considerable rarity, and it was probable that the influence of heredity in the general population was manifested as an average predisposition of low intensity.

Meanwhile the question had been approached by Tyzzer,[2] who called attention to the relative frequency of growths in the descendants of those mice which had fallen prey to malignant tumors. The number which had occurred in three families derived from three female mice with spontaneous growths indicated that tumor development was influenced by inherited qualities.

Murray,[3] without wishing to minimize the value of Tyzzer's careful work, indicated that the neoplasms recorded by that author belonged mainly to types in which the malignancy was not very pronounced. The majority of the tumors were cyst-adenomata of the lung, a variety which it would be hazardous to regard in all cases as malignant, and the multiple lymphomata, which were second in frequency, could not be separated satisfactorily from conditions resembling diffuse hyperplasia, although the type did include new growths of undoubted

[1] *Fourth Sci. Report, Imperial Cancer Research Fund*, London, 1911, Introduction, p. xvi.
[2] *Jour. Med. Research*, 1907–1908, N.S., xii, 199.
Jour. Med. Research, 1909, N.S., xvi, 479.
[3] *Fourth Sci. Report, Imperial Cancer Research Fund*, London, 1911, 115.



J. R. Ford, del.

Hypertrophic mammary nodule. Increased number of acini, on the whole of fairly normal appearance, with increased cellularity of the stroma. Concretions in the lumina. At one point the acini have assumed atypical forms, and there are signs of pressure in the surrounding tissues. No other sign of active growth. $\times \frac{56}{1}$.

J. R. Ford, del.

Hypertrophic mammary nodule from another mouse. The epithelium of the acini is distinctly atypical, in that the cells are larger than normal and more crowded, so that several layers are found lining some acini. This appearance is not due to obliquity of the section. A few mitoses are discoverable. $\times \frac{166}{1}$.

malignancy. Furthermore, while cyst-adenomata of the lung occurred in males and females with equal frequency, multiple lymphomata had been found among Tyzzer's material much more often in females, and a serious source of error was introduced when the frequency of tumors as a whole was reckoned on males and females together.

Jensen [1] was able to bring to maturity among the descendants of a cancerous female about fifty mice in four or five generations, none of which, however, developed a tumor. Another mouse with spontaneous cancer bore four young, one of which developed an enormous intra-abdominal round cell sarcoma. Two males of this litter used for breeding gave rise to four or five hundred descendants in six or seven generations, and even though many of their offspring died at an early age during epidemics, several tumors of the usual type developed among them.

Influence of Inflammation

Haaland,[2] in trying to account for the multicentric origin of mammary tumors in mice, examined serial sections of the mammæ in both cancerous and non-cancerous animals. The normal condition of this gland after the era of physiological activity had passed appeared to be one of more or less atrophy, accompanied by sclerotic changes in the connective tissue and arteries. Chronic inflammation of the interstitial tissue, either diffuse or localized, was very common, as was dilatation of the ducts with its consequent cyst formation. The epithelium bounding these cavities was usually that of the normal duct or acinus flattened by pressure of the contents, although occasionally the cysts were lined by epithelial cells of the squamous type.

In addition to the lesions just mentioned, all stages of nodular hypertrophy of the mammary epithelium were met with, varying from a slight increase in the number of normal acini in one lobe, to the formation of definite nodules. All gradations might exist between this condition and malignant growth, and, indeed, there were times when it was impossible to decide between the two. The frequent association in the same gland of all degrees of nodular hypertrophy with veritable

[1] *Zeitschrift f. Krebsforsch.*, 1908–1909, vii, 285.

[2] *Fourth Sci. Report, Imperial Cancer Research Fund*, London, 1911, 30.

new growths, made it probable that the lesion bore some relation to tumor development, and it was intimated that the nodes might be either the base upon which cancerous proliferation started, or true tumors from their very inception. While nodular hypertrophies were

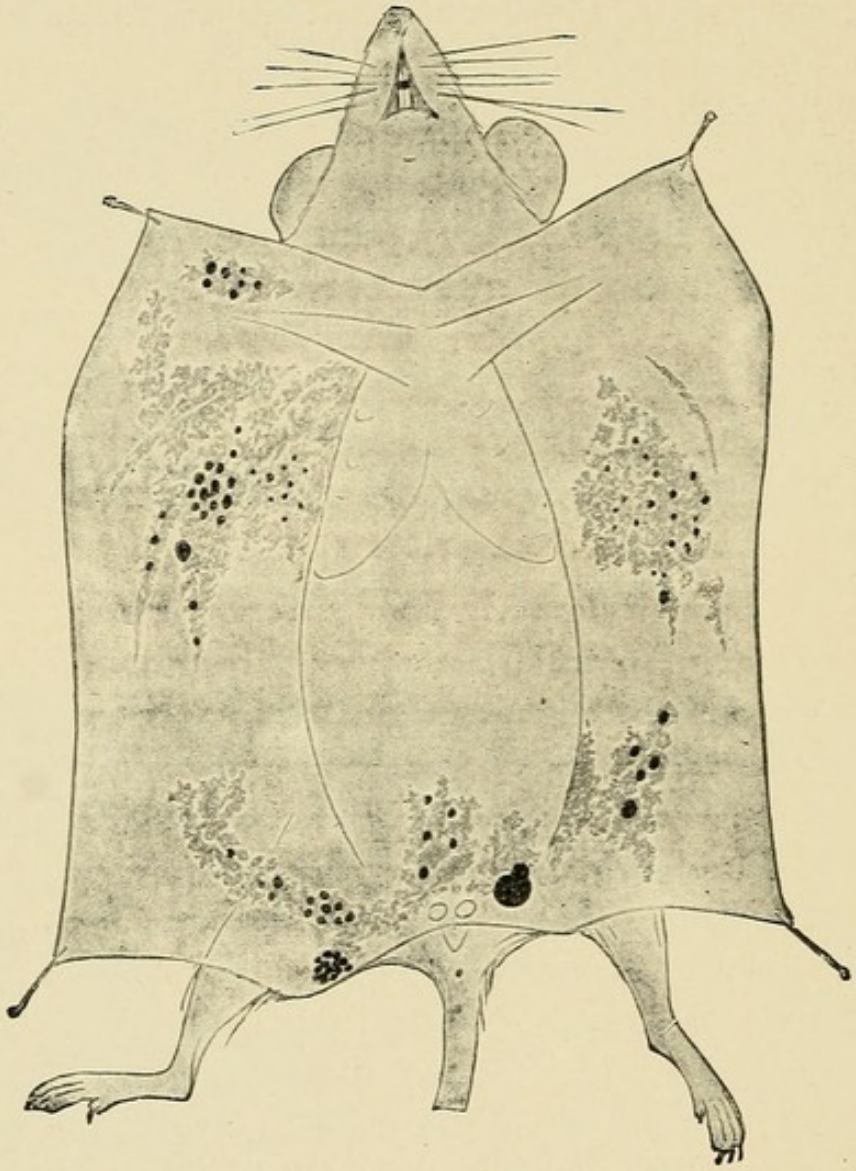
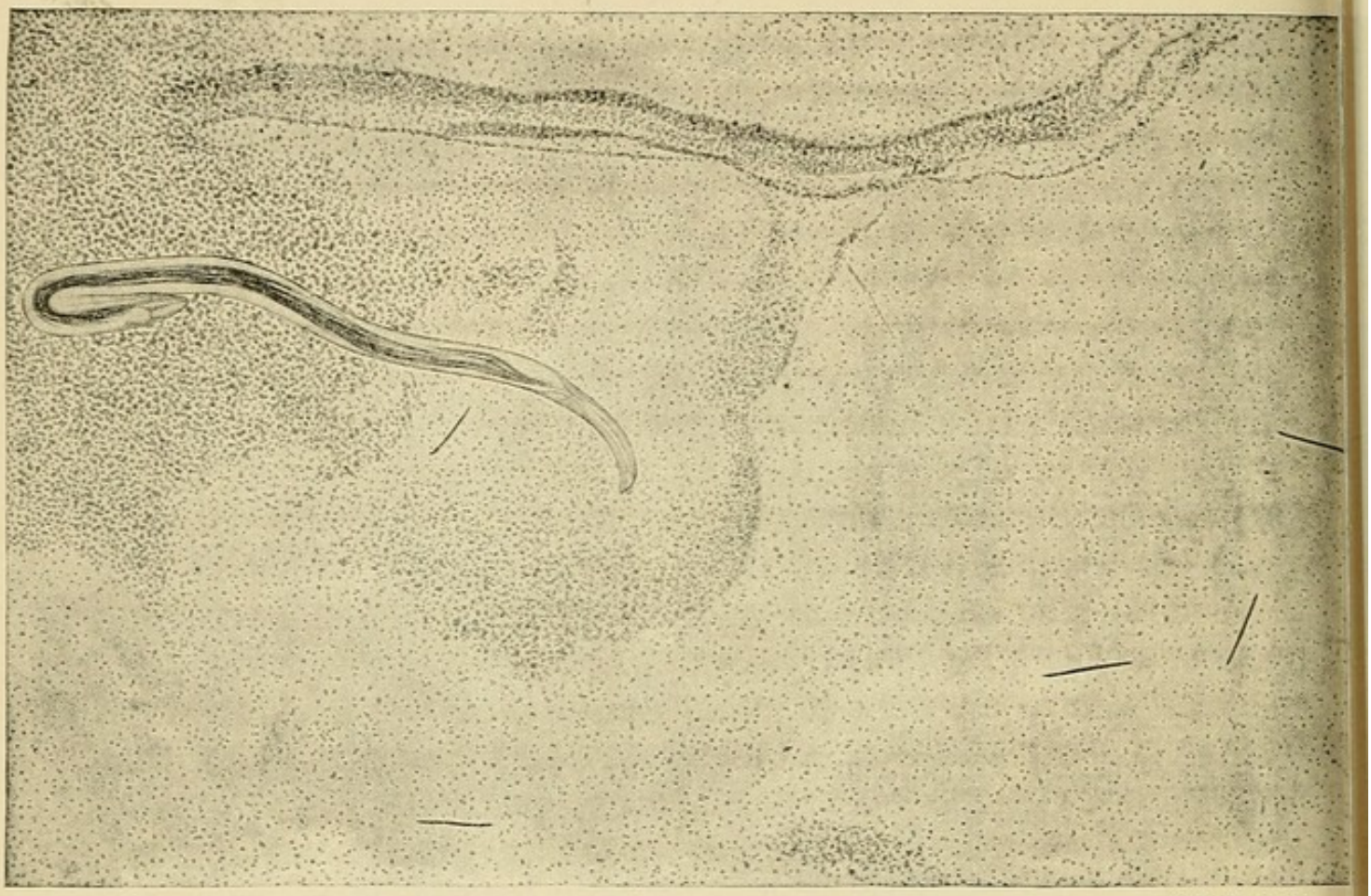


FIG. 10.— Multiple minute hypertrophic nodules in the mammæ, reflected with the skin. The figure also represents the zone free from mammary gland, chosen for autologous inoculation.

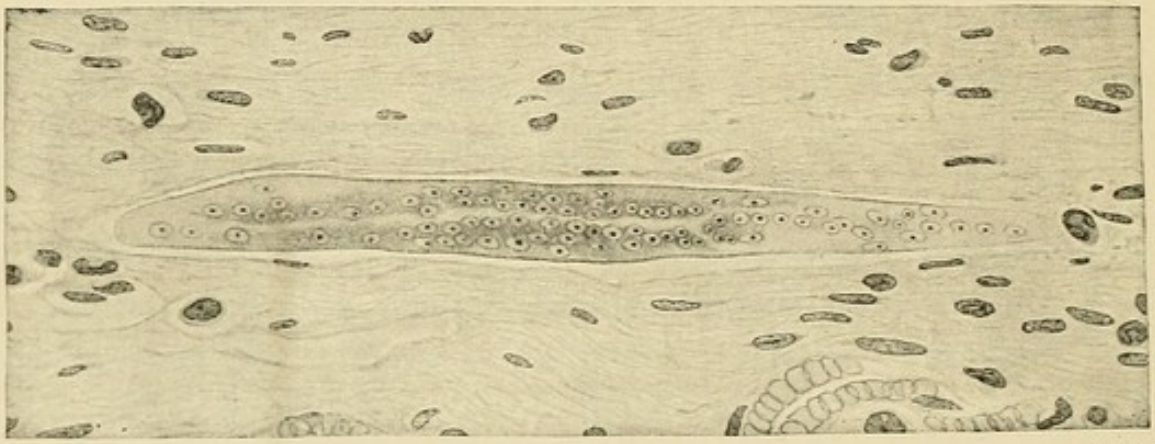
found most commonly in mice with cancer in another mamma, Haaland proved that they were not secondary deposits by demonstrating in serial sections their normal connection with the mammary ducts.

The inflammatory and sclerotic conditions in the connective tissue were important because many cases of tumor development suggested that the process had taken place in an organ diffusely diseased, and, moreover, because the nodular hypertrophy just discussed was



J. R. Ford, del.

Stretched and stained subcutaneous tissue of old normal female mouse. A nematode is seen, containing large embryos; five extruded embryos in the subcutaneous tissue at a considerable distance from the mother worm. $\times \frac{25}{1}$.



J. R. Ford, del.

Oblique section of a nematode embryo free in the interstitial mammary tissue of an old normal female mouse. $\times \frac{525}{1}$.

a process too far advanced to be investigated ætiologically. It was therefore necessary to examine the mammary glands of old non-cancerous mice. When this was done, inflammatory changes in the connective tissue were discovered to be of common occurrence, and often associated with the presence of nematodes, such as had previously been found by Borrel in young tumors and their surroundings, and in the lungs, mammæ, and mediastinal lymph nodes of tumor-bearing mice.

In his own cases, Haaland detected the parasites in the subcutaneous tissue of a large proportion of old normal mice from different breeders, in addition to finding them, also, in the pulmonary vessels and once in the pleura. They were usually few in number and discoverable only after long search, although as many as half a dozen or more had been found in one mamma. The worms were all females containing large numbers of eggs or embryos, and the possibility of liberation of the latter in the subcutaneous tissue was demonstrated by their discovery in that location.

That the nematodes were capable of setting up an intense inflammation was shown, not only by the zone of leucocytes surrounding areas where they had remained, alive, for some time (encapsulated?), but by still other signs marking their path. When they lay dead in the tissues a vigorous leucocytic and lymphocytic reaction supervened, and phagocytosis proceeded until the worm finally became unrecognizable.

These characteristic lesions accompanying the parasites affected the loose connective tissue alone, and the mammary gland was involved only indirectly and in so far as it was embedded on all sides in this tissue. In several instances the inflammatory condition co-existed with nodular hypertrophy, an association which suggested that the two might be connected and referred to a common cause — the presence of nematodes in the subcutaneous tissue. In half a dozen males examined, the worms were found in the same proportion as in females, while evidence from young or adult mice was still scanty.

The observations of Murray [1] on carcinoma of the liver in cows gain an enhanced interest in connection with Haaland's remarks. These growths Murray found very common, and almost invariably asso-

[1] *Veterinary News*, 1910, vii, 563.

ciated with a severe biliary cirrhosis due to infection with the *Distoma hepaticum*. The new growths did not arise in the bile ducts, however, but in the parenchyma, and the irritation of the parasites acted only as an indirect cause.

The observations of both authors together are of further significance when taken in conjunction with the well-known frequency of epithelial growths of the rectum and bladder of man in the presence of infection with the *Bilharzia haematobia*.

Ætiology Theoretically Considered

Ehrlich [1] advanced the following explanation of malignant growth, couched in terms of the side-chain theory. The division of nutrient material throughout the body was regulated by the number of receptors possessed by the various cells, as well as by the avidity of these receptors, so that immoderate growth, like that of a tumor cell, could be achieved only through equipment with receptors having an avidity for foodstuffs relatively higher than the normal. When, as often happened, a primary tumor of the mouse was implanted unsuccessfully into hundreds of other mice, this failure could mean only that the receptors of the transplanted tumor cells possessed an avidity no higher than the average obtaining in the cells of these mice. Hence the origin of a growth must be conditioned, not by an increase in the avidity of its own cells, but by a general lowering of that of the receptor apparatus of the entire body, and the tumor cells possessed, therefore, a relatively but not an actually higher avidity than the normal cells of the organism.

The growth of a transplantable tumor in normal mice was, however, in the opinion of Murray,[2] more a result of the adaptability of its cells to new surroundings. He found that tumors transplanted into spontaneously affected animals which had been relieved by operation became established in consequence of a great power to accommodate themselves in new hosts, and that they shared the food supply in common with the normal tissues without starving them. When a spontaneous

[1] *Zeitschrift f. aerztliche Fortbildung*, 1906, iii, 210.
Arb. a. d. Königl. Inst. f. Exp. Therap., 1906, Heft i, 86.
[2] *Third Sci. Report, Imperial Cancer Research Fund*, London, 1908, 108.

growth recurred it increased in size more rapidly, as a rule, than did the transplanted tumor, even though its energy of growth (as tested by inoculation into normal animals) was much inferior to that of the propagable neoplasm. This would be quite inexplicable on the assumption that a difference in avidity for food was the factor principally determining their relative rates of proliferation, but became at once intelligible when the importance of adaptability was considered. The recurrent spontaneous tumor was in its native environment and the food materials presented to it were those to which it had always been accustomed, wherefore adaptation was not required, and successful competition with a transplantable growth was possible.

The relation of a spontaneous growth to the animal bearing it could not be described so simply as Ehrlich had supposed, merely by postulating a different avidity for food in accordance with the side-chain theory. The relative affinities for nourishment exhibited by normal tissue and tumor must remain vaguely speculative, as long as the avidity of malignant cells was measured solely by their ability to grow and comparisons made with normal adult cells no longer engaged in active proliferation.

Haaland [1] exchanged grafts between two animals spontaneously affected, each tumor being at the same time inoculated into its bearer and into normal mice. In the course of these experiments two cancroids were transplanted into about a thousand young mice, and although six hundred and eighty-six of them lived more than four weeks, growth did not take place in a single instance. According to Ehrlich's hypothesis, the body cells of the spontaneously affected mice in which tumors of such low avidity had been allowed to grow should be possessed of a still lower degree of avidity, and such animals should be, in consequence, more susceptible to the transplantation of other tumors. But this was not the case. Grafts were exchanged between the two animals in which these cancroids arose, but no growth was obtained in either; moreover, the two mice were refractory even to a tumor of higher avidity. These experiments, and others of a similar nature, indicated that the conditions for tumor growth were much more complex and specific than could be explained by supposing a difference of avidities. "The conditions for which two histologically indistinguishable tumours are adapted

[1] *Fourth Sci. Report, Imperial Cancer Research Fund*, London, 1911, 62.

are so different that they can be only very rarely exchanged without the malignant mode of growth ceasing altogether."

Bashford [1] represented Ehrlich's hypothesis diagrammatically, and discussed the question as follows: —

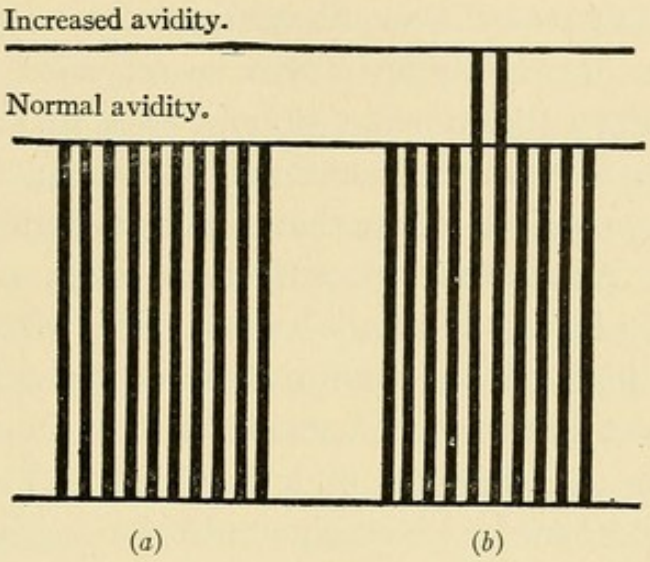
"The side-chain theory as applied to cancer, if stripped of its special technical terminology, amounts simply to assuming that the cells of some malignant new growths take up food-stuffs more rapidly and more energetically than do normal tissues. By virtue of this property such tumours can be transplanted to fresh hosts; once established and growing in these, such tumours can prevent a secondary inoculation or metastases from growing by virtue of the rate with which food-stuffs are withdrawn. A subsidiary assumption is made that this starvation of a natural or artificial metastasis may be effected by deprivation of special food-stuffs. Great importance is attached to the assumption that it is possible to increase artificially the avidity of the tumour-cells for food-stuffs; in other words, to increase the rapidity of cell-division.

"Following the practice observed in the Second Scientific Report, where all the more important theories of cancer were diagrammatically depicted, Ehrlich's hypothesis, formulated since then, may also be reduced to a diagram without suggesting that he would approve it as correctly reproducing all the details of his elaborate argument.

"A number of lines of equal length may represent, as in the accompanying diagram, the normal avidity of the body. Departures from the normal may be depicted by other lines exceeding or falling short of one passing through the tops of those representing the normal. Thus in the accompanying diagrams (*a*) and (*a'*) will represent the normal and (*b*) will represent the simplest departure from it, namely, an increased avidity; but this assumption requires no further consideration, since, if it were true, then on the basis of Ehrlich's hypothesis every tumour should be easily transplanted into normal animals. This is not the case, and Ehrlich dismisses this assumption, which was put forward by that distinguished pathologist, the late Professor E. Albrecht. Instead of assuming retention of normal avidity by the body, and increased avidity by the tumour-cells, Ehrlich assumes that as a rule the body-cells lose in avidity with increasing age (*b'*), (*c*), (*d*), while the tumour cells retain the normal (*b'*) or do not lose it in a degree

[1] *Fourth Sci. Report, Imperial Cancer Research Fund*, London, 1911, 188.

equal to that lost by the body (*c*). In some cases it is assumed that Albrecht's view (*b*) may hold, or that while the body-cells lose in avidity, the tumour-cells may exceed the normal (*d*), and it is further as-



Diagrammatic representation of Albrecht's hypothesis.

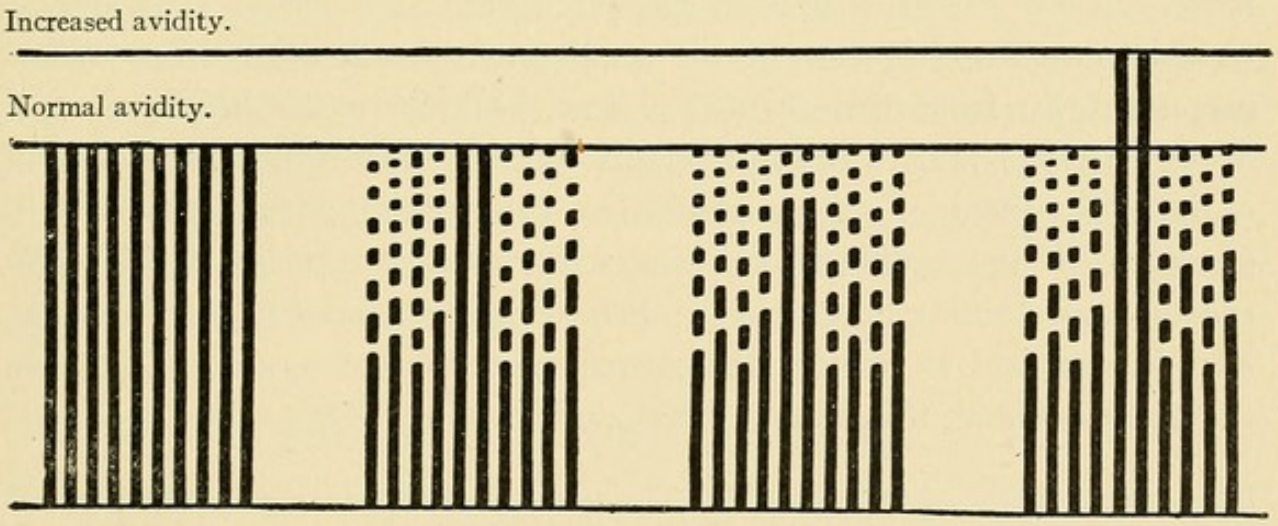


Fig. 11. — Diagrammatic representation of Ehrlich's hypothesis as deduced by him from his transplantation experiments on mice.

sumed that this exaggerated avidity, when not present at the first transference to normal animals from the animal in which the growth arose, can be artificially induced by a particular experimental procedure, viz. artificial selection of the rapidly growing tumours and forced *passage*.

"This hypothesis assigns a very important etiological part to a constitutional change ensuing with advance in years. The ingenuity and the simplicity of the conception are striking. However, sufficient account has not been taken of the differences existing between spontaneous and propagated cancer, as consistently emphasized from this laboratory, since the hypothesis was based upon the unfavourable results of removing a spontaneous tumour from its native environment and transplanting it into normal animals, without giving due consideration to any other possible factor than the assumption of a differential of cell-avidities. At the time when the hypothesis was formulated, the investigations conducted in this laboratory had already fully demonstrated that the hemorrhagic mammary tumours could be transplanted with ease, whereas Ehrlich had concluded that they were practically untransplantable and therefore of low avidity. The transplantation of spontaneous tumours had been shown to give better results in young than in old animals, and, therefore, the peculiar age-incidence of cancer was bound up with the inception and not with the continued growth of cancer. The fact that old animals did not yield a soil uniformly more suitable for growth than did young animals, led as a matter of course to an investigation of the quality of the soil which mice suffering naturally from cancer offered for the growth of their own and other tumours, and to the investigation of the most important question of individuality. . . ." A glance at the following table ". . . will show how it is possible to contrive experimental tests of the hypothesis, by ascertaining how tumours behave in animals of normal avidity, and in animals in which departures from it are assumed to have occurred because they have developed cancer. The table, embody-

	Mice with Spontaneous Cancer			Normal Mice
	A	B		— 0/35
Spontaneous Tumour A . .	+	—		+ 3/35
" " B . .	—	+		
	C	D	E	
" " C . .	+	—		— } 0/1000
" " D . .	—	+		— }
" " E . .	—	—	+	+ 4/26

ing some of Haaland's experiments with five mice and their spontaneous tumours, summarizes the kind of results obtained. . . . In the first experiment, tumour A, when tested in normal mice, does not grow, therefore the avidity of mouse A is below normal. Tumour B grows in normal mice, therefore it must have a greater avidity than tumour A, and a still greater avidity than the body-cells of mouse A; but tumour B will not grow in mouse A. It follows that the hypothesis is inadequate to explain the failure of transplantation in this case. In the second experiment, two mice, C and D, have been picked out whose tumours would not grow when tested on a thousand animals of normal avidity; it follows that tumours C and D were growing in mice C and D in spite of an avidity very much below the normal, and also that mice C and D had an avidity even lower in the scale. Nevertheless, a tumour E, which had so high an avidity that it grew easily in mice of normal avidity, was not able to grow in mice C and D, of which the avidity has been proved to be so low. This is a *reductio ad absurdum*. It follows from these, and from many similar or slightly different experiments, that Ehrlich's atreptic hypothesis is inadequate to explain the growth of cancer either when transplanted into a fresh host, or when growing in the animal in which it arose, and it may be inferred is equally inadequate to explain the cause and nature of cancer. Without denying that cancer-cells may have *inter se* a different avidity for food-stuffs, the explanation of the development, nature, and prevention of cancer is not to be sought along the lines indicated by Ehrlich's atreptic hypothesis."

HISTOLOGY

During the first few years of the investigation of mouse tumors it was customary to speak of "Jensen's tumor," or of "Borrel's tumor," an expedient which could from the nature of the case, however, be but temporary. As the number of cases under observation grew larger it became desirable, and even necessary, that some sort of classification be attempted. It was found possible to make one, partially satisfactory at least, and, furthermore, that this would follow, although somewhat roughly, those suggested for tumors in man.

Michaelis[1] was, perhaps, the first to attempt to classify the spon-

[1] *Med. Klin.*, 1905, i, 204.
Zeitschrift f. Krebsforsch., 1906, iv, 3.

taneous tumors, arranging them under three heads. The first two were the *simple alveolar carcinoma*, which was sometimes tubular, and the *adeno-carcinoma*, often signalized by the formation of cysts and papillæ. In those of the third type, the parenchyma was divided into alveoli, as in the first group, but within each alveolus the cells were wreathed about lumina in single layers, conferring somewhat the appearance of a sweat gland on cross-section. The acini were not in contact, however, but separated by irregularly arranged alveoli. This type, unnamed by Michaelis, corresponds to the *malignant adenomata.*

Apolant[1] suggested the following comprehensive classification for the mammary tumors of the mouse: —

I. *Adenoma.*
   (*a*) *Adenoma simplex.*
   (*b*) *Cyst-adenoma simplex.*
   (*c*) *Adenoma cysticum œdematosum s. haemorrhagicum.*
   (*d*) *Cyst-adenoma papilliforme.*

II. *Carcinoma.*
   (*a*) *Carcinoma simplex alveolare*, including *cysto-carcinoma haemorrhagicum.*
   (*b*) *Carcinoma papillare*, including *fissure-forming carcinoma.*

The poverty of the normal mammary gland as concerned a well-developed stroma, made it understandable that connective tissue neoplasms had not so far been discovered in it. The mammary tumors of the mouse were epithelial growths, the ground type of which was represented by the adenoma simplex. By the term *ground type* Apolant wished not only to convey the idea that the adenoma was at once the most simple of all growths and the one most closely allied to the structure of normal mamma, but further, to emphasize its genetic relation to other epithelial tumors, for in spite of a great variety in structure he had seen no growth that could not be traced back to the simple adenoma.

The *adenoma simplex* in its adult condition was more or less lobular in microscopic structure, separated by a capsule from the normal gland and, as a rule, but poorly provided with stroma. The round or oval acini corresponded in general to the normal acini of the mamma, although they lay more closely together and were not

[1] *Arb. a. d. Königl. Inst. f. Exp. Therap.*, 1906, Heft i, 11.

arranged in groups within the lobules. The cells, arranged in one layer, were usually small, approximately cubical in shape, and were furnished with a relatively large nucleus rich in chromatin. Mitoses were infrequent and never of pathological type. The acini, like those of the normal mamma, usually contained a colloid-like material staining with acid dyes, and either filling the lumina entirely or irregularly retracted from their walls. Altogether, the histological picture imitated very closely that of a parenchymatous goiter. The blood supply was usually in proportion to the amount of stroma, although some areas might be well provided with vessels while others in the same tumor were supplied but poorly. In very young tumors, below the size of a lentil, a lobular structure was not usually demonstrable, and although these were separated to some extent from the normal gland by strands of connective tissue, at other points there was such a gradual transition that it was impossible to differentiate between the newly formed acini and the mamma itself. The distinction was rendered more difficult since neither the form of the alveoli nor that of its cells departed very far from the normal type. As these immature growths were distinguished from the true adenomata by the possession of ducts which were traceable into those of the normal gland, they might with more justice be described as local hypertrophies than as tumors in the strict sense of the word. Nevertheless, a sharp distinction between the two was impossible, and not infrequently both forms were present at one and the same time, the hypertrophic moiety still in intimate connection with the mamma, the adenomatous, on the other hand, surrounded by a connective tissue capsule. Occasionally a number of mitoses greater than was ever associated with true adenoma could be discovered in the cells of local hypertrophic areas, and in such cases careful examination would nearly always disclose regions where the glandular epithelium, having suddenly taken on atypical growth, was progressing directly toward carcinoma without the intervention of an adenomatous stage.

Secondary alterations, to which the adenomata always succumbed after any considerable duration, might affect either the parenchyma or the stroma. As an outcome of the former there resulted a *cyst-adenoma simplex*, and of the latter, an *adenoma cysticum œdematosum s. haemorrhagicum.*

The change in the parenchyma resulting in the evolution of a *cyst-adenoma simplex* was a dilatation of the acini with the formation of retention cysts. The cubical cells lining these cavities were sometimes greatly flattened by pressure and when the process took place in adjacent alveoli the series of occasionally intercommunicating chambers thus produced recalled vividly the structure of lung as seen under the low power.

The *adenoma cysticum œdematosum s. haemorrhagicum* was marked in its early stages through the separation of certain still normal alveoli from their fellows by an edematous stroma, or later, by final destruction of the isolated alveoli from increase of the edema. The edema varied according to whether stasis in the tumor involved more particularly the blood or the lymph vessels. In the former case, dilated capillaries traversed the growth, often conferring the appearance of a cavernoma, and it was not unusual for the vessel-wall to rupture, giving rise to a more or less extensive hemorrhage. Generally, however, the lymph channels were primarily or concomitantly affected, and an edema was produced which evolved the lesions just described, before stasis in the blood vessels became apparent.

The fate of the adenomata was thus a varied one, and depended upon whether secondary degenerations ran their course in the parenchyma or in the interstitial tissues. Although the lesions just discussed could exist separately to a certain degree, they were so combined in their later stages that discrimination was impossible.

The essential characteristic of the *cyst-adenoma papilliferum* lay in this, that in contradistinction to the types previously described, cellular proliferation, keeping pace at least with the dilatation of the cysts, or more usually outstripping it, created a papillary structure. This rather rare tumor owed its peculiar architecture to the energetic stroma development. As the cysts dilated, their form became irregular from introversions of their walls, while at the same time the lining epithelium threw out tiny buds which, aided by the growth of new connective tissue, gradually developed into delicate papillæ. In the presence of very vigorous cell growth these papillæ could project lateral branches, although Apolant had never seen any very intricate pattern produced in this way.

The *carcinoma simplex alveolare* originated from adenomata

which had undergone neither cystic nor hemorrhagic transformation, and the malignant change was initiated either in circumscribed or more extended localities. There occurred in the midst of the ordinary adenomatous tissue one or more sharply defined areas, where the epithelial cells formed a solid nest without any trace of a lumen. Mitoses were seldom lacking even in this earliest stage, and their presence afforded a further distinction between the young carcinoma and the surrounding adenoma. Serial sections demonstrated such entire isolation of areas of carcinomatous transformation from one another, that their origin could be considered multicentric. The malignant change seldom developed without the occurrence of distinct morphological alterations, such as a gradual increase in the size of the cells. These elements assumed a characteristic vesicular appearance, while with the increment of protoplasm there was associated an enlargement of the nucleus. Hyperchromatic and other atypical mitoses were abundant in this tumor type as they were in all other rapidly growing mouse carcinomata. As a rule the growth of new blood vessels was unable to keep up with the rapid proliferation of the parenchyma, whence more or less extensive areas of degeneration occurred in the central portions of the tumor even at an early stage of development. Nevertheless, there were alveolar carcinomata which possessed an adequate blood supply, and these one might be inclined to class among the endotheliomata on account of the seemingly close relation between blood vessels and tumor cells, were it not that an endothelial origin had never yet been indubitably demonstrated for any mouse tumor.

Closely connected with the alveolar variety, and a derivative of it rather than a separate type, was the *cysto-carcinoma haemorrhagicum*. This tumor was, in fact, an alveolar carcinoma that had developed upon a cystic or hemorrhagic adenoma, and a typical example at the height of development had the appearance of a transformed adenoma, with cystic dilatation of the alveoli and extensive hemorrhages into the interstitial tissue. An adenomatous structure was no longer to be recognized in the outlying parts, and the cystic or hemorrhagic portions were surrounded by cells arranged in the most disorderly manner. In later stages the columns of tumor cells isolated by blood spaces gradually succumbed until finally, the proliferative power of

the cells exhausted, the whole process terminated in a cyst filled with thick, brown, bloody débris. The relatively benign nature of this variety was indicated further by a noteworthy lack of mitoses, a condition which was in sharp contrast to the great number seen in true alveolar tumors.

Carcinoma papillare was a much less common growth than alveolar carcinoma. While in the latter type an acinous arrangement of the cells with lumen formation occurred but rarely, in that now under discussion the presence of lumina was a constant characteristic. If the lumina retained their original size the structure of the tumor corresponded very closely to that of malignant adenoma, but cystic enlargement produced the true papillary carcinoma. So long as the growths contained no papillæ they might be termed *fissure-forming adeno-carcinomata*, for the lumina, instead of being round or oval, were narrow and protracted or, at other times, tortuous or triangular. The epithelial cells at first inclosed these openings in a single layer but evinced their carcinomatous character, notwithstanding this arrangement, by mitosing freely and furthermore, by joining with neighboring groups to form an extensive reticulum. Large solid cell nests were not often encountered because of the frank tendency toward lumen formation, and the stroma was developed to a slight extent only. While this fissure-forming variety might persist in pure form, transitions between it and the true papillary type were more usual. The process was quite analogous to that which ran its course in the papillary cyst-adenomata. Coincidently with dilatation of the acini their lining epithelium extruded processes into the lumina, and these gradually developed into long branched papillæ. The papillary adeno-carcinomata, besides being evolved from tumors of the fissure-forming type as just described, occasionally originated in simple adenomata through a sudden widening of the lumina followed by a vigorous ingrowth on the part of the epithelium.

Although the several varieties above described were the most characteristic forms of mouse carcinoma, Apolant pointed out that the account as given was by no means complete, for malignant tumors of uniform structure were very rare, and the multitude of types was so enriched by manifold combinations that an accurate description of them all was hardly possible.

According to Murray,[1] the histological variations presented by mammary carcinomata could be referred with but few exceptions and by easy gradations to an acinous ground type which, in its turn, led directly to the structure of the normal mammary gland; furthermore, the parenchyma of any single tumor might exhibit several of these modifications simultaneously or successively. Although purely acinous growths were extremely rare, spontaneous tumors without any trace of an adenomatous arrangement were seldom met with. Apolant had inferred, from the association of the adenomatous with other structural types, that alveolar carcinomata and adeno-carcinomata arose directly from preëxisting adenomatous portions, but Murray was of the conviction that the course of transformation was usually in the opposite direction, that is, that an area originally alveolar became split up into acini by penetrating connective tissue and capillaries. Still, the converse process, by which the acinous was transformed into the alveolar structure, was frequently seen, but its interpretation in the primary tumor was more difficult.

Edematous and hemorrhagic changes in the stroma were more apt to occur in those tumors where an extremely delicate connective tissue was associated with thin-walled blood vessels. Fluid exudate accumulated between the walls of the capillaries and the adjacent stroma, so that the vessels appeared to be suspended in wide spaces filled with a light flocculent coagulum. With this condition there was generally associated a dilatation of the capillaries, pointing to a partial stasis of the circulation as the primary change. Hemorrhage into the widened lymph spaces surrounding the vessels frequently occurred, and when the acini of the parenchyma had been dilated into cysts such blood extravasations easily passed through their attenuated walls and distended the cavities. The thin trabeculæ of parenchyma intervening between adjacent cysts were often much compressed when the dilatation was extreme, and the vascular endothelium might be destroyed for long distances — a condition which, particularly in old growths, produced the appearances that had led v. Hansemann and others to place many of the mouse tumors among the endotheliomata.

[1] *Third Sci. Report, Imperial Cancer Research Fund*, London, 1908, 69.

Apolant's classification was fully indorsed by Gierke [1] who, however, emphasized the fact that it was purely histological and that it dealt with tumors biologically identical and varying only slightly in their microscopic appearance. As regarded the conversion of adenomatous into carcinomatous structure, Gierke was of the opinion that Apolant had been in error, and that exactly the reverse evolution took place, the acinous portions arising from the alveolar. This would correspond to a differentiation (maturation) of the tissue of the tumor, and was comparable with the formation of Pflüger's primordial "egg tubes" in the ovary, or with the development of the embryonic or strumous follicles of the thyroid gland. As Apolant had stated, variations in the rate of growth determined, to a certain degree, variations in architecture, and this observation made it easier to understand the significance of histological structure. The epithelial cells would differentiate faultily when growing at a rapid pace and would, in consequence, assume a garb histologically more malignant than that which they possessed when growth was slower. And yet, such relations between structure and growth rate did not always obtain, and there was a whole series of other factors involved, including the behavior of the connective tissue and the general constitution of the animal itself.

CLINICAL COURSE

A primary growth of the mouse pursues much the same course as does a neoplasm in the human subject. Spontaneous absorption may take place and has, in fact, been recorded by Ehrlich [2] as an occurrence not particularly rare. Murray,[3] on the other hand, thought that while absorption was not uncommon as a localized process affecting small areas, the disappearance of an entire tumor was a very unusual event, and although he had frequently observed temporary arrest of growth and in some cases even an actual diminution in size, the usual course was a progressive increase in the dimensions of the nodule. The rate at which growth took place was variable, the most rapid occurring

[1] *Beitr. zur path. Anat.*, etc., (Ziegler), 1908, xliii, 336.
Third Sci. Report, Imperial Cancer Research Fund, London, 1908, 124.
[2] *Arb. a. d. Königl. Inst. f. Exp. Therap.*, 1906, Heft i, 82.
[3] *Third Sci. Report, Imperial Cancer Research Fund*, London, 1908, 101.

among the hemorrhagic carcinomata in which sudden increments (although sometimes due to an extravasation of blood) not infrequently supervened as the result of an almost explosive proliferation of the parenchyma.

Haaland [1] recorded a few mice which had been able to rid themselves of sporadic growths. In one of these animals, although the absorption of a spontaneous tumor was under way, another was growing progressively at the same time and producing large metastases in the lungs and liver. In this case, therefore, the disappearance of a sporadic neoplasm was due to local conditions affecting the tumor cells themselves rather than to the intervention of general constitutional changes.

Examined under the microscope, disappearing nodules were found to contain a fair amount of healthy tumor tissue showing no signs of active proliferation and surrounded by a very sclerotic connective tissue. At the periphery, and scattered through the growth, were numbers of large phagocytes, some of which were filled with brownish granules while others possessed a vacuolated protoplasm like that of the phagocytes seen in spontaneously regressing propagable tumors. These cells appeared not only as a reaction zone outside the alveoli, but also within them, replacing by degrees the parenchymal cells. The resemblance of the picture to that accompanying the absorption of transplantable tumors lay chiefly in the presence of phagocytes and sclerotic connective tissue, whereas the infiltration of small round cells usually encountered in propagable tumors undergoing absorption was less marked.

Discussing the effect of a spontaneous growth upon the animal bearing it, Murray [2] said that the weight of the mouse generally increased slowly with the growth of the tumor until ulceration or hemorrhage supervened. A diminution in weight then set in, and was always a symptom of the gravest import, for seldom did an animal survive more than two weeks after its inception.

The result of operative removal of sporadic growths was discussed by Murray as follows: —

"When a large tumour is removed by operation from a mouse, the

[1] *Fourth Sci. Report, Imperial Cancer Research Fund*, London, 1911, 51.
[2] *Third Sci. Report, Imperial Cancer Research Fund*, London, 1908, 103.

animal loses weight in excess of that represented by the tumour and blood loss. This is gradually regained in the succeeding week or two weeks, and then the weight remains constant. Minor variations of a half to one gram unless progressive from week to week are of no importance. Inter-current illnesses are always accompanied by loss of weight, sometimes considerable. Should recurrence take place, the animal at first increases in weight, as already noted. Loss in weight generally indicates an approaching lethal issue, either from too great nutritive demands by the recurrent tumour, or from respiratory embarrassment due to extensive pulmonary metastases. The frequency with which recurrence supervenes after apparently complete extirpation, is surprising when the encapsulated appearance of these tumours to the naked eye is borne in mind. Early operation, as in man, gives the best promise of lasting freedom from recurrence. Out of 48 animals operated on . . . recurrence took place in 23, two recurring three times, six twice, and the remainder once. The interval of survival between the first operation and death averaged three to six weeks in the later operations, and in five cases was more than 100 days. . . . The number of recurrences and the length of survival are not strictly comparable with similar data from the human subject, since the prolongation of life is of prime importance in man, but in the case of the mouse other considerations than the prolongation of life have such importance that the animal may have to be sacrificed before death would have occurred."

Murray's clinical investigations were continued by Haaland[1] on a material consisting of three hundred and fifty-three primary growths, occurring in two hundred and eighty-eight mice. Roughly speaking, 90% of the tumors were adeno-carcinomata of the mamma corresponding very closely in their histology to the descriptions of Apolant and Murray.

Most of the ablations were performed as early and as completely as possible, but the difficulty of total removal was increased by the wide distribution of the mammary apparatus and the relatively large size of the tumors. Among one hundred and seventy-four operated mice ninety-six exhibited recurrence which, in two-thirds of the cases, took place before the end of the sixth week, and in one-fourth more

[1] *Fourth Sci. Report, Imperial Cancer Research Fund*, London, 1911, 49.

between the sixth and tenth weeks. Only isolated instances occurred later than this.

RELATION BETWEEN TUMOR AND HOST

The relation between a spontaneous tumor and the animal bearing it was one of the first problems to receive attention.

Loeb [1] found that an adenoma of the mammary gland in a white rat would grow in the rat herself but not in other rats, and Loeb and Leopold [2] extended this experiment to include the transplantation of one of the common mixed mammary tumors of the dog, which they had discovered in a spaniel. Grafts were inoculated into three other dogs (two spaniels and a fox terrier), but did not grow, while all of the pieces implanted into the original animal remained alive, neither increasing nor decreasing in size.

Bashford [3] succeeded in implanting two mice with their own spontaneous tumors, even though inoculations into normal animals were in vain. In a third experiment the grafting of a growth into the animal herself was fruitless, but fragments grew in two out of one hundred and fifty-six healthy mice as well as in one of the two referred to above as having given a positive result upon inoculation with their own tumors.

Bashford, Murray, and Cramer [4] concluded tentatively in the following year, however, that mice in which tumors had originated were not much more susceptible to other grafts than normal animals, and that the subject of a primary tumor could be inoculated only exceptionally with its own neoplasm. This latter conclusion, as will be seen on a succeeding page, does not agree with the results of more extended investigation by Bashford and his colleagues.

Borrel and Petit [5] inoculated a cancer of the horse into the animal in which the growth had arisen, and into a normal horse. Of four auto-inoculated grafts two were successful, although all transplantations failed in the normal animal.

[1] *Jour. Med. Research*, 1902, N.S., iii, 46.

[2] *Jour. Med. Research*, 1907–1908, N.S., xii, 299. [3] *British Med. Jour.*, 1906, ii, 208.

[4] *Proc. Roy. Soc.*, Series B, 1907, lxxix, 170.
Third Sci. Report, Imperial Cancer Research Fund, London, 1908, 323.

[5] *Bull. de l'Inst. Past.*, 1907, v, 1033.

Apolant [1] ingrafted eight mice each with its own spontaneous tumor and succeeded in obtaining growth in six of them. Eighty normal controls, on the other hand, afforded only three daughter tumors, all of them from the same sporadic growth. Of two inoculations into other spontaneously affected mice, one was fruitful and one was not, and the tumor successfully transplanted had been inoculated with a negative outcome into the animal in which it arose. This seemed to Apolant to speak for a considerable predisposition on the part of spontaneously affected animals toward the implantation of other tumors, the more so because such mice were always old, and in old animals the outcome of inoculation was usually much lower than in young.

Ribbert [2] inoculated several fragments of a fibroma into a dog from which it had just been removed, and at the end of four months found that each had attained the size of a walnut. Transplantation of grafts from these secondary tumors into the dog itself was again successful, but further attempts carried out in another dog were without result.

The question of the relative suitability of an animal for the implantation of its own tumor was very fully investigated by Haaland.[3]

"Out of 59 epithelial tumours of varied histology, 57 have grown on transplantation of the cells into the same spontaneously affected animal. Only two cases have been negative; of these, one should not be counted, for the mouse was ill practically the whole time it was under observation, and died four weeks after the inoculation. The other negative case occurred in a mouse surviving for 26 weeks after inoculation without exhibiting any growth at the point of inoculation. Of non-epithelial tumours there are 4, three spindle-cell sarcomata positive on re-inoculation, the fourth a melanotic tumour of peculiar structure and of very low power of growth; no evident increase was noted of the grafts re-inoculated simultaneously in four different places into the mouse in which it had arisen, although the tumour cells remained alive for four months.

"In the cases where the grafting has been carried out subcutaneously, the period elapsing between implantation and the appearance of a palpable nodule showing increase in size is as follows: —

[1] *Zeitschrift f. allg. Physiol.*, 1909, ix, Sammelreferat, 80.

[2] *Centralbl. f. allg. Path.*, etc., 1910, xxi, 625.

[3] *Lancet*, 1909, ii, 1588. *Fourth Sci. Report, Imperial Cancer Research Fund*, London, 1911, 57.

End of 1st week 6
" 2d " 23
" 3d " 13
" 4th " 8 (in two of these cases the inoculated tumour remained stationary for a long time — in one case for eleven weeks — then grew slowly)
End of 5th week 3
" 6th " 1 (large dose)
" 7th " 1 (" ")

"On the whole the rapidity of growth of the graft corresponds to the rate of growth of the primary tumour, and the same individual characteristics are retained in the histological picture. Two different tumours of the same mouse may exhibit different powers of growth when re-transplanted into the mouse itself. Sometimes, however, it is surprising how rapidly a graft from an apparently stationary haemorrhagic tumour may grow. In the great majority of cases the graft is already developing into a new tumour 2–3 weeks after inoculation into the same spontaneously affected mouse. This is on the whole considerably sooner than spontaneous tumours develop when first transplanted into normal mice, and the subsequent rate of growth is much more rapid than that observed in normal animals. This result is most easily accounted for by a large number of cells surviving transplantation, and the circumstance of their being more in concord with their surroundings."

The general result of Haaland's experiments was thus that autoplastic inoculation of a spontaneously attacked mouse was nearly always fruitful, except in the case of tumors of very low growth power. Cells re-introduced into the organism to which they were native found all the necessary conditions for continued existence and growth, but ingrafted into other spontaneously affected mice or into normal ones, were in a less favorable situation, and inoculation was accordingly not so often successful. This difference between the animal in which the tumor originated and any other animal has been well expressed by Bashford, Murray, and Cramer:[1] —

"The influence of the individuality, *i.e.* the sum total of changes

[1] *Proc. Roy. Soc.*, Series B, 1907, lxxix, 184.
Third Sci. Report, Imperial Cancer Research Fund, London, 1908, 337.

due to the past life of the organism, will be to make any mouse different from all others, and these differences will increase the longer the animal lives. The difficulty of obtaining success in the primary transplantation of spontaneous tumours would be accounted for by supposing that the new animals provide an environment to the cancer cells so strange, that they cannot survive the interruption to their nutrition. Their failure to grow does not necessarily imply that they would fail to proliferate in their new hosts if the conditions to which they had been accustomed could be immediately supplied in the experiment. Cells which have lived and become accustomed to the body fluids of one mouse for, say, two years, may easily die or fail to adapt themselves when transferred to the bodies of new animals. The frequency, in our experience, of large metastases in animals spontaneously affected is in marked contrast to the difficulties in obtaining growth in normal animals, and harmonises well with this view."

As Haaland did not neglect to indicate, his experiments suggested not only the advantages to be gained by the surgeon in employing tissues from the same individual in all transplantations but, furthermore, the peril to that patient in whom cancer cells were disseminated throughout an operation wound. On the other hand, they demonstrated the relatively insignificant danger to be apprehended from cancer cells introduced into individuals other than the one in which they had taken on malignant growth.

CHAPTER VIII

TUMORS OF A NATURE STILL UNDECIDED

A REVIEW of the three following growths has been relegated to a separate chapter because, as the best pathological judgment of the day is still divided over the question of their character, it seemed inexpedient to combine the results following their investigation with those that have been gained through the examination of tumors which have been almost universally accepted as malignant.

TRANSMISSIBLE LYMPHO-SARCOMA OF THE DOG

The transmissible "lympho-sarcoma" of the dog is, briefly, a tumor which occurs on the genital organs of this animal as the result of infection during coitus. Histologically it is indistinguishable from a small round cell sarcoma, while in its clinical course it reproduces many characteristics of the true tumors; the different opinions held by various authorities concern chiefly the manner in which the growth develops after having been introduced into a new host.

The first investigator to examine the nature of this tumor appears to have been Novinsky,[1] who reported its inoculation and the consequent production of nodules identical in structure with the primary growth, which was described as a myxo-sarcoma. Metastatic deposits were not found.

Wehr[2] considered the growth a carcinoma. He observed the spontaneous absorption of transmitted tumors, and in one instance the presence of metastases in the retroperitoneal and cervical lymph nodes, and the spleen.

Duplay and Cazin[3] endeavored for several years to transmit

[1] *Med. Vestnik*, St. Petersburg, 1876, xvi, 289. Cited by Sticker, *Arch. f. klin. Chir.*, (v. Langenbeck), 1906, lxxviii, 774.

[2] *Verhandl. d. deutschen Gesellsch. f. Chir.*, 1889, xviii, Teil ii, 86.
Arch. f. klin. Chir., (v. Langenbeck), 1889, xxxix, 226.

[3] *Atti dell' xi Congr. med. internaz.*, Rome, 1894, ii, Pat. gen., etc., 103.

various neoplasms from one dog to another, but sixty animals all proved refractory. Positive results were obtained, however with the growth now under discussion, which the authors described as resembling very closely the granulomata. A possible metastasis was noted in one case.

Geissler [1] recorded the presence of nodules throughout the body in a dog bearing a transmitted tumor, but whether or not they were genuine metastases it was impossible to say, because preservation had miscarried. The tumor was not the typical epithelial carcinoma found in man.

In the subsequent discussion of this paper v. Hansemann expressed his conviction that the tumor was not a carcinoma, although he would not deny either that it was malignant or transmissible.

Smith and Washbourn [2] described in detail the clinical course of the tumor as it occurred sporadically in dogs, and furnished numerous instances of its natural transmission. So far as structure was concerned the growths were sarcomata. In two autopsies carried out upon sporadically affected dogs no secondary deposits were found in the viscera, although metastasis had taken place in the inguinal lymph nodes. In no case where the disease was sporadic did regression of the tumor occur. In a later article the authors [3] gave an account of successful inoculation in thirteen out of seventeen dogs; and while most of these were young, one old animal at least had not proved refractory to the growth. A few dogs appeared to be naturally immune and resisted several successive attempts at transference, while animals which had been able to rid themselves of their growths were invariably refractory to subsequent inoculation.

A large part of the investigation of the dog tumor has been prosecuted by Sticker,[4] who has consistently held that the growth was a

[1] *Verhandl. d. deutschen Gesellsch. f. Chir.*, 1895, xxiv, Teil i, 87.

[2] *Trans. Path. Soc. London*, 1897, xlviii, 310.
Jour. Path. and Bact., 1898, v, 99.

[3] *British Med. Jour.*, 1898, ii, 1807.

[4] *Zeitschrift f. Krebsforsch.*, 1903–1904, i, 413.
Zeitschrift f. Krebsforsch., 1906, iv, 227.
Arch. f. klin. Chir., (v. Langenbeck), 1906, lxxviii, 773
Berl. klin. Woch., 1907, xliv, 486.
Deut. med. Woch., 1907, xxxiii, 867.

round cell sarcoma; for in addition to its microscopic structure, the recurrence, the infiltrative growth, the formation of metastases in the regional lymph nodes, and the fact that the cells were able to attain the general circulation were all evidences of its sarcomatous nature.

The cells differed from the normal lymphocytes of the dog by their larger size, by the possession of a distinct nucleolus, and by their staining reactions. At an early stage of growth the elements of the tumor lay tightly compressed and there was almost no intercellular tissue to be found, although at a later period a delicate reticular connective tissue made its appearance. In older portions of the nodules dilated capillaries were present, and the rupture of their walls produced occasional areas of hemorrhage.

Cells exposed to a temperature of − 14° C. for twenty-four hours, or heated at 50° C. for two hours, were still capable of proliferation, but the power to grow was abolished in those which had been kept at − 11° C. for twenty-five days. The fact that no tumors resulted from the introduction of crushed cells, or of filtered or centrifuged emulsions, excluded in all probability an extracellular parasite as an etiological factor, and it had been found impossible to demonstrate an organism by means of the usual bacteriological methods.

Very few dogs were possessed of a natural immunity and implantation could be performed in practically any part of the body, while in two out of three foxes tested, transmission was accomplished. Grafts in dogs produced no inflammatory reaction in their neighborhood, and the new tumor was not formed through participation of the surrounding connective tissue. Metastasis took place by way of the blood or lymph streams, but not very frequently in either case. Examination of the blood of tumor-bearing dogs showed an increase in the number of polymorphonuclear leucocytes and a decrease in that of the lymphocytes and eosinophile leucocytes, while in the presence of dead tumor material large mononuclear lymphocytes (macrophages) could be demonstrated. Spontaneous healing occurred in about 16 % of all dogs with transmitted tumors, and in some cases the rate of diminution was very striking — as in one where a tumor one hundred and seven days old, and as large as a hen's egg, vanished within fourteen days.

Dogs in which tumors were regressing or had entirely disappeared were always immune to re-inoculation, and the blood of these resistant animals had in two cases brought about a cessation of growth and partial regression in two subcutaneous tumors, although in common with the serum of normal dogs it had no effect upon tumor cells *in vitro*.

Animals bearing progressively growing tumors were re-inoculable, but not during the period immediately following the establishment of the first tumor. The tissues surrounding a growth constituted a *tumor zone*, and the rest of the body an *immune zone* within which the development of a second graft was inhibited. This condition existed, however, for a certain period of time only, and as the tumor increased in size it extended its sphere of influence at the expense of the immune zone, which grew progressively smaller until finally the organism produced no more immunizing material. A second phase then commenced, during which there supervened the widespread occurrence of metastases. These periods Sticker called the *pre-metastatic* and the *metastatic* stages, respectively.

White [1] thought that although these growths had been frequently referred to as sarcomata, they differed from that type of neoplasm in the human subject. In the first place, they were highly contagious, while secondly, they never infiltrated the surrounding tissues, gave rise but rarely to metastases, and were very slow in their growth. They had, furthermore, certain analogies with the infectious diseases, such as the high degree of contagiousness and the long incubation period. Still, the author did not believe that the contagiousness of these growths could safely be used in support of the argument for the parasitic nature of malignant disease, since the infective agent might be the tumor cell itself. The fact that the tumors could not be transferred to any animal except the dog pointed to the conclusion that transmission was an example of implantation rather than of infection. White had been informed that the disease was occasionally inherited.

Bashford, Murray, and Cramer [2] believed that in spite of the histological similarity between this tumor and the sarcomata there

[1] *British Med. Jour.*, 1902, ii, 176.

[2] *Sci. Reports, Imperial Cancer Research Fund*, London, 1905, No. 2, Part ii, 33. *Berl. klin. Woch.*, 1905, xlii, 1434.

Infective venereal tumor of dog's vagina. Primary growth. Transformation of connective tissue corpuscles into tumor cells. $\times \frac{400}{1}$.

Infective venereal tumor of dog. Graft removed 48 hours after transplantation. Degeneration of introduced tissue and proliferation of new capillaries. $\times \frac{400}{1}$.

Infective venereal tumor of dog. Graft removed 4 days after transplantation. Transformation of connective tissue corpuscles into tumor cells. Mitotic figures in many of the latter.

were several facts which militated against its inclusion under that class of growth. In the examination of a case of natural infection it was found that where the tumor adjoined the areolar tissue of the host there was apparent, unless growth had been so rapid as to produce marked pressure, a gradual alteration of the surrounding connective tissue corpuscles. While these cells were normally elongated and flattened structures with densely staining nuclei, a gradual transition occurred between this type and that characteristic for the parenchyma of the fully developed tumor. In the course of this transformation the cytoplasm of the connective tissue cells became more voluminous and the nuclei of these elements increased in size, but stained more faintly. Although additions to the tumor mass occurred by this conversion, the increase in size of the growth was mainly due to the active division of cells which already possessed the distinctive characteristics of tumor elements; hence in its later stages the tumor grew almost entirely from its own resources, closely imitating in this respect a true malignant new growth.

When grafts were examined after removal at varying intervals following implantation, it could be seen that the establishment of a new tumor was accomplished by a transformation of the connective tissue cells at the inoculation site into elements indistinguishable from those of the primary growth. The introduced tumor cells, however, degenerated very rapidly, only a few retaining their vitality for three or four days.

Another reason against including this tumor among the sarcomata was the observation that it never appeared naturally in animals before sexual congress, and was uncommon in old age. It showed, therefore, an age incidence different from that of sarcoma, the curve for which was similar to that of carcinoma.

Bashford and his collaborators were inclined to describe the tumor as a connective tissue reaction educed by a living virus still undiscovered, notwithstanding the fact that the growth resembled the neoplasms in its histological features, local mode of origin, partial increase from its own resources, and a limit of transmission within one species.

The transfer of this tumor to dogs the authors had found nearly always successful, no matter what breed was inoculated. A small

proportion of the transmitted growths regressed and ultimately disappeared, and in cases where this had occurred, as well as in dogs bearing large tumors, re-inoculation had succeeded.

Beebe and Ewing [1] discussed several diseases of the dog which might readily be confounded with lympho-sarcoma, and suggested that more than one malady had been included in the investigations of those who had worked with dog tumors, and that certain conflicting observations might find herein an explanation. They had been unable exactly to classify the growth While it most nearly resembled a large cell lympho-sarcoma, the elements were polygonal more often than round; moreover, the fine reticulum of a lympho-sarcoma was missing, the growth was much less malignant than large cell lympho-sarcoma in man, the arrangement of the cells in alveoli without lumina was sometimes distinct, the protoplasm was nearly homogeneous, and a mucinous discharge from the cut surface of the tumor was characteristic. The general histological features suggested the diagnosis of alveolar sarcoma or endothelioma.

The crucial point which these authors attempted to settle was the mode of origin of the transmitted tumor — whether the nodules resulted from the proliferation of inoculated cells or were produced by stimulation of the surrounding connective tissue elements. For the purpose of deciding this question, grafts were removed at intervals of from one to twenty-one days. After two days peripheral islands of tumor cells, chiefly normal, were found in contact with small blood vessels which seemed to belong to the original tumor, since they contained healthy red blood cells, as though circulation had been restored in them. Most of these islands were sharply separated from the host's tissues by fibrin or leucocytes. After three days the graft had become well fused with the surrounding subcutaneous tissue, and the peripheral islands of intact cells were usually incorporated into a definite layer surrounding the necrotic center. In this zone many blood vessels were to be found, some of which must have belonged to the graft, as they were too large to have developed in three days. The outer margin of the layer was sharply separated by strands of fibrin from the neighboring tissues, and no trace could be found of the transformation of fibroblasts, or any other elements of the host, into tumor cells. Many of

[1] *Jour. Med. Research*, 1906, N.S., x, 209.

the parenchymal cells of the graft were undergoing mitosis at this stage. In six-day specimens areas were occasionally encountered in which a mingling of fibroblasts and tumor cells had taken place, but no evidence could be discovered of the conversion of fibroblasts into malignant cells. Such areas were, however, rare, and all but small portions of the circumference of the graft was from the very first separated from the host's tissues by fibrin, fat, or connective tissue. In by far the greater part of the growing mass the authors found it impossible to conceive how the malignant cells could have been derived from the elements of the host.

They were forced to the conclusion, therefore, that the infectious lympho-sarcoma of dogs was a true malignant neoplasm, the cells of which showed, perhaps, a greater capacity for independent existence and infectivity than was seen in any other known tumor process.

The same authors,[1] in a discussion of the biology of the tumor cell, gave a preliminary account of a series of experiments designed to test the growth power of the cells of this tumor in various media. In physiological saline solution death supervened after forty-eight hours at room temperature. The serum of a rabbit immunized against pure nucleo-proteid prepared from the growth caused pronounced agglutination, but preserved the cells considerably longer than did salt solution. In sterile defibrinated blood from a dog with growing tumors the cells remained alive for as long as seventy-two hours, and at the end of this period they were apparently still capable of growth. At the end of forty-eight hours they presented figures highly suggestive of karyokinesis. When the medium was changed every twelve hours, viability was preserved for as long as ninety-six hours, although no evidence of cell division was discernible after forty-eight. In all the experiments, the blood from dogs with growing tumors seemed to be a more favorable medium than that from animals in which the tumor had regressed.

Because of the location of the primary growths, and the transmissibility of the dog tumor, Apolant[2] considered it a granuloma rather than a true malignant growth, and suggested as a possible etiological factor a virus analogous to that of lues.

[1] *British Med. Jour.*, 1906, ii, 1559.

[2] *Handbuch d. path. Mikroorganismen*, Kolle u. Wassermann, Jena, 1907, erste Ergänzungsband, Heft 2, 446.

Wade [1] recorded the transference of the growth to two foxes and a number of dogs. In normal animals of the latter species transmission was invariably successful, but those which had been able to rid themselves of a tumor were no longer susceptible.

Nodules excised at various intervals after introduction showed that the tumor was formed both from the cells inserted and the elements of the surrounding connective tissue, the latter, under the influence of the tumor cells, remaining immature and acquiring the characteristics of tumor cells instead of developing into finished fibroblasts.

Examination of a receding tumor demonstrated a smaller number of mitoses than that generally seen in this growth, a peripheral laminated border of fibrous tissue, and several hemorrhages. A nodule removed from a dog in which a number of tumors had just disappeared, was surrounded by a thick, laminated border of dense, fibrous tissue containing many polyblasts and damaged tumor elements, while in the central part there was found an enormous number of polyblasts, and an occasional degenerating parenchymal cell.

The lymphocyte and its derivatives having been found to play such an important part in the life cycle of the tumor, examination of the blood was undertaken, and daily estimations made in eight animals during a period of two months showed that establishment and growth were accompanied by a steady increase in the percentage of lymphocytes in the circulating blood.

The kidneys of twenty-three animals were examined. Sixteen were dogs which had been inoculated with portions of the tumor, five were animals into which a filtrate had been injected, one was a dog that had recovered from a sporadic growth, and one a fox in which a tumor had developed as the result of implantation. In all these animals, with but three exceptions, evidence of inflammatory change was present, the apparent age of the lesion corresponding closely with the length of the period during which the virus had been exerting its action. The kidneys of six apparently healthy dogs showed, on the contrary, no sign of inflammation.

Discussing the classification of the dog tumor, Wade expressed a preference for the name *infective sarcoma*, in the belief that the

[1] *Jour. Path. and Bact.*, 1908, xii, 384.

growth lay in the borderland between the infective granulomata and the true neoplasms.

v. Dungern [1] attempted to elucidate the nature of the tumor by a method employed in conjunction with Coca[2] in earlier investigations of a hare sarcoma. This tumor was able to proliferate in rabbits also, where it produced antibodies against hare blood, a proof that the tissue growing in the rabbit had been derived from hare cells. A similar procedure applied to the dog tumor growing in the fox showed that in all probability the nodule was composed of elements produced by the latter host, rather than of cells from the dog. The growth was accordingly not a true blastoma, but a granuloma, and presumably the product of a micro-organism. However, this conception could not be used to support directly a theory of the parasitic etiology of neoplasms, for in spite of a similarity in histological structure the type of growth exhibited by the dog tumor was enough to distinguish it from the true blastomata. Still, it was evident that there could exist an infective agent able specifically to excite the growth of certain cells and, therefore, that the distinction between the genuine blastomata and the granulomata need not be necessarily absolute.

As for therapeutic investigations upon the dog tumor, Sticker [3] brought about partial regression through the inoculation of immune serum, and cure by the repeated intravenous inoculation of live tumor cells. Salvarsan also exerted a definite curative action, but the effect of atoxyl was only temporary. Bergell and Sticker [4] announced the disappearance of a tumor after treatment with liver ferments. Beebe [5] and Crile and Beebe [6] were able to cure tumor-bearing dogs by bleeding them as completely as possible and transfusing them with the blood of immune dogs, while Beebe and Tracy [7] found that certain bacterial toxins exerted a destructive action upon the tumors.

[1] *Münch. med. Woch.*, 1912, lix, 238.
[2] *Zeitschrift f. Immunitätsforsch.*, etc., Orig., 1909, ii, 395.
[3] *Zeitschrift f. Krebsforsch.*, 1906, iv, 269, 310.
Berl. klin. Woch., 1908, xlv, 1391.
Centralbl. f. allg. Bakt., etc., Erste Abt., Orig., 1911, lix, 464.
[4] *Deut. med. Woch.*, 1907, xxxiii, 1521.
[5] *Jour. American Med. Assoc.*, 1907, xlix, 1492.
Jour. Med. Research, 1910, N.S., xvii, 389.
[6] *Proc. Soc. Exp. Biol. and Med.*, 1906–1907, iv, 118.
Jour. Med. Research, 1908, N.S., xiii, 385.
[7] *Jour. American Med. Assoc.*, 1907, xlix, 1493.

Gaylord [1] reported the successful treatment of the growths by the injection into them of uncoagulated blood from immune dogs, suggesting that the apparent importance of preventing the blood from clotting indicated that leucocytes might play an important rôle in preserving the immune characteristics of the injected serum.

THYROID ADENO-CARCINOMA OF THE TROUT

Bonnet [2] was among the first to describe this disease, not, however, as a malignant growth. Among the trout in a hatchery at Torbole on the Gardasee he observed an epidemic which in four months and a half destroyed not less than three thousand fish. In the trout affected, tumors appeared on the floor of the mouth and the gills, grew with great rapidity, and determined the death of the fish. In two cases Bonnet found soft, bluish red, smooth nodules on both sides of the tongue at the first and second gill-arches, and similar, although smaller, confluent tumors ventrally situated on the gill-arches, while in three others the lesions were limited to the gills, and in one case were unilateral. Microscopic examination, which offered "nothing characteristic," showed an epithelial, often tubular structure, and demonstrated the presence of bodies resembling lymphoid cells, which Bonnet took for gregarines.

Purvis,[3] in 1888–1889, discovered columnar cell carcinomata affecting the pharynx in two trout from the Solway fisheries in Scotland. According to Murray [4] the last epidemic of the disease in the British Isles occurred in 1888.

The disorder was next reported from New Zealand by Scott, Ayson, Gilruth, and Wilkie.[5] Scott discussed a form of tumor prevalent among the American brook trout in the New Zealand hatchery, which, originating in the middle line of the ventral wall of the pharynx, extensively involved the gill-arches. Upon microscopic examination Scott found that there took place at first a proliferation of the cells in

[1] *Jour. American Med. Assoc.*, 1909, lii, 411.

[2] *Bayerische Fischereizeitung*, 1883, No. 6, 79. (As this publication was inaccessible in this country, Bonnet's account has been taken from Pick's excellent monograph.)

[3] Cited by Bashford and Murray, *Sci. Reports, Cancer Research Fund*, London, 1904, No. 1, 9.

[4] *Third Sci. Report, Imperial Cancer Research Fund*, London, 1908, 52.

[5] *Reports of the New Zealand Department of Agriculture, Division of Veterinary Science*, 1891 and 1901–1902; cited by Pick.

the acini (the organ was not mentioned), and secondarily a destruction of boundaries which permitted an outgrowth of cells, and that these elements, infiltrating the stroma, finally produced a true carcinoma. Ayson encountered three cases of this "gill disease" in 1890 among American brook trout, and later among rainbow and other trout. The disorder occurred invariably in certain hatcheries and always among certain kinds of salmonoid fish. Gilruth received from Ayson three rainbow trout, each with a reddish soft tumor the size of a large walnut and involving the first and second gill-arches. The smaller alveoli were clothed with cylindrical or cubical epithelium, while the larger were lined with a single layer of columnar epithelium and contained cubical, more or less degenerated elements in the center. The capsule and the stroma carried wide, thin-walled blood vessels, and hemorrhages were frequently encountered.

Marianne Plehn [1] described the disease in the trout and the char, several affected specimens of which had been discovered in hatcheries and sent into the laboratory during the preceding few years. The tumors were in the neighborhood of the lower jaw, involving both mouth and throat, and were firm while still of small size, but became softer as the process advanced. In fish bearing tumors of considerable size the jaws were not infrequently forced apart, a condition which led to grave interference with both respiration and the intake of nourishment. These growths, which microscopic examination showed to be adeno-carcinomata, arose in the thyroid and presented at first a structure simulating the normal gland, the cancerous nature of the affection remaining masked until a later period when it displayed itself by invasion and destruction of bone and muscle.

Hofer [2] described the disorder under the name of malignant goiter, or adeno-carcinoma of the thyroid, and reported instances among lake trout living in a state of nature in the Mondsee. Of the causation of this rare disease nothing was known, and no parasite had been discovered which could be brought into etiological relationship with it.

Jaboulay [3] studied six trout with thyroid adeno-carcinomata invading

[1] *Allg. Fischerei-Zeitung*, 1902, xxvii, 117.
[2] *Handbuch der Fischkrankheiten*, Munich, 1904, 191.
[3] *Lyon méd.*, 1908, cx, 335.
Province méd., 1908, xix, 186.

all the tissues in the neighborhood of the gland and, in an advanced stage, involving even distant organs. In the opinion of this author the disease, which was both hereditary and contagious, was the outcome of infection with myxosporidia.

Pick and Poll [1] described a thyroid tumor of the trout, glandular in structure throughout its early stages, but possessing the characteristics of a medullary carcinoma when more advanced.

Pick himself [2] published a much fuller description of the disease about two years later. That it was not of equal distribution was shown by the fact that, in spite of reported epidemics in other parts of the world, the disorder was totally unknown in North and Middle Germany, while in the southern part of the country only sporadic cases had been encountered. The tumors were indubitably carcinomata with the power of infiltrating and destroying neighboring tissues, although they varied in structure from parenchymatous goiter to medullary or scirrhous carcinoma. Histological appearance was, however, no guide to clinical behavior, and those growths which looked most benign were not infrequently found to infiltrate most seriously. Pick had never seen any metastatic deposits, if one doubtful instance were excepted, although he confessed that the search had not been by any means so careful as that which had been prosecuted upon neoplasms of the mouse. The tumors originated in the thyroid gland, as was clearly shown by their location and, above all, by their architecture, and no variety of salmonoid fish living in hatcheries was exempt, although the growths were often curiously limited to one species in a hatchery, as when, for example, where *Salmo iridens* and *S. fontinalis* were both kept, only the former was affected. On the other hand, in a neighboring hatchery neither might be spared. Again, certain ponds would be found to contain affected fish while in others the trout remained free from the disorder. The disease attacked chiefly fish over two years old, and from 2% to 7% of those exposed fell victims to it.[3] Pick did not see any necessity for implicating parasites in the etiology of the disease or for assuming that the carcinoma, as

[1] *Berl. klin. Woch.*, 1903, xl, 547.

[2] *Berl. klin. Woch.*, 1905, xlii, 1435, 1477, 1498, 1532.

[3] According to Plehn (*Travaux de la deuxième Conférence internat. pour l'Étude du Cancer*, Paris, 1911, 227) 70% of the fish in a pond may be affected.

such, was endemic. Reasoning from what was known of human cases it was very probable that in the trout the primary lesion was a simple endemic goiter evolved, perhaps, by some condition of the water, and that the malignant proliferation was secondary to this lesion.

v. Hansemann [1] expressed the opinion that, in spite of the absence of metastases, there could be but little doubt of the carcinomatous nature of the tumor which Pick had described.

Plehn [2] was unable to accept the water of the hatchery as an etiological factor, for only one of two kinds of trout in the same pond might be affected, while, furthermore, the disease might disappear after having involved a hatchery for several years, and this without any change having been made in the water. To explain the causation of the disease there remained, in addition to a parasitic hypothesis, the assumption that all affected fish were the descendants of a few groups of parents, and that hereditary influence was a powerful predisposing factor.

Gaylord [3] published a preliminary description of an epidemic among two-year-old brook trout and brown trout. At the hatchery in question, water was supplied from a spring issuing from a hillside and emptying into a pond, to be piped from there to a small reservoir and finally through a series of tanks. Carcinoma of the thyroid had been discovered among the fish in this pond two years previously, and a year later the pond was emptied and re-stocked with young fish. One of the tanks fed from the water passing through this pond, and holding nearly four thousand two-year-old brook trout raised from eggs procured at a hatchery where the disease was not known to exist, contained seven hundred fish in various stages of the disorder. In an adjoining tank, which had no connection whatsoever with the one in question, there were two hundred brown trout reared from eggs hatched on the premises; of these, from 3% to 4% were affected. The infected fish had at no time come into direct contact with those in the upper pond, where it was known that the disease had existed, neither had the brook trout and the brown trout been at any time in contact with each other. Gaylord believed that the conditions discovered in the hatchery under discussion pointed very strongly to the infectious nature of this form of cancer, and to the suggestion

[1] *Berl. klin. Woch.*, 1905, xlii, 1542. [2] *Zeitschrift f. Krebsforsch.*, 1906, iv, 560.
[3] *Jour. American Med. Assoc.*, 1909, lii, 411.

that the contagion was water borne. It was possible that in this instance the feeding of liver was in some way connected with the outbreak, for in another hatchery, where this material had been replaced by chopped sea fish, the disease had disappeared entirely, although formerly it had been endemic.

Continuing his report, Gaylord [1] described a fish with carcinoma of the thyroid and a similar growth on the lower jaw, a distribution which made it plain either that the tumor could metastasize or that it was transplantable. Analysis showed that trout occupying ponds which received water from those containing cases of the disease might become affected, although a great many, and more particularly hybrids, were immune. The disease attacked fish both large and small and, especially in the former, spread rapidly, eroding the bone, destroying the cartilage, and infiltrating the muscle. The tumors showed varying characteristics, frequently retaining the alveolar type with colloid or, again, showing a structure strictly adenomatous; but in all cases there were areas which presented the features of solid carcinoma.

In the discussion of this paper Stockard [2] pointed out that many of the infiltrative phenomena in the affected thyroid might be due to the fact that the gland was not encapsulated, and that small follicles often appeared among the muscle fibres and loose tissues of the branchial region. On the same occasion Gudernatsch offered an account of the normal thyroid gland of the *Teleostei*. This was not a compact, uniform organ as it was in mammals, but was broken up into numerous single follicles, the distribution varying not only with the species but with the individual. The follicles were generally most densely packed around the ventral aorta and its branches to the gills, while toward the periphery the arrangement became less close until the follicles lay completely separated. Their distribution extended as far as the neighboring structures would allow and they even invaded other tissues, reaching out laterally along the gill arteries and sometimes even penetrating the gills. A more detailed description of this gland in the fish may be found in two papers by Gudernatsch [3] and in the article by

[1] *Jour. American Med. Assoc.*, 1910, liv, 227.
[2] *Jour. American Med. Assoc.*, 1910, liv, 227.
[3] *Jour. Morphol.*, 1910, xxi, 709.
Johns Hopkins Hosp. Bull., 1911, xxii, 152.

Maurer,[1] who was the first fully to describe the diffuse arrangement of the thyroid gland in the fish, although Baber [2] had previously mentioned a few detached vesicles in the thyroid of the skate.

According to Ewing,[3] while the diffuse condition of the gland did not affect the conclusion that genuine thyroid tumors did arise in some fish, it indicated that the local extensions of these growths were not always to be regarded as signs of metastasis and malignancy. He [4] considered it highly improbable that the disease was parasitic in its origin.

Gaylord [5] thought that the non-encapsulation of the gland would not account for the infiltration of vessel walls, bone, and cartilage seen when the growth had reached the status of carcinoma. He had found the disease endemic in probably not less than 75% of the hatcheries containing salmonoids throughout the United States, and epidemic from time to time, and had observed a certain variation in the severity of the disease and in the characteristics of the lesions in different epidemics. Thus, in one hatchery, more than 50% of the tumors presented the structure of carcinoma, while in an epidemic encountered the following year in another locality the growths were more like simple goiters. If the disease started in the uppermost tanks, it progressed with the course of the water through the lower ones, and the percentage of infected fish increased from above downward; but where it occurred low down it had never been found to proceed upward against the stream. About 50% of fish taken from infected pools and placed under better hygienic conditions would recover, although spontaneous cure occurred also in tanks where the disease was in progress. Fish in which tumors had disappeared did not acquire the disease a second time, and entire groups had been observed to resist attack, but whether through possession of natural immunity or through a condition of resistance conferred by previous recovery, had not yet been determined. Muddy water from the most infected ponds produced goiter in dogs after five weeks and caused marked enlargement of the thyroid in rats.

[1] *Morphol. Jahrb.*, 1886, xi, 129.
[2] *Philosoph. Trans. Roy. Soc. London*, 1881, clxxii, 580.
[3] *Jour. American Med. Assoc.*, 1910, liv, 228. [4] *Arch. Internal Med.*, 1908, i, 176.
[5] *Travaux de la deuxième Conférence internat. pour l'Étude du Cancer*, Paris, 1911, 787.

Marine and Lenhart [1] undertook experiments designed to discover any possible connection between the ordinary goiter (active thyroid hyperplasia) of fish and animals, and the so-called cancerous affection of the thyroid. For the reasons that young fish were more affected than old, that the hyperplasia was checked by the addition of Lugol's solution to the water in the tank, that the removal of fish to an open brook effected a cure, that the clinical incidence of tumors was in close relation with the supply of water, and finally, because the severity of the disease as determined by histological examination was also directly related to the water supply, these authors could not accept the prevailing opinion that the disease was true cancer. On the contrary, they believed it to be an extreme illustration of endemic goiter, the end stage of which was cretinism. All phases of the lesion reacted to iodine, the mild degrees more rapidly than the severer, and the disorder in its earlier stages underwent involution after from two to three weeks of treatment, although at later periods one or two months might be necessary. The iodine reaction was a specific test for functional hyperplasia, since it did not occur in the case of true tumors. As the authors had been unable to find any point in the course of the disease under consideration at which it did not yield to iodine, they concluded that there was no stage which could be looked on biologically as cancer. Although they had been unable to transmit the disease by grafts from one fish to another, they did not consider that their technic had reached a degree of perfection sufficient to permit the conclusion that the affected tissue was not transplantable. They had never observed secondary growths, with the exception of two doubtful tumors in the gills and in the lower lip, but it was questionable whether nodules in these locations might not be due to hyperplasia of existing anlagen. There was no evidence that the disorder was either infectious or contagious, but much in favor of the view that it was the symptomatic manifestation of a metabolic and nutritional disturbance.

There were three major conditions which, in some way still obscure,

[1] *Jour. Exp. Med.*, 1910, xii, 311.
Johns Hopkins Hosp. Bull., 1910, xxi, 95.
Bull. No. 7, Dept. Fisheries, Pennsylvania, Harrisburg, 1910.
Jour. Exp. Med., 1911, xxii, 455.
Bull. No. 8, Dept. Fisheries, Pennsylvania, Harrisburg, 1911.

influenced the thyroid growth: Limited water supply, overcrowding, and overfeeding with a highly artificial and incomplete food. The water of the hatchery was not intrinsically goiter-producing, because fish would not develop the disease unless at least the factor of overfeeding with an incomplete food were in operation, and because they recovered if the overfeeding and overcrowding were corrected, even though remaining in the same pond. Therefore it seemed probable that food was the major factor in bringing about some fault of nutrition favorable to goiter development, although it was impossible to suggest what elements in the diet were implicated.

That the thyroid was an extremely labile organ, reacting quickly to relatively slight variations in the body metabolism and even showing a slight daily histological change, has been frequently emphasized by these authors;[1] and while they found that thyroid changes did not take place in fish so rapidly as in mammals, the range of histological variation was nevertheless equally wide.

Gaylord[2] was able to substantiate the action of iodine upon the thyroid as described by Marine and Lenhart, and found that affected fish kept in running water to which potassium iodide was continuously added in a concentration of one to five million, showed distinct histological changes after a few days. They were similar to those described by the authors just cited, and consisted in evidences of cessation of active proliferation on the part of the glandular disease, flattening of the epithelium, and the presence of colloid within the alveoli. But Gaylord discovered that bichloride of mercury in the same concentration would produce all these alterations more effectively and in less time, and described a tumor of seven millimeters diameter which disappeared completely in the course of forty days' exposure to mercury. Hence he concluded that the action of iodine upon the thyroid was not specific. In the observations that the disease was of epidemic occurrence, that its incidence increased with the flowing of water from pond to pond, that it was "created" in certain ponds and that it was not a result of the original constituents of the water, that fish recovered spontaneously and were then immune, that the disorder could be markedly influenced and perhaps cured by iodine and mercury, both

[1] *Johns Hopkins Hosp. Bull.*, 1911, xxii, 217.

[2] *Travaux de la deuxième Conférence internat. pour l'Étude du Cancer*, Paris, 1911, 789.

well-known antiseptics, Gaylord saw evidence to support the contention that the disease was infectious.

TRANSMISSIBLE SARCOMA OF THE FOWL

Fujinami and Inamoto [1] described in 1910 a spontaneous connective tissue neoplasm of the hen, probably a myxoma or a myxo-sarcoma, which was so readily transplantable that inoculation had been almost invariably successful. Spontaneous absorption was relatively common and seemed to depend upon some special condition of the host. In the case of transplanted tumors metastases were discovered in the skin, eyelid, lung, heart, liver, kidney, and intestinal wall. At the time of writing, the neoplasm had reached its twentieth generation without any essential change in its histology having occurred, and there was no evidence to support the hypothesis of parasitic etiology. Growth power was abolished by boiling, but not by a two hours' exposure to 50° C. or −15° to −20° C., or by grinding the cells in a mortar with sand. The action of antiseptics for from one to eighty minutes was fatal.

Another transmissible tumor has been described since then by Rous [2] in a young barred Plymouth Rock hen of light color and pure blood. First noticed when the bird was about fifteen months old and approximately two months before she was brought into the laboratory, the neoplasm had developed slowly until, when first seen by Rous, it appeared as a large, irregularly lobular mass projecting sharply from the right breast. This growth was removed by operation and frag-

[1] *Verhandl. der japanischen path. Gesellsch.*, 1911, erste Tagung, 114.

[2] *Jour. Exp. Med.*, 1910, xii, 696.
Jour. American Med. Assoc., 1910, lv, 1805.
Jour. American Med. Assoc., 1911, lvi, 198.
Proc. American Philosophical Soc., 1912, li, 201.
See also Rous and Murphy.
Jour. American Med. Assoc., 1911, lvi, 741.
Jour. Exp. Med., 1912, xv, 119.
Jour. Exp. Med., 1912, xv, 270.
Jour. American Med. Assoc., 1912, lviii, 1938;
and Rous, Murphy, and Tytler.
Jour American Med. Assoc., 1912, lviii, 1751.
Jour. American Med. Assoc., 1912, lviii, 1840.

ments of it were at once inoculated with a trocar into the opposite breast and the peritoneal cavity of the host, and into two other hens from the same setting of eggs. Upon section the tumor was found to have undergone widespread coagulation necrosis at the center, although it had a margin of translucent, rather friable, yellowish pink tissue with a glistening, finely striated surface. Microscopically the tumor suggested sarcoma. Thirty-five days after implantation, the host was dead of intraperitoneal growths, and in the breast of one of the inoculated fowls a large nodule had developed.

Microscopic examination of the original growth and of the nodules occurring elsewhere in the host following implantation, showed them to be sarcomata composed of loose bundles of spindle cells, crossing in every direction and separated from the smaller blood vessels only by endothelium, a structure which was reproduced with great fidelity in all the growths of subsequent generations. Intercellular fibrils were demonstrable with Mallory's phosphotungstic acid stain, but they were rare in the more cellular portions of the growth. The areas of necrosis seemed to be dependent in general upon insufficient vascularization, although hemorrhages from thin-walled blood vessels were occasionally responsible for their production. In spite of a fairly well marked capsule the growth showed a certain tendency to infiltrate, not so actively, however, as did its daughter tumors. Repeated bacteriological examination yielded negative results, and the growth was not transmissible to pigeons, ducks, rats, mice, guinea-pigs, or rabbits.

The daughter tumors throve at first only in intimately related fowls of the pure stock in which the spontaneous neoplasm had been discovered, but later transplantations into similar fowls of pure blood from another source were successful, and the tumor finally came to grow in about 80 to 100% of pure bred, light-barred Plymouth Rocks, and in an occasional individual that showed by its plumage the slight admixture of some darker strain. Contrasted with this outcome was that obtained in the first four generations, where the number of successful transplantations was but three in twelve. It was never possible to propagate the tumor in any breed except Plymouth Rocks, and proliferation was especially active in young birds. The re-inoculation of fowls that had proved refractory to implantation was uniformly

unsuccessful. Growth became so vigorous in the later generations that a fragment of from one to two millimeters in diameter might give rise within three weeks to a nodule having an average diameter of about eight millimeters, and retrogression of a developed tumor, fairly frequent at first, became a rare event save in cases where the host was ill. While the growths obtained from the first inoculation required seventy-one days to reach an average diameter of about four centimeters, and to affect seriously the health of the host, tumors belonging to later generations, and the product of a similar mode of inoculation, attained an average diameter of about nine centimeters three weeks after the implantation of a fragment two millimeters in diameter, and frequently determined a fatal issue in from twenty-six to thirty days. In the seventh generation, the average time required after inoculation for the development and dissemination of secondary nodules was approximately one-half of that demanded in the third.

Metastasis, which occurred by way of the blood stream and but rarely through the lymphatics, was more frequently observed in the later than in earlier generations, and secondary nodules were encountered less often in the heart than previously, but more commonly in the liver, while the spleen, exempt until the seventh generation, contained secondary deposits at that period in two cases out of twenty-five, and was thereafter frequently involved.

In a study of early stages it was found that during the first forty-eight hours the tumor fragment, unchanged in appearance, occupied a rent in the host's tissues surrounded by exudation and a moderate accumulation of polymorphonuclear leucocytes. The connective tissue of the host was edematous at the torn margin, its blood vessels dilated, and toward the end of the period under description fibroblasts and macrophages appeared. During the third day the graft often united at one or more points with the host's tissues, and although it still remained unvascularized, proliferation was in active progress, cell division taking place chiefly by amitosis, and the fragment was not infrequently much increased in size. Its loose structure facilitated nourishment by a direct interchange of fluids, but unless the graft were broken or of very small size necrosis took place at its center. The polymorphonuclear leucocytes commenced to disappear at this time and fibroblasts, macrophages, and an abundant supply of newly

formed capillaries took their place. In some fowls small round cells, histologically identical with the lymphocyte, collected in limited number, localizing at points where the implantation had become attached to the surrounding connective tissue, but in very susceptible hosts this, like all cellular reaction, might be absent. By the fourth day or later the fragment had increased greatly in size, vascularization was accomplished, and establishment of the nodule might be considered perfect. Even before this period, however, tumor cells were often discovered invading the tissues of the host at points where these were in contact with the graft, proliferating strands of spindle cells extending into the normal structures and utilizing for their support the blood vessels and connective tissue already present. The activity of the neoplastic cells, once the tumor had become established, was startling, and as many as 46% had been found in process of segmentation at one time, of which 42% were in amitotic and 4% in mitotic division. It was not necessary for the development of tumor fragments that they be brought directly into contact with the connective tissue of the host, for grafts placed in the peritoneal cavity might establish themselves in spite of its endothelial lining, proliferating sometimes in a way to suggest cell cultures in a fluid medium and at length obtaining a blood supply from the underlying tissues.

In naturally immune fowls, or in those with acquired resistance, the phenomena in action about the tumor fragment did not differ during the first forty-eight hours from those in susceptible hosts. Following this period death of the graft took place in one of two ways. (*a*) The fragment often remained for many days unvascularized and almost unattached to the surrounding tissues, and underwent a gradual necrosis in which the marginal cells were the last to succumb. These elements might live for a week or ten days and might even multiply at first to some extent, but they did not invade the connective tissues at those points where they were in contact with them. Meanwhile leucocytes disappeared; fibroblasts and macrophages were substituted, lymphocytes in small numbers infiltrated the zone of living tumor, and by the end of two weeks, as a rule, the graft was wholly necrotic and reorganization under way. (*b*) More frequently the implantation increased in size, invaded the tissues of the host, and became vascularized with the same rapidity as in susceptible fowls, but imme-

diately upon its obtaining a foothold a lymphocytic reaction supervened in the surrounding tissues, so intense in character that even by the fifth day the graft might be embedded in a mass of small round cells six to eight times its own diameter. A certain degree of infiltration of the fragment occurred during this period, and despite its blood supply the tumor tissue was already degenerated. These two sets of phenomena leading to the death of the implanted fragment might both be discovered in one host, an observation suggesting that their incidence was largely determined by local conditions.

The death of the implantations could not be ascribed to their failure to acquire a supporting and vascular stroma, as Bashford and Russell had assumed in explanation of the death of grafts in hosts with acquired resistance, for in by far the greater number of unsuccessful transplantations vascularization actually occurred, although it existed for a brief period only. The authors agreed with DaFano regarding the significance of the lymphocyte for immunity. In the case of the fowl, however, the lymphocytic response was more vigorous than in mammals, possibly for the reason that the lymphocyte not infrequently constituted over 40% of the white cells in normal individuals and, furthermore, because this type of cell responded to certain processes more actively in the fowl than in mammals. No general lymphocytosis accompanied the local reaction surrounding the tumor.

It was found that the sarcoma could be transmitted after an emulsion had been passed through a Berkefeld filter (No. 5 medium) impermeable to *B. prodigiosus*, or by means of the dried and powdered neoplastic tissue. Growths resulting from the inoculation of a filtrate were much longer in making their appearance than where grafts had been deposited in the ordinary way, not a trace of tumor being palpable for from ten days to three weeks, while subsequent proliferation went on more slowly than in control tumors.

The growths following upon injection of a filtrate, which were first noticeable as minute nodules at some point in the needle track, developed in only a small number of inoculated fowls; but when the causative agent was introduced in the form of dried powder suspended in Ringer's solution the sarcomata appeared as more or less diffuse masses at the site of injection, and developed in a much larger pro-

portion of the inoculated fowls. These observations led Rous and his collaborators to assume that the causative agent required for its action a cell proliferation or derangement such as that induced by the needle prick or the presence of dried tissue, and experiment proved this hypothesis tenable. A number of susceptible fowls having been injected in one breast with a large quantity of active filtrate and in the other with an equal amount of filtrate to which had been added a little sterile washed diatomaceous earth, it was found that in the latter case a larger number of growths developed than where the filtrate had been injected alone, and also, that in the one instance the tumor regularly arose as a diffuse mass owing to simultaneous proliferation from many foci, whereas in the other it slowly appeared as a small discrete nodule in the needle track.

The importance of injury was demonstrated again in the observation that when large quantities of an active Berkefeld filtrate free of foreign particles were injected into the circulation, only four tumors resulted among seventeen chickens, while in contrast to this proportion, seven out of twenty developed growths after a little diatomaceous earth had been added to the filtrate. Apart from these figures, however, even the site of those tumors arising after the injection of filtrate free of foreign particles demonstrated the importance of cell derangement, for in three cases the growth had its primary seat in the functioning ovary, an organ where injury and proliferation are of daily occurrence, while in the fourth the growth was in a liver where the authors felt that some focal derangement must have been present.

Significant though trauma might be, its absence would not of itself suffice to explain the striking lack of infectivity displayed by the sarcoma under ordinary circumstances. Thus, for three years over twelve hundred chickens, many of them bearing the sarcoma, had been confined at one time or another in relatively close quarters. To some of them fresh sarcomatous tissue had been fed, and many others must have been contaminated with the dried tumor, in which it was known that the causative agent would remain alive for over seven months, and yet, although trauma and other types of injury had been frequent among the chickens so exposed, not one had developed the sarcoma except after direct inoculation. Furthermore, the growth was not naturally endemic among fowls.

The nature of the other factors conditioning tumor development had not yet been determined, but it was evident that they were both local and general. It was still inexplicable why, even with the agent in its active form, and with the factor of injury supplied, many of the fowls injected with a large amount of filtrate should fail to develop a growth, and why the tumors following intravenous injection were seldom primarily multiple, despite the numerous injuries everywhere caused by the infusorial earth.

The conditions governing the appearance or non-appearance of growths possessed considerable interest in view of the close resemblance of the tumor to some malignant mammalian neoplasms, not only in regard to growth and general behavior, but also in the obvious lack of infectivity under ordinary circumstances; and that injury should have a share in determining tumor development was noteworthy in view of the importance of this factor as a contributory cause of mammalian sarcomata, including those of man.

To determine whether pulmonary metastases were invariably due to the proliferation of transported cells, or whether they might not sometimes follow localization of the causative agent as such, a suspension of fresh tumor in Ringer's solution was injected intravenously. It was found, however, that secondary deposits arose uniformly by a survival and growth of the inoculated tumor cells, and this observation, no less than the one that visceral metastases occurred by far most frequently in the lungs, led to the assumption that secondary nodules were a product of the proliferation of cells sieved out of the circulation by the pulmonary capillaries in the usual way. But to explain the frequent involvement of the lungs there might be entertained the alternate hypothesis that the extrinsic agent, as such, engendered tumors more easily in these than in other organs. That this was not true, however, was demonstrated by the outcome of intravenous injections of a Berkefeld filtrate containing the causative agent, for in only two out of eleven fowls developing the growth after such treatment was the sarcoma primary in the lungs, despite the fact that the agent had been directly carried there. In seven of the cases diatomaceous earth had been added to the filtrate and had lodged for the most part in the pulmonary capillaries, producing injuries such as had been found to favor the action of the agent. Although it was thus

certain that metastases were referable in general to a development of cell emboli, there still remained the question whether secondary tumors might not occasionally be caused by the filterable agent as such, for that this was able to enter the circulation was shown in some instances by the appearance of a sarcoma after the injection into susceptible hosts of large quantities of centrifugalized plasma from fowls moribund with metastasizing growths. The general importance of injury in determining the action of the agent led the authors to attempt the achievement of secondary tumors through trauma, and under conditions which would show them to be independent of transported cells in their origin. Tissue derangements in tumor-bearing fowls were produced by the injection of Scharlach R or diatomaceous earth, or by means of incisions allowed to heal by secondary intention, but numerous experiments gave results almost entirely negative, the sarcoma failing to localize at the seat of injury. In only a single case was there suggested the direct action of the extrinsic agent, where, in a chicken bearing a very large sarcoma, but with no discoverable metastases in the viscera, a small secondary growth was found post-mortem at the site of injection of diatomaceous earth. The heart was not examined for a patent foramen ovale. Two other instances of localization at the point of injury were less significant, since there was demonstrated the presence of a large number of metastases in the lungs and elsewhere. A fourth case came to attention during autopsy on a fowl with a large inoculated tumor and many metastases in the lungs, the remainder of the body having been spared with the exception of the oviduct, which contained an inspissated egg and exhibited a reactive thickening and increased vascularization of its walls. Whether in any or all of these tumor-bearing fowls the extrinsic agent, as such, had been active in evolving a growth at the site of an injury, the authors were unwilling to declare.

The conditions governing the curious relationship between the tumor and its cause were of great interest. How did it happen that the sarcoma, although ultimately dependent upon an extrinsic agent, was dominated in its behavior by the cells composing it? In answer to this question the author suggested several replies. The production of a neoplastic change by the causative factor might take place with such extreme slowness, as compared with the proliferation of cells

which had once become neoplastic, that growth might seem to occur entirely through the multiplication of the cells in an initial focus. Tumors did doubtless result wholly in this latter way in many cases, because of a second peculiarity of the agent — its dependence on a special set of conditions in order that it might initiate neoplastic change. The necessity for these conditions would go far to explain the failure of the agent to take an active part in the dissemination of the tumor throughout the body, granting that the agent was present in the circulation before the fowl was moribund. The possibility of the existence of immune processes effective against the agent when it was separated from the cells should also be kept in mind, although the evidences of resistance thus far recognized had been directed against the cells themselves, as in the case of mammalian tumors.

Regarding the nature of the filterable agent causing the sarcoma, the most important question for decision was whether it was living, to which the most direct reply would be to grow and transfer it in culture; but investigation in this direction had not been particularly successful. Differential filtration showed that in a dilute tumor extract the agent would pass through Berkefeld filters which would hold back at the same filtration *B. fluorescens liquefaciens*, an organism measuring 0.5 micron by 1.0 to 1.5 micra, while on the other hand, Chamberland bougies (F) were impermeable. Were it not known that filters of fine texture would hold back complex proteins these findings might indicate that the agent was organized, and perhaps even visible, although repeated attempts to demonstrate it with special stains or with the dark field had been in vain.

The agent would retain its activity in dried tissue for seven months, and for at least one month in tissues placed in 50% glycerine, undergoing gradual attenuation, however, in both instances. Repeated rapid freezing and thawing, which reduced the tumor to a pulp, did not greatly lessen the activity of the associated agent, although the resistance of the latter to heat was but little greater than than of the neoplasm itself. The sarcoma, when submitted to a heat of 50° C. for fifteen minutes, failed absolutely to grow *in vitro* where its proliferation was ordinarily very active, in spite of the fact that it often gave rise to tumors when inoculated into susceptible fowls, even after exposure to 53° C. Whether growth *in vitro* was a real index of viabil-

ity was uncertain, but the ability of the filterable agent to withstand a temperature of 50° C. for fifteen minutes was demonstrated a second time by the production of tumors with tissue which had been dried, ground, and heated after suspension in Ringer's solution. Material that had been raised to a temperature of 55° C. for fifteen minutes, however, never developed into a tumor. In sarcomatous tissue autolyzing at the temperature of the chicken's body (41° C.), the agent remained active for less than forty-eight hours, while toluol and chloroform in the proportions employed to prevent bacterial growth during autolysis destroyed it in less than two hours, as did 50% alcohol or 2% phenol. Unlike the virus of poliomyelitis the agent could not resist exposure to 0.5% phenol, and like the animal organisms, in distinction to most of the vegetable (v. Prowacek), it was rapidly destroyed by bile and by saponin in high dilutions. Although no single attribute among those determined would suffice to show the nature of the agent, its characters were in general those ordinarily associated with micro-organisms.

It was found that the sarcoma could be transplanted into the developing embryos of barred Plymouth Rock eggs or into the membranes, either by the deposition of tumor fragments or by injection of a cell-free filtrate. That growth under these conditions was excellent was shown by the observation that, in tumors of the embryo, more cells on the average were in process of division at one time than in those of the adult.

To regulate the amount actually implanted in any tissue was, however, difficult, for that which it was intended to place in an extra-embryonic membrane largely escaped at either side, while that implanted in the embryo itself not infrequently followed the needle to the surface. The majority of the growths occurred in the outer membrane (fused chorion and allantois) for the obvious reason that this was necessarily pierced in reaching other structures, so that its inoculation could hardly be prevented during withdrawal of the needle. Tumors were discovered also in the fused allantoic and amniotic membranes and the extra-embryonic body cavity, according to the point at which puncture had been undertaken. Although no attempt was made to plant the growth in special regions of the embryo, since the instability of the latter under pressure of the needle rendered the procedure difficult, by indiscriminate implantations growths had

been obtained in the chest wall, heart, liver, peritoneal surface of the gizzard, and the soft tissue of the thigh and of various other parts, all appearing in the track of the needle. In the adult fowl it was not always necessary for the development of tumor fragments that they be brought directly into contact with connective tissue, since pieces introduced into the peritoneal cavity might establish themselves despite the endothelial lining, but it was not so in the extra-embryonic membranes of the chick. Here a layer of ectoderm or endoderm but one cell thick constituted an absolute protection against surface implantation, and only where a mesodermal layer was exposed, as, for example, on the outer side of the allantoic membrane, did such implantations occur. When incubation was far advanced at the time of inoculation, and the chick was allowed to hatch, tumors that had developed in the outer membranes were cast off with them. Growths in the yolk-sac were drawn with it into the body where they continued to develop.

Among one hundred and forty-seven inoculated eggs, one or more tumors resulted in one hundred and eight; but of the thirty-nine negative attempts, inoculation had been done at a very early period in seventeen, in nine others a Berkefeld filtrate alone had been introduced, and in five, dried material, leaving but eight that gave negative results after the most effective method of transplantation, as compared with one hundred and eight positive results from inoculations of all sorts. Histologically the tumors differed but little from those in adult fowls; although the structure of the sarcoma was much looser and the spindles might be so attenuated as to suggest myoma, necrosis was a rare occurrence, and infiltration seldom well marked except where the growths lay in structures which, like the heart or liver, opposed a certain amount of resistance. Regional metastases, presumably distributed by the lymphatics, were fairly common in the outer allantoid membrane, assuming the form of small nodules near the main growth and along the larger vessels supplying it. Remote metastases, however, had never been encountered, probably because of the structure of the membrane. By using the outer allantoid membrane as the injection site, and employing the growths found there after seven days, the authors had succeeded in transplanting the sarcoma to three successive sets of eggs, only to lose it on the fourth transfer by the death of all the embryos. The same event terminated

more quickly two other series. During continued propagation in the egg no change was noted in the behavior of the tumor, and inoculation back into the adult yielded the characteristic sarcoma. While the growth did not proliferate at all in pigeons or ducks, and grew but slowly in chickens of a variety other than that in which it originally occurred, with the embryos of these alien fowls the results were quite different; thus, in four of nine pigeon eggs inoculated and developing, and in six of sixteen duck eggs, tumors were obtained. Inoculations were made into the membranes alone, and the resulting growths resembled those arising in similar situations in Plymouth Rock eggs, which was true also of the tumors developing in the eggs of an alien variety of chicken (Brahma). When portions were transplanted to adult pigeons or ducks, implantation was always unsuccessful. The growth occurring in the eggs of pigeons or ducks was not analogous to the brisk temporary proliferation often seen in alien adults, because there, even in so short a time, an immense accumulation of lymphocytes took place about the graft, which itself showed well marked degenerative changes. In the embryo, however, neither phenomenon was present.

Certain embryos failed to develop tumors, a circumstance probably due entirely to chance causes rather than to the possession of natural immunity, for extensive search failed to disclose in any instance those histological signs about and within the graft which the authors were accustomed to associate with the display of resistance in the adult.

On the whole, young embryos (tenth day of incubation) seemed to offer a more favorable soil for tumor growth than adult fowls, and Rous and his collaborators could see no reason against extending this conclusion to embryos at even earlier periods of development, since the less striking results obtained before the tenth day could be ascribed in large part to the method of inoculation and to the relative unfitness of the host to nourish the implanted tissue, because of small size and simple structure.

CHAPTER IX

THERAPEUTICS

THE discovery that cancer was not at all of infrequent occurrence among animals and that it could be transplanted from one to the other, placed within the hands of the biologist a means of investigating this disease at once so easily available and so inexhaustible as to encourage the hope that the scourge could not continue much longer to defy those who were attempting its subjugation. Indeed, the conquest of transplantable cancer has frequently seemed an accomplished fact, but only because it was not fully appreciated that such tumors often undergo spontaneous regression. Hope has run the higher, too, since a sharp distinction has not always been drawn between an autochthonous new growth on the one hand, and, on the other, a propagable neoplasm situated in alien soil. Thus many observers have failed to realize that, even though it should prove possible to influence detrimentally the growth of a transplanted neoplasm, there would be no reason to assume that the curative agent would be equally efficacious against the proliferation of cancer cells in the organism to which they are native. However, investigators have begun to perceive the distinction, and to appreciate that the cure of spontaneous cancer offers this therapeutic puzzle: To find a method of damaging a certain area of native cells while at the same time their neighbors within the organism are left undisturbed.

Although Jensen [1] at first believed that the serum of rabbits treated with cancer could bring about the disappearance of transplanted tumors in mice, he [2] wrote later that when he had credited this serum with curative power it had not been known that propagable tumors were prone to retrogress independently of any treatment whatsoever. While he now realized that spontaneous healing would explain most of these

[1] *Centralbl. f. Bakt.*, etc., erste Abt., Orig., 1903, xxxiv, 30.

[2] *Zeitschrift f. Krebsforsch.*, 1908–1909, vii, 281.

earlier cures, it would not account for the disappearance of several very large tumors in his series, and he did not despair, therefore, of the final discovery of a specific cytotoxic serum against mouse cancer.

v. Leyden and Blumenthal [1] practised an analogous method in dogs, successfully treating a cancerous animal with the serum of a rabbit that had been injected with dog cancer. Another was almost entirely cured of carcinoma by the inoculation of juice expressed from a dog carcinoma. Furthermore, alleviation of the disease in man was said to have been brought about in several instances after the injection of fluids expressed from human cancer, a result which, according to v. Leyden,[2] was confirmed by later investigations.

Clowes [3] treated mice bearing propagable tumors with the serum of mice in which Jensen's carcinoma had been absorbed, and found that while small growths were restrained in development or even cured, tumors of more than three or four grams in weight were not influenced in any way. Commenting upon these results, Clowes [4] said that the inhibitory effect could scarcely be interpreted to mean anything but that the serum contained an antibody directly antagonistic to the development of neoplastic cells; and since it had been shown that the serum in question possessed neither hemolytic nor cytolytic characteristics, it appeared probable that the effect was exerted directly upon some intracellular virus rather than upon the cell itself.

Bashford,[5] on the contrary, found that although the action of immune serum outside the organism had in some cases deprived tumor cells of their power to grow, no definite evidence of such action had been obtained in the living body, while neither Borrel [6] nor Bridré [7] was able to affect tumors in mice by injecting the animals with the serum of sheep

[1] *Deut. med. Woch.*, 1902, xxviii, 637.

[2] *Zeitschrift f. Krebsforsch.*, 1907, v, 164.

[3] *Johns Hopkins Hosp. Bull.*, 1905, xvi, 130.
Med. News, 1905, lxxxvi, 477.
British Med. Jour., 1906, ii, 1550; see also Gaylord, Clowes, and Baeslack, *Med. News*, 1905, lxxxvi, 91.

[4] *British Med. Jour.*, 1906, ii, 1552.

[5] *British Med. Jour.*, 1905, ii, 96.
Lancet, 1905, ii, 104.

[6] *Bull. de l'Inst. Past.*, 1907, v, 607.

[7] *Ann. de l'Inst. Past.*, 1907, xxi, 774.

or fowls previously treated with mouse cancer. Furthermore, Bridré could see no conclusive outcome from inoculating tumor-bearing mice with fresh macerated cancer, dried or heated tumor being even less successful.

Lewin [1] recorded the failure of all attempts to obtain a curative serum by the injection of mouse cancer into animals of other species. On the other hand, the serum of rats in which tumors had regressed spontaneously produced a distinct retardation of the growth of tumors in other rats, although the cases were too few to be convincing.

Walker,[2] arguing that certain phenomena proper to reproductive cells took place also in those of malignant growths, and that tumor cells seemed to be, like reproductive cells, out of coördination with the rest of the body, attempted to cure transplanted carcinomata in mice by means of the serum of rats inoculated with mouse testis. It was found that tumors in animals so treated grew with only one-third the rapidity evinced by those in control mice injected with normal rat serum, and, furthermore, that favorable results could be obtained also when tumor-bearing mice were treated with the serum of rats inoculated with the same growth. The results in all cases seemed to be much less satisfactory where large tumors were treated than where the growths were small.

Gay [3] found that when rats bearing the Flexner-Jobling adeno-carcinoma were re-inoculated with the same growth not less than three or four weeks after the primary implantation, the second graft failed to grow, and in about 50% of the animals the original tumor was cured and metastases were prevented.

Freytag [4] inoculated tumor mice with the serum of immunized rabbits, with mouse blood, or with the blood or serum of alien species. Although regressive changes were evident within the tumors, complete disappearance had never been observed.

The condition of *parabiosis*,[5] introduced by Sauerbruch and

[1] *Zeitschrift f. Krebsforsch.*, 1907–1908, vi, 308.

[2] *Lancet*, 1908, ii, 797, 1299, 1474.
Lancet, 1910, i, 990.
Lancet, 1911, i, 1275.

[3] *Boston Med. and Surg. Jour.*, 1909, clxi, 211.

[4] *Zeitschrift f. Krebsforsch.*, 1910–1911, x, 157.

[5] Albrecht and Hecht (*Centralbl. f. allg. Path.*, etc., 1909, xx, 1039) were led to the pre-

Heyde,[1] was utilized by Rous[2] for the demonstration of the presence or absence of antibodies in the circulation of rats that had been three times unsuccessfully inoculated with the Flexner-Jobling adenocarcinoma. Such resistant rats were united in parabiosis to rats bearing the same tumor, but the union had not the slightest effect upon the course of the growths, neither retarded development nor retrogression having been observed.

Uhlenhuth, Haendel, and Steffenhagen[3] prepared a rat antiserum by injecting rabbits with rat serum. Half a cubic centimeter was introduced into rats three times at six day intervals, beginning just after tumor inoculation, but in spite of these attempts to confer passive resistance, vigorous tumor development took place. The serum of a rabbit that had been injected intraperitoneally with tumor emulsion was equally ineffective, as were organ extracts and the serum of cattle.

Blumenthal[4] treated rats with autolyzed transplantable sarcoma. Tumors the size of a hen's egg were made to disappear after one treatment, in spite of the fact that nodules larger than a pigeon's egg had never been known to recede in untreated rats.

Lane-Claypon and Starling[5] having postulated the existence of *hormones*,[6] or substances able to stimulate normal growth, A. and H. Grünbaum[7] suggested that, given an excess of a hormone together with a lesion or irritation of the tissue complementary to the hormone,

liminary conclusion that parabiosis in animals otherwise normal exerted a striking inhibitory effect upon tumor growth as well in unilateral as in bilateral inoculation of the parabiotic pair. Such tumors as grew in parabiotic animals were retarded — a circumstance which the authors considered analogous to immunization with blood, embryonal tissue, and skin. A short time after the separation of parabiotic animals with young tumors, a distinctly rapid and vigorous growth of the tumors set in, which might be explained by the removal of inhibitory factors.

Goldmann (*Beitr. zur klin. Chirurgie*, [v. Bruns], 1911, lxxii, 82), however, was unable to convince himself that parabiosis, as such, had any inhibitory effect upon tumor growth.

[1] *Münch. med. Woch.*, 1908, lv, 153.
Zeitschrift f. exp. Path. u. Therap., 1909, vi, 33.

[2] *Proc. Soc. Exp. Biol. and Med.*, 1909–1910, vii, 12.
Jour. Exp. Med., 1909, xi, 810.

[3] *Arb. a. d. Kaiserl. Gesundheitsamte*, 1911, xxxvi, 491.

[4] *Med. Klin.*, 1910, vi, 1982.

[5] *Proc. Roy. Soc.*, Series B., 1906, lxxvii, 505; see also Starling, *Lancet*, 1905, ii, 579.

[6] For a refutation of Starling and Lane-Claypon's results, however, see Frank and Unger, *Arch. Internal Med.*, 1911, vii, 812.

[7] *Jour. Path. and Bact.*, 1911, xv, 289.

unlimited growth might take place. When immune rats were inoculated with rat sarcoma and parotid gland together, the result indicated that the gland was able to assist the growth of sarcoma in animals otherwise insusceptible, while, on the other hand, removal of the parotid gland from a tumor-bearing rat was followed by the occurrence within the nodule of fatty and fibrotic changes, although growth continued progressively. Other experiments with the parotid gland, or the ovary, were indecisive.

The same authors [1] found it possible to effect the regression of small transplanted rat tumors by injecting antivenom horse serum, and found further [2] that by the simultaneous administration of adrenalin they could bring about the disappearance of still larger growths in about 70% of the animals inoculated.

Beard [3] recorded the successful treatment of Jensen's mouse tumor with trypsin, and ascribed it to destruction of the less powerful ferment of the cancer, *malignin*, by the more active trypsin.

Vidal [4] had observed the arrested evolution of tumors in four patients with a temperature above 40° C. In order to be sure that this was not a quadruple coincidence Vidal daily exposed tumor-bearing mice to a temperature above that of the normal organism, and found that they lived longer than their controls and that their tumors were the seat of degenerative changes. In a bitch the subject of spontaneous lympho-sarcoma the "zone of Richet" was punctured, in consequence of which the temperature rose to 40.8° C., while the tumor diminished rapidly in size, finally to disappear.

The action of X-rays upon the new growths of animals has been investigated by several observers. Clunet [5] found slight hyperplasia and accentuation of the cystic structure in transplantable and spontaneous carcinomata of mice exposed to these rays, and discovered at a later period still more serious lesions which were coincident with macroscopic alterations. Marie and Clunet,[6] in summing up the changes which they had observed both in human carcinomata and in those of

[1] *Lancet*, 1911, i, 879.
[2] *Lancet*, 1912, i, 644.
[3] *British Med. Jour.*, 1906, i, 140.
[4] *Travaux de la deuxième Conférence internat. pour l'Étude du Cancer*, Paris, 1911, 302.
[5] *Recherches exp. sur les Tumeurs malignes*, Paris, 1910, 250.
[6] *Travaux de la deuxième Conférence internat. pour l'Étude du Cancer*, Paris, 1911, 160.

the mouse, described a latent stage signalized by the occurrence of a large number of abnormal cells and followed by one of cell death. In the third stage groups of necrotic cells were replaced by polymorphonuclear leucocytes and young connective tissue, while in the fourth there occurred the formation of a scar, which for a long time inclosed in its meshes cells of "diminished vitality."

Contamin [1] found that the younger the growth the more readily could it be brought to absorption with X-rays, and that irradiation before inoculation exerted a direct action upon malignant cells, affecting more their growth energy than their ability to withstand transplantation.

As for the effect of radium, Apolant [2] reported that many mice bearing transplantable carcinomata had been entirely cured, while in the remainder the tumors had been very materially reduced in size. During the earlier periods of healing there were to be found in the connective tissue a series of changes presenting all the signs of inflammation, among which the presence of large fibroblasts was especially noticeable. These cells penetrated the tissues in all directions, and wandering cells continued to the interior of the alveoli instead of halting at the edges; the alveoli themselves were continually under subdivision by advancing fibroblasts, and the epithelial cells within them lost their sharp contours in the process and came to resemble a syncytium. The changes were essentially similar to those described in man, in that there was a disappearance of epithelial elements and an overgrowth of connective tissue. In Apolant's opinion it was hardly to be doubted that radium possessed a specific action upon the malignant cells because these elements were entirely absorbed under its influence, while those in a tumor undergoing spontaneous regression were, on the contrary, transformed into necrotic masses which never underwent absorption.

Bashford, Murray, and Cramer [3] also described the splitting up of alveoli into smaller cell groups by proliferating connective tissue, but denied that radium had any selective action upon tumor cells. On the contrary, when the connective tissue proliferation was at its

[1] *Bull. de l'Assoc. franç. pour l'Étude du Cancer*, 1910, iii, 160.

[2] *Deut. med. Woch.*, 1904, xxx, 454, 1126.

[3] *Sci. Reports, Imperial Cancer Research Fund*, London, 1905, No. 2, Part ii, 56.

height tumor cells could be found in active division and, furthermore, tumors could be successfully transplanted even after a prolonged exposure to radium. As great engorgement of the blood vessels, and even hemorrhage, were prominent features in growths that had been treated, the attempt was made by these authors to produce bleeding by other agencies in order to determine its significance in the earlier stages of the radium reaction. For this purpose mice with growing tumors were injected with one cubic centimeter of a one to ten thousand solution of adrenalin in the hope that the great rise in blood pressure would cause an extravasation of blood from the capillaries of the tumor, since they possessed neither vasomotor nerves nor the muscular mechanism by which these act. Mice dying within the first twelve hours after injection showed widespread hemorrhages into the lung and into the substance of the tumor. Three animals survived these large doses of adrenalin, and in one of them the growth, which by the fifth day had diminished in size, presented the same histological appearance as resulted from the action of radium.

Reicher [1] reported that he had succeeded in curing transplantable sarcomata and carcinomata in rats and mice through injection of adrenalin into the tissues surrounding the growths. Microscopic examination showed a central necrosis surrounded by a secondary inflammatory reaction.

Spiess,[2] in continuation of his previous investigations upon the effect of anesthesia on inflammation, injected anesthetics into the sarcomata or carcinomata of mice, employing for the purpose nirvanin, novocaine, and a third substance ("337") not upon the market. In a preliminary experiment the last-named proved to be the most active, and the other two were accordingly discarded. Seventy-eight mice in all were treated with "337," their tumors varying from the size of a pea to that of a hazel-nut, and of the seventy-four appearing in the final reckoning, fifty-two were favorably influenced; of these, twenty-two were entirely cured, sixteen showed great, and fourteen less, though still distinct, improvement. The more malignant were the tumors, the less favorable was the outcome, and only tumors of slowly growing strains could be cured. That spontaneous healing need not be taken

[1] *Deut. med. Woch.*, 1910, xxxvi, 1356.
[2] *Zeitschrift f. Krebsforsch.*, 1907, v, 81.

into account was shown by the different course of events in treated mice, by the observation that control tumors grew rapidly and led to the death of the animals bearing them, and finally, by the fact that animals which had been cured were not refractory to re-inoculation.

Uhlenhuth and Weidanz,[1] investigating the action of atoxyl on mouse tumors, found that growth was so much more rapid in treated animals than in their controls that after a month's medication the tumors were nearly twice as large. Pyocyanase, on the contrary, when injected directly into rat sarcomata, brought about the disappearance of tumors even after they had attained the size of a walnut.

Uhlenhuth, Haendel, and Steffenhagen[2] employed quinine, sodium taurocholate, ox-gall, antiformin, arsenophenylglycin, ferments, adrenalin, alcohol, and the application of cold. None of these was so effective as pyocyanase which, however, was found to be poorly borne by rats.

Beck[3] investigated the action of arsenic, atoxyl or some of its preparations, and quinine, finding them all without effect. More favorable results followed the use of certain substances prepared from bacteria or their metabolic products (if injections were made directly into the tumor), and filtrates and extracts from *B. prodigiosus* and *S. pyogenes aureus* were employed with success although other bacterial preparations, as tuberculin, were without curative action.

v. Wassermann, Keysser, and Wassermann[4] outlined the difficulty of attacking the malignant cell somewhat as follows, employing the terminology of Ehrlich. While in treating the protozoal diseases the aim of the investigator was to prepare a medicament which would be parasitotropic but not organotropic, the problem in cancer was to obtain a material which should be organotropic, but whose action should be limited to a certain area in the organ — the tumor cells.

To affect the very malignant neoplasms of the mouse it would be necessary either to employ an unusually powerful agent or to find a means of attacking the nucleus, the most vital point of the tumor cell; it would be necessary, in other words, to discover a nucleotropic

[1] *Arb. a. d. Kaiserl. Gesundheitsamte*, 1909, xxx, 444.

[2] *Centralbl. f. Bakt.*, etc., erste Abt., Ref., 1910, xlvii, Beiheft, 159.
Arb. a. d. Kaiserl. Gesundheitsamte, 1911, xxxvi, 492.

[3] *Zeitschrift f. Krebsforsch.*, 1910–1911, x, 153.

[4] *Deut. med. Woch.*, 1911, xxxvii, 2389.

substance. This would have to be at the same time a material capable of exerting its curative effect when introduced by way of the blood stream, for all agents, when locally employed, lacked the necessary elective power to make their own way to the neoplastic cell. Gosio [1] had discovered that tellurium and selenium were reduced by living bacteria as black and red precipitates respectively, and the present authors found that in the case of the cancer cell this reduction, *in vitro* at least, was most intense about the nuclei. It was then essential to know whether the specific reaction would occur in the living organism. While preparations of selenium or tellurium, injected into a tumor, were found to have a destructive effect upon its cells, this was especially true of tellurium. But as these compounds remained entirely inert after introduction into the body through the blood stream because they had not succeeded in reaching the malignant growth, it was necessary, in the words of the authors, to construct a railway by means of which the selenium and tellurium could be carried to their destination. Because in mouse tumors the blood supply was limited almost entirely to the periphery, it was necessary to use for this purpose a rapidly diffusible substance, and after many trials it was found that eosin fulfilled the requirements. This dye, combined with selenium and injected into the circulation of mice bearing transplantable growths, produced an immediate reddening of the entire animal, although no effect upon the tumor was noticeable until three daily inoculations had been given. After the third treatment a distinct softening was demonstrable, while after the fourth the tumor felt more like a fluctuating cyst than a solid nodule; and after this condition had been obtained absorption set in until, within ten days, the cure was complete. Often, however, recovery was not so uneventful and, as was especially the case with large tumors, the mice were killed by a toxemia resulting from the absorption of their own growths. Two mice with spontaneous tumors had also been cured, and four strains of transplantable carcinoma and one propagable sarcoma had all proved equally susceptible to treatment. Tumors in control mice, on the contrary, continued to grow progressively. Recovered animals had been kept under observation for months, but no suggestion of recurrence had been observed. In mice autopsied dur-

[1] *Zeitschrift f. Hyg.*, etc., 1905, li, 65.

ing the first few days of treatment the growths were stained an intense red as contrasted with the surrounding tissues, showing that the neoplastic cells had exerted a specific attraction upon the injected material. As the eosin-selenium compound was extremely toxic, and as it was unfortunately necessary to give almost the lethal dose in order to achieve a cure, many mice perished merely as the result of the injection.

v. Hansemann,[1] in conjunction with the three authors previously mentioned, discussed the histological appearance of treated tumors, and found evidence to support the selective action of selenium upon the nuclei. Examinations of the organs of injected mice showed no serious lesion attributable to the treatment.

All four investigators united in declaring that the treatment was still in the experimental stage, and that it marked rather the introduction of a new era of chemotherapy than the discovery of a cure for cancer.

In discussing the work of v. Wassermann and his collaborators Klemperer [2] said that he also, in conjunction with Emil Fischer, had been able to cause the regression of mouse tumors with selenium.

Walker [3] began a series of experiments with selenium immediately after the publication of v. Wassermann's article. The first preparation to be investigated was a combination of colloidal selenium and eosin, but this proved to be entirely without effect upon the transplantable tumors of rats and mice. It was, however, non-toxic, in contrast to the highly poisonous preparation of the German investigator. Combinations of selenium and eosin of doubtful composition were also tried, but although they were highly poisonous none of them showed the slightest selective action upon tumor cells.

Neuberg and Caspari,[4] starting with the observations that tumors were characterized by increased fermentive activity, particularly autolysis (in the widest sense of the word), and that certain substances like radium and compounds of the heavy metals in colloid form were capable of increasing autolysis *in vitro*, tried to influence tumor growth by intravenous injections of compounds of the heavy metals. They were able to find several materials possessed of an affinity for malignant cells — compounds of gold, platinum, silver, rhodium, ruthenium,

[1] *Berl. klin. Woch.*, 1912, xlix, 4.
[2] *Deut. med. Woch.*, 1912, xxxviii, 89.
[3] *Lancet*, 1912, i, 1337.
[4] *Deut. med. Woch.*, 1912, xxxviii, 375.

iridium, lead, and especially copper and tin. Other substances, arsenic and vanadium, also exerted an action upon autolysis, although a less certain one. Sulphur, and iodine and its compounds were, on the contrary, entirely inert. In a later article Neuberg, Caspari, and Löhe [1] added compounds of antimony, mercury, cobalt, osmium, and palladium to the list of active materials. Microscopic deposits of the metals employed were to be found in the tumors of animals that had been subjected to treatment.

The attack of these elements was extraordinarily sudden, beginning, sometimes after as short an interval as one minute, with extreme congestion, which was shortly followed by the hemorrhage initiating the cure. Within the first twenty-four hours a tumor of considerable size might become almost entirely softened, while repeated injections resulted in an entire liquefaction and absorption of the nodule. Several strains of transplanted tumors, as well as sporadic growths, proved equally amenable to treatment, and as spontaneous recoveries from the transmissible growths employed were very rarely encountered, the authors rejected the possibility that healing might not have been the result of their treatment. The materials used were all toxic and, as in v. Wassermann's experiments, the effective dose lay very near the lethal amount, wherefore these observers were unwilling to forecast the relation of their investigations to the treatment of cancer in the human subject.

The question of chemotropism in neoplastic tissue has been reviewed by von den Velden.[2] Simple though the conception might be that one material could act as a pilot or a railway to direct or transfer another substance to its destination, the discovery of such a combination was an entirely different matter. The union must not be so unstable as to be broken down in the stomach, the tissues, or the blood, nor yet so firm that the active principle could be deterred from exerting its effect through remaining coupled after arrival at its goal. The clearest instance of specific affinity was offered by iodine, and was best expressed negatively by saying that fat, as well as the central nervous system with its large lipoid content, was practically barren of iodine. Oswald Loeb had shown, nevertheless, that iodine could be made to penetrate both fat and the central nervous system if it were first com-

[1] *Berl. klin. Woch.*, 1912, xlix, 1405.

[2] *Berl. klin. Woch.*, 1912, xlix, 825.

bined with a lipoid. Thus was afforded an example of an exogenous factor with power to alter the distribution of a chemical substance within the body. Contrasted with this instance there were certain endogeneous factors. A change in the relative bulk of the various tissues might act in this way, as was demonstrated by the difference in what occurred when a material soluble in lipoids was administered to a very thin or a very fat person. The endogenous factors were most distinct, however, under pathological conditions, as Jacoby had shown by his work on salicylic acid. Thus, in a rabbit with experimentally produced general sepsis, salicylic acid would be taken up in large amounts by the joints although previously they held none of this drug; furthermore, Oswald Loeb and Michaud had shown that in turpentine abscesses and in the tuberculous eyes, lymph nodes, and lungs of guinea-pigs and rabbits, there occurred a very appreciable storing of iodine when potassium iodide had been introduced into the body. von den Velden himself had been able clearly to demonstrate iodine in metastatic carcinomata of the liver and pancreas after introducing into a moribund cancer patient one gram of sodium iodide for every twenty kilograms of body weight, although the tissues themselves in which the secondary nodules were embedded contained no iodine. This finding had, furthermore, been confirmed by Takemura, who found that mouse carcinomata and rat sarcomata were capable of storing up iodine, while Blumenthal had discovered a tropism for neoplastic tissues in the case of arsenic.

The affinity of various chemicals for certain organs had been referred by Oswald to changes in the diffusion processes caused by alterations in the colloid structure of the membrane and peripheral portions of the cells; and as, in the opinion of that author, similar alterations were to be found not only in inflammatory processes but in new growths as well, the "membrane problem" thus suggested became of such wide significance that it might transfer the entire field of investigation to the realm of physical chemistry.

Therapeutic experiments have been undertaken not infrequently in man also, certain of them inspired without doubt by the early endeavors of Héricourt and Richet [1] to obtain an immune serum from

[1] *Compt. rend. de l'Acad. des Sc.*, 1895, cxx, 948.
Compt. rend. de l'Acad. des Sc., 1895, cxxi, 567.

animals injected with human cancer, although others, falling within the domain of immunity, have been attempts to transfer to man such knowledge as has been gathered by observation upon the lower animals. The investigations which have been prosecuted in the laboratory within the last decennium have, however, yielded no result germane to therapeutics other than to show that an animal can be made resistant to the transplantation of malignant cells from another organism, and the fallacy of applying these findings to the treatment of tumors autochthonous in origin and firmly established, has been indicated by Bashford [1] as follows, in a discussion of the refractory condition in animals: "It is necessary to point out emphatically, that nothing but harm can result from ignoring the fact that the above experiments only indicate the possibility of rendering normal mice unsuitable for the growth of experimental cancer. They have not enabled us to arrest the progress of experimental tumours with certainty, far less to affect the cure of the disease occurring naturally."

Fichera [2] attempted to cure cancer in rats and in the human subject by injecting autolyzed rat embryo in the one case, and autolyzed human embryo in the other. Twenty-five patients with tumors of various kinds continued treatment long enough to allow the method a fair trial. While nine of them showed no improvement whatever, in the remainder there occurred arrest of development, involution, or sometimes temporary disappearance of the growth.

Coca and Gilman [3] injected a series of patients, each with his own carcinoma after it had been thoroughly ground and emulsified, and found the results encouraging. A footnote stated that a carcinoma of the same kind taken from another individual might be substituted.

In a later article, however, Coca, Dorrance, and Lebredo [4] concluded from a study of seventy-nine cases that, except for the rapid relief of cachectic symptoms, they had seen no evidence that the injection of tumor tissue exerted any specific influence over malignant growths.

Rovsing,[5] who introduced heated tumor emulsions, gained the im-

[1] *British Med. Jour.*, 1906, ii, 209.

[2] *Tumori*, Turin, 1911; abstr. in *Bull. de l'Inst. Past.*, 1911, ix, 272; see also *Lancet*, 1911, ii, 1194.

[3] *Philippine Jour. Sci.*, 1909, iv, B, 391.

[4] *Zeitschrift f. Immunitätsforsch.*, etc., Orig., 1912, xiii, 543.

[5] *Travaux de la deuxième Conférence internat. pour l'Étude du Cancer*, Paris, 1911, 562.

pression that although this treatment was without effect upon carcinomata it might possibly prove valuable against sarcomata.

Hodenpyl [1] offered a preliminary report regarding the treatment of cancer by means of ascitic fluid withdrawn from a patient in whom both primary and recurrent carcinomata had disappeared. The fluid was tested first upon mice bearing transplantable carcinomata, and when it had been found that these growths diminished in size or even disappeared, the experiment was extended to include forty-seven inoperable cases in man. In general the effect of the injection was to cause a temporary redness, tenderness, and swelling in the neighborhood of the growth, this reaction being followed by necrosis of the tumor and a decrease in size or, in some instances, by complete disappearance.

Ill and Miningham [2] obtained ascitic fluid from a somewhat similar case and introduced it into more than thirty patients with malignant disease. Although not a single case was cured, pain was mitigated, hemorrhage was decreased or entirely stopped, and there occurred a transient gain in weight. The psychic element was eliminated from this striking improvement in the general condition by the fact that only one patient knew the nature of her disorder.

A useful résumé of the serum therapy of cancer, which contains nearly one hundred and fifty references, has been compiled by Vidal,[3] while Coca [4] has but recently prepared a critical review of the "vaccination" treatment for human cancer in its relation to the experimental data bearing upon normal tissue and tumor immunity.

[1] *Med. Record*, 1910, lxxvii, 359.
[2] *Jour. American Med. Assoc.*, 1912, lix, 497.
[3] *Travaux de la deuxième Conférence internat. pour l'Étude du Cancer*, Paris, 1911, 293.
[4] *Zeitschrift f. Immunitätsforsch.*, etc., Orig., 1912, xiii, 524.

CHAPTER X

GENERAL SUMMARY

ALTHOUGH the literature of experimental cancer may seem to be a maze of conflicting statements, there are nevertheless a few threads which may be fixed upon and followed through. These are represented by a number of findings which, having received the support of a majority of those engaged in cancer research, may be accepted as probably reliable. Nevertheless, it has happened occasionally that even the most competent observers have come to diametrically opposed conclusions in spite of the most careful work, a situation suggesting that differences still unrecognized may have existed between the animals used in the several experiments. The mouse is not a test tube where so much of one substance and so much of another will always produce so much of a third, but is, on the contrary, a highly complex organism exhibiting protean reactions. What takes place, therefore, in one mouse may not happen in the next — nay, what happens in two groups of ten mice each may not, and often does not, occur in a third series.

Malignant growths have been found throughout the animal kingdom down to, and including, the fish — except that no certain instance has yet been reported among the reptiles. By far the greater amount of attention has, however, been bestowed upon the disease in the mouse, not only on account of the ease with which this animal can be procured and the cheapness and facility with which it can be kept under observation, but also because the subcutaneous situation of its tumors makes for early and ready recognition. The fact that information has been accumulating chiefly in regard to mouse cancer must not, therefore, be understood to imply that the findings might not hold good for malignant new growths of other species, were it convenient or possible to investigate them in the same way. There can be scarcely a doubt that the neoplasms of the mouse are entirely comparable to

those of man, since they exhibit continuous, infiltrative, growth, no less than the power to metastasize and to recur after incomplete removal.

Evidence has been adduced that there is some hereditary influence at work, making mice of recently cancerous ancestry more prone to develop cancer than are those in which the taint is more remote. But whether or not this observation may be applied to man it is at present impossible to determine, nor would it be safe to venture an opinion until data as accurate as those relating to the mouse become available.

It has been ascertained that almost any spontaneous new growth of the mouse can be transplanted into other mice, and that although the first inoculation may not be attended by any great degree of success, propagation generally becomes easier in succeeding generations. Why this should be so is not known; some observers ascribe it to an increase of virulence on the part of the tumor cell, while others see in it merely an increasing power of this element to adapt itself to altered conditions. It has been the experience of the majority of investigators that for transmission to be effective, living and intact cells must be introduced into an animal of the same species as that in which the primary tumor took its origin, or at least into a species very closely related.

In this connection there has been discovered a phenomenon of the highest importance. It is that cancer is more readily transplantable into the animal in which it arose spontaneously than into any other. This, and the complementary observation that an animal cannot be immunized in any manner yet at command against the inoculation of its own primary tumor, should never be lost sight of in evaluating descriptions of therapeutic experiments, for it demonstrates beyond reasonable doubt that cancer cells proliferating in an animal to which they are not native are doing so under a certain disadvantage. A procedure, therefore, able to turn the tide against them might still be ineffectual when applied against malignant cells more favorably situated in an animal to which they are autochthonous. Furthermore, the almost uniform success with which the cancer cell can be ingrafted in the organism within which it first acquired malignant properties, as contrasted with any other organism, militates very strongly against any parasitic theory of tumor genesis. At the same time, the observa-

tion that the bearer of a tumor offers a particularly favorable soil for the cells of that neoplasm will intimate to the surgeon the necessity for a continuance of the most extreme care against the distribution of cancer cells throughout the operation wound, a precaution already suggested in the past by the frequency of local recurrence following the excision of malignant tumors. Secondary growth along the needle track after exploratory puncture of a tumor furnishes an even more striking example from human pathology of this extreme susceptibility of the individual to inoculation with his own neoplasm. Finally, these considerations demonstrate how minimal is the chance of the transfer of cancer from one individual to another in the ordinary course of events.

The proliferation of a tumor after its introduction into another host is an example, not of infection, but of transplantation; the parenchymal cells of the resulting growth are derived, not from the elements of the new host, but from those of the transferred graft. These cells are supported and nourished by a stroma which, built up by the connective tissues and blood vessels of the new organism, is well on its way toward completion by the third or fourth day following transplantation.

Propagable tumors are subject to fluctuations in growth energy. They may grow progressively, or disappear spontaneously. Even under optimum conditions, however, the proliferative power of the cancer cell falls far short of that possessed by the bacteria, and perhaps even of that displayed by the cells of the developing embryo. The key to the nature of the malignant cell lies, therefore, not in the explanation of augmented growth power, but rather in an elucidation of the capacity of this cell for continuous growth.

A tumor under cultivation usually retains unaltered the characteristics of the primary growth. While most transplantable neoplasms show a marked deviation from the normal structure of the organ which gave them origin, this is by no means an invariable rule, since complete loss, or even latency, of differentiation is not necessary for continuous growth, and tumors which, under the microscope, are distinguishable from their mother tissue only with difficulty, have been proved capable of unlimited propagation. So far, then, as the retention of normal structure is a criterion of innocence its significance

has been lost, a situation parallel to that obtaining among certain tumors of the thyroid gland and the ovary in man.

The cells of a few transplantable carcinomata have been found capable of causing a transmutation of the stroma in the course of which, acquiring malignant properities, it becomes indistinguishable from other sarcomata of the mouse.

Mice in which tumors have undergone partial or complete absorption are refactory to subsequent transplantation, either of the same or of another growth. This condition of resistance is brought about also by the absorption of living and intact cells of the same species, but not those of the animal itself. Immunity, once induced, cannot be transferred passively to other animals either by the cells or the body fluids, although it is probably these agents which are responsible for the spread of the condition throughout the organism within which it has been engendered. And while there is a certain amount of evidence in favor of the fact that natural resistance may be the outcome of hereditary transmission, there is none to show that acquired immunity can be so transferred.

Immunity has been attributed to a lack of the specific nutritive materials necessary for tumor growth. On the other hand, it has been described as a suppression of the stroma reaction which, under more favorable conditions, would have undertaken the support of the tumor in its new host. The latter supposition has more evidence in its favor.

It must be frankly confessed that so far, at least, the study of cancer, instead of affording an understanding of the nature of the disease, has but opened up new problems which were formerly not even conceived. Hence earnest students have had to content themselves with slowly and laboriously collecting data, in the hope that at some future time these may be combined into a coherent whole. Slow at the best, this is nevertheless the only way in which the goal can be surely attained, for the empirical method, in spite of its centuries of effort, has produced nothing nearer to a cure than surgical removal. The hope of substituting some plan of specific treatment for this crude and only too often inefficient means, rests on the fact that in many mice transplanted tumors, and in a few, spontaneous tumors, have vanished completely, although it must be admitted that we have not yet the slightest insight into the mechanism by which this occurs. Still, the

balance must be very delicate, the margin between continuous proliferation and cessation very narrow, and the newer conceptions of malignant growth encourage the hope that at some time, far in the future though it may be, the nature of this difference will be unriddled, and the dream of effectual interference with incessantly proliferating cells will become an actuality. William H. Woglom (1913)

INDEX OF AUTHORS

ADAM, on homoplastic transplantation, 42

ADAMI, on cancer houses, 43; on cancerous degeneration, 12

ALBERTS, on heteroplastic transplantation, 51

ALBRECHT and HECHT, on age and susceptibility, 130; on amyloid degeneration, 95; on parabiosis, 258 (footnote); on pregnancy and susceptibility, 138; on race and susceptibility, 136; on sarcoma development, 122; on Scharlach R, 37; on the specificity of immunity, 143.

ALIBERT, on homoplastic transplantation, 43

APOLANT, on the action of radium, 261; on athrepsia, 163, 166; on autoplastic transplantation, 224; on classification, 214; on the frequency of cancer in mice, 199; on the genesis of mouse tumors, 185, 186, 187; on the hare tumor of *v. Dungern*, 156; on hypersensibility, 182; on immunity with alien tissues, 148; on immunity and histology, 107; on infiltrative growth, 192; on lactation and ætiology, 200; on transmissible lympho-sarcoma of the dog, 233; on the malignancy of mouse tumors, 193; on sarcoma development, 112, 113; on spindle-shaped epithelial cells, 106; on spontaneous absorption, 90; on the stroma of hemorrhagic tumors, 109; on sweat glands in the mouse, 185; on the transplantation of tumor mixtures, 79, 171; on variations during transplantation, 105; on virulence, 62; see also EHRLICH and APOLANT

APOLANT and EHRLICH, on the transplantation of tumor mixtures, 79

APOLANT and MARKS, on immunity with autologous tissues, 150

ARNDT, on Egyptian medicine, 1

ASKANAZY, on pregnancy and growth, 28; on the production of tumors, 27

AUBERTIN, see MARIE and AUBERTIN

AYSON, on thyroid adeno-carcinoma in the trout, 237

BABER, on non-encapsulation of the thyroid, 241

BAESLACK, on infiltrative growth, 191; see also GAYLORD, CLOWES, and BAESLACK, and CLOWES and BAESLACK

BASHFORD, on acquired immunity, 139, 144; on ætiology, 6, 9, 210; on age and susceptibility, 129; on autoplastic transplantation, 223; on the distribution of immunity, 153; on dosage, 69, 73; on the duration of immunity, 154; on growth rate, 84; on health and susceptibility, 137; on heredity and ætiology, 201, 204; on histological structure, 109; on immunity with alien tissues, 143, 148; on immunity with devitalized tissues, 149 (footnote); on the infectivity of cancer, 198; on inoculation site, 76; on race and susceptibility, 132; on sarcoma development, 111; on the specificity of immunity, 142, 147; on spontaneous absorption, 91; on the stroma of hemorrhagic tumors, 109; on the technic of inoculation, 70; on treatment, 257, 268; on variations during transplantation, 100, 101; on virulence, 65

BASHFORD and MURRAY, on age and susceptibility, 129; on early stages, 59; on the genesis of mouse tumors, 186; on heredity and ætiology, 202; on heterotypical mitosis, 18; on infiltrative growth, 191; on the malignancy of mouse tumors, 192; on metastasis, 190; on pregnancy and susceptibility, 137; on race and susceptibility, 132; on the re-inoculation of tumor-bearing animals, 167; on the zoölogical distribution of cancer, 184

BASHFORD, MURRAY, and BOWEN, on age, race, and susceptibility, 76; on diminished transplantability, 78; on fluctuations in growth energy, 86; on spontaneous absorption, 91

BASHFORD, MURRAY, and CRAMER, on acquired immunity, 139; on adaptation, 225; on autoplastic transplantation, 223; on the clinical course of transplanted tumors, 92; on the distinction between tumors and inflammatory swellings, 78; on the distribution of immunity, 153; on the duration of immunity, 154; on early stages, 59; on fluctuations in growth energy, 85; on the frequency of cancer in mice, 199; on variations during transplantation, 99; on the histology of receding tumors, 91; on infiltrative growth, 191, 192; on the transmissible lympho-sarcoma of the dog, 230; on metastasis, 190; on the optimum conditions of transplantation, 88; on phagocytosis in spontaneous absorption, 92; on the propagation of hemorrhagic tumors, 72; on race and susceptibility, 132; on the relative importance of soil and graft, 72; on sarcoma development, 110; on the specificity of immunity, 142; on spontaneous absorption, 91; on the development of spontaneous tumors during immunity, 128; on the stroma of hemorrhagic tumors, 109;

on the action of radium, 261; on tumors of the mouse, 188; on virulence, 66
BASHFORD, MURRAY, and HAALAND, on alternations in biological qualities of the tumor cell, 75; on athrepsia, 167; on the hereditary transmission of immunity, 158; on hypersusceptibility, 180; on immunity in tumor-bearing animals, 151; on passive resistance, 156; on sarcoma development, 115; on the specificity of immunity, 142; on the development of spontaneous tumors during immunity, 128
BASHFORD, MURRAY, HAALAND, and BOWEN, on adaptation, 65; on growth energy, 89; on race and susceptibility, 132; on the technic of inoculation, 70
BASHFORD and RUSSELL, on athrepsia, 168; on spontaneous absorption, 90
BEARD, on ætiology, 18; on treatment, 260
BECK, on treatment, 263
BECKTON, on Altmann's granules, 193; see also COLWELL and BECKTON
BECKTON and COLWELL, on Altmann's granules, 194
BECKTON and RUSS, on Altmann's granules, 194
BEEBE, on treatment, 235; see also CRILE and BEEBE
BEEBE and EWING, on the transmissible lympho-sarcoma of the dog, 232, 233
BEEBE and TRACY, on treatment, 235
BEHLA, on homoplastic transplantation, 42
BENEKE, on kataplasia, 16
BENSLEY, on Altmann's granules, 194
BENTHIN, on atypical epithelial growth, 36
BERGEL, on a fat-splitting ferment in lymphocytes, 37
BERGELL and STICKER, on treatment, 235
v. BERGMANN, on autoplastic transplantation, 45
BILLROTH, on heteroplastic transplantation, 51
BIRCH-HIRSCHFELD and GARTEN, on the production of tumors, 22
BLUMENTHAL, on treatment, 259; on the deposition of arsenic, 267; see also v. LEYDEN and BLUMENTHAL
BONNET, on thyroid adeno-carcinoma of the trout, 236
BORREL, on athrepsia, 165; on immunity, 140, 146; on the infective nature of tumors, 195; on the interval after which growth becomes apparent, 77; on metastasis, 190; on parasites and ætiology, 195, 196; on the percentage of success, 71; on squamous cell carcinoma, 188; on treatment, 257; on transplantation, 57
BORREL and PETIT, on autoplastic transplantation, 223
BORRMANN, on ætiology, 13
BORST, on ætiology, 13
BOWEN, see BASHFORD, MURRAY, and BOWEN, and BASHFORD, MURRAY, HAALAND, and BOWEN
BOYCOTT, on uterine tumors in the rabbit, 22
BRIDRÉ, on athrepsia, 166; on immunity, 140, 144, 146, 154; on the interval after which growth becomes apparent, 77; on parasites and ætiology, 196; on passive resistance 156; on pregnancy and susceptibility, 138; on the premetastatic stage, 152; on the resistance of the cancer cell, 82; on treatment, 257; on the technic of inoculation, 68
BRIDRÉ and CONSEIL, on parasites and ætiology, 196
BRODIE-MILLS, on horn core, 6
BROSCH, on atypical epithelial growth, 22
BROWN, on the nucleus, 3
BUDD, on homoplastic transplantation, 41
BURGESS, on the stroma reaction in immunity, 176
BURKHARDT, on Altmann's granules, 194
BURROWS, on tissue cultivation *in vitro*, 124, 126; see also CARREL and BURROWS
BUSCHKE, on age and susceptibility, 129
BUTLIN, on autoplastic transplantation, 44, 46; on homoplastic transplantation, 39

CALKINS, on rhythms of growth, 88
CARMALT, on the ameboid motion of cancer cells, 5
CARREL, on tissue cultivation *in vitro*, 125, 126
CARREL and BURROWS, on tissue cultivation *in vitro*, 124, 126
CASPARI, see NEUBERG and CASPARI, and NEUBERG, CASPARI, and LÖHE
CASPER, on the zoölogical distribution of cancer, 184
CAZIN, see DUPLAY and CAZIN
CELSUS, on classification, 1
CHALMERS and PERRY, on ætiology, 6
CHISHOLM, on the blood of rats bearing transplantable sarcoma, 96
CLOWES, on the hereditary transmission of immunity, 157 (footnote); on immunity, 141, 143; on race and susceptibility, 132; on reinoculation, 164; on the resistance of the cancer cell, 81; on spontaneous absorption, 90, 138; on the technic of inoculation, 71; on treatment, 257; see also GAYLORD and CLOWES, and GAYLORD, CLOWES, and BAESLACK
CLOWES and BAESLACK, on age, race, and susceptibility, 76; on the distinction between tumors and inflammatory swellings, 78; on dosage, 73; on immunity, 139, 141, 155; on the interval after which growth becomes apparent, 77; on spontaneous absorption, 91; on the stimulation of growth energy, 68
CLUNET, on the histology of tumors after exposure to X-rays, 260; on sarcoma development, 123; on sarcoma developing after ex-

posure to X-rays, 30; on the development of spontaneous tumors during immunity, 128; see also MARIE and CLUNET
COCA, on treatment, 269; see also v. DUNGERN and COCA
COCA, DORRANCE, and LEBREDO, on treatment, 268
COCA and GILMAN, on treatment, 268
COHNHEIM, on ætiology, 8
COLWELL, see BECKTON and COLWELL
COLWELL and BECKTON, on Altmann's granules, 194
CONSEIL, see BRIDRÉ and CONSEIL
CONTAMIN, on the action of X-rays, 261
COPEMAN and HAKE, on the acidity of the gastric contents in tumor-bearing animals, 97
CORNIL, on autoplastic transplantation, 49
CRAMER, on metabolism in tumor-bearing rats, 97; see also BASHFORD, MURRAY, and CRAMER
CRAMER and PRINGLE, on metabolism in tumor-bearing rats, 98
CRILE and BEEBE, on treatment, 235
CRITZMANN, on the relation between twin births and cancer, 18
CUÉNOT and MERCIER, on the hereditary transmission of immunity, 159; on the hereditary transmission of susceptibility, 158; on pregnancy and susceptibility, 138; on race and susceptibility, 136

DAVIDSOHN, on amyloid degeneration in the white mouse, 96
DETON, on the genesis of mouse tumors, 187
DORRANCE, see COCA, DORRANCE, and LEBREDO
DUDGEON, see SHATTOCK, SELIGMANN, and DUDGEON
v. DUNGERN, on immunity, 155; on the transmissible lympho-sarcoma of the dog, 235; on passive resistance, 155
v. DUNGERN and COCA, on immunity, 172; on a transmissible sarcoma of the hare, 235
DUPLAY and CAZIN, on heteroplastic transplantation, 52; on the transmissible lympho-sarcoma of the dog, 227
DUPUYTREN, on heteroplastic transplantation, 50

EBERTH and SPUDE, on the genesis of mouse tumors, 185
EDELFSEN and HENSEN, on growth and pregnancy, 98
EHRENREICH and MICHAELIS, on the zoölogical distribution of cancer, 184
EHRLICH, on the resistance of the cancer cell, 82; on inoculation site, 77; on the percentage of success, 71; on the technic of inoculation, 69; on the stimulation of growth energy, 68; on the stroma reaction, 61; on increase of virulence, 62; on the stroma in hemorrhagic tumors, 109; on sarcoma development, 110, 113; on passive resistance, 156; on immunity, 140, 141, 154, 159; on the transplantation of tumor mixtures, 79; on pregnancy and susceptibility, 138; on sex and susceptibility, 137; on the limits of transplantation, 129; on athrepsia, 159, 169; on spontaneous absorption, 220; on ætiology, 208; on chondroma in the mouse, 188; see also APOLANT and EHRLICH
EHRLICH and APOLANT, on age and susceptibility, 130; on the frequency of cancer in mice, 199; on sarcoma development, 109, 111, 112, 113; on spontaneous carcinoma sarcomatodes, 188
EHRLICH, H., see KRAUS, RANZI, and H. EHRLICH
v. EISELSBERG, on a transplantable sarcoma of the rat, 54
EWING, on autoplastic transplantation, 44, 48; on thyroid adeno-carcinoma of the trout, 241; on tissue cultivation *in vitro*, 126; see also BEEBE and EWING

FALLOPIUS, on diagnosis and treatment, 3
DA FANO, on the relation of plasma cells and lymphocytes to immunity, 176; on the distribution of immunity, 153
FARMER, MOORE, and WALKER, on heterotypical mitosis, 17
FÉRÉ, on the growth of transplanted tissues in brooding hens, 28
FICHERA, on pregnancy and susceptibility, 28, 138; on treatment, 268; on the production of tumors, 28
FIEBIGER, on the zoölogical distribution of cancer, 184
FINSTERER, see PFEIFFER and FINSTERER
FIRKET, on a transplantable sarcoma of the rat, 55
FISCHER, on atypical epithelial growth, 30
FISCHER, E., on treatment, 265
FLEISCHMANN, see MICHAELIS, FLEISCHMANN, and PINCUSSOHN
FLEXNER, on heteroplastic transplantation, 52 (footnote)
FLEXNER and JOBLING, on adeno-carcinoma in the rat, 188; on the duration of immunity, 155; on hypersusceptibility, 178; on immunity, 139, 140; on inoculation site, 77; on metastasis, 191; on the premetastatic stage, 153
FOLLIN, on heteroplastic transplantation, 51
FRAENKEL, on the production of tumors, 22
FRANK and UNGER, on hormones, 259 (footnote)
FREUND, on pregnancy and growth, 29; on the production of tumors, 27
FREYTAG, on amyloid degeneration, 95; on treatment, 258
FUJINAMI and INAMOTO, on a transplantable tumor of the fowl, 244

GALEN, on ætiology and treatment, 2
GARTEN, see BIRCH-HIRSCHFELD and GARTEN
GAY, on hypersusceptibility, 180; on passive resistance, 156; on the premetastatic stage, 153; on race and susceptibility, 136; on the transplantation of metastases, 79; on treatment, 258
GAYLORD, on hypersusceptibility, 179; on passive resistance, 157; on the resistance of the cancer cell, 81; on thyroid adeno-carcinoma of the trout, 239, 240, 241, 243; on treatment, 236
GAYLORD and CLOWES, on cancer cages, 197; on dosage, 73; on spontaneous absorption, 91, 92, 139
GAYLORD, CLOWES, and BAESLACK, on spontaneous absorption, 138
GEISSLER, on the transmissible lympho-sarcoma of the dog, 228
GIERKE, on age and susceptibility, 130; on athrepsia, 162, 168; on classification, 220; on metastasis, 191; on race and susceptibility, 134; on histology and malignancy, 107; on sex and susceptibility, 137; on the specificity of immunity, 142; on the stroma reaction, 61; on the technic of inoculation, 70
GILMAN, see COCA and GILMAN
GILRUTH, on thyroid adeno-carcinoma of the trout, 237
GOLDMANN, on parabiosis, 259 (footnote); on inoculation site, 77
GOSIO, on the reduction of selenium and tellurium by bacteria, 264
GOUGEROT and LAROCHE, on the production of tumors, 29
GRAWITZ, on slumber cells, 18
GREISCHER, on atypical epithelial growth, 35
GRÜNBAUM, A. and H., on treatment, 259, 260
GUDERNATSCH, on non-encapsulation of the trout thyroid, 240
GUELLIOT, on homoplastic transplantation, 40

HAALAND, on age and susceptibility, 130; on autoplastic transplantation, 209, 224; on the resistance of the cancer cell, 83; on the frequency of cancer in mice, 200; on health and susceptibility, 137; on hypersusceptibility, 181; on immunity with devitalized tissues, 144, 149; on immunity in tumor-bearing mice, 151, 152; on inflammation and ætiology, 205; on lactation and ætiology, 200; on metastasis, 114, 190; on operative removal and recurrence, 222; on passive resistance, 156; on pregnancy and susceptibility, 137; on race and susceptibility, 133; on the relative resistance of tumors to heat, 82; on sarcoma development, 113, 118; on the specificity of immunity, 142; on spontaneous absorption, 221; on squamous cell carcinoma, 188; on the technic of inoculation, 68, 69; on the transplantation of tumor mixtures, 79; on tumors of the mouse, 188; see also BASHFORD, MURRAY, and HAALAND, and BASHFORD, MURRAY, HAALAND, and BOWEN
HAENDEL, see UHLENHUTH, HAENDEL, and STEFFENHAGEN
HAHN, on autoplastic transplantation, 48
HAKE, see COPEMAN and HAKE
HAMBURGER, on autoplastic transplantation, 46
HANAU, on atypical epithelial growth, 21; on a transplantable carcinoma of the rat, 53
HANES, see LAMBERT and HANES
v. HANSEMANN, on ætiology, 7; on the analogy between human and mouse cancer, 193; on anaplasia, 14, 16; on atypical mitosis, 14; on the genesis of mouse tumors, 185, 189; on heterotypical mitosis, 17; on the transmissible lympho-sarcoma of the dog, 228; on metastasis, 103, 193; on sarcoma development, 111 (footnote); on the selective action of selenium, 265; on thyroid adeno-carcinoma of the trout, 239; on heteroplastic transplantation, 52
HARRISON, on tissue cultivation *in vitro*, 124
HAUSER, on ætiology, 7, 12, 13
HECHT, see ALBRECHT and HECHT
HELMHOLZ, on atypical epithelial growth, 32
HENSEN, see EDELFSEN and HENSEN
HÉRICOURT and RICHET, on treatment, 267
HERTWIG, on ætiology, 9
HERTWIG and POLL, on athrepsia, 165; on immunity, 141; on race and susceptibility, 132, 133; on the technic of inoculation, 71
HERXHEIMER and REINKE, on the relation of lipoids to cell division, 37
HERZOG, on a transplantable sarcoma of the rat, 188; on pregnancy and susceptibility, 137
HEYDE, see SAUERBRUCH and HEYDE
HIGUCHI, on immunity, 147, 148
v. HIPPEL, on the production of tumors, 27
HIPPOCRATES, on cancer of the breast, 1
HODENPYL, on treatment, 269
HOFER, on thyroid adeno-carcinoma of the trout, 237
HOOKE, on the cellular structure of cork, 3

ILL and MININGHAM, on treatment, 269
INAMOTO, see FUJINAMI and INAMOTO

JABOULAY, on thyroid adeno-carcinoma of the trout, 237
JACOBY, on the deposition of salicylic acid, 267
JANEWAY, on ætiology, 14
JENNY, on the histology of *Hanau's* rat carcinoma, 53
JENSEN, on acquired immunity, 139; on diet and susceptibility, 134; on early stages, 58; on the genesis of mouse tumors, 185; on heredity and ætiology, 205; on the interval after which growth becomes apparent, 77; on

race and susceptibility, 130, 131; on the resistance of the cancer cell, 80; on selection, 141; on spontaneous absorption, 90; on the technic of inoculation, 68; on treatment, 256; on a transplantable carcinoma of the mouse, 56; on a transplantable sarcoma of the rat, 131, 188; on a transplantable tumor of the beet, 184; on transplantation, 71
JENTZER, on pregnancy and growth, 28
JOACHIM, on Egyptian medicine, 1
JOBLING, on the premetastatic stage, 153; on the re-inoculation of tumor-bearing animals, 168; on heteroplastic transplantation, 52; see also FLEXNER and JOBLING
JOBSON, see LOEB and JOBSON
JOLLY, on tissue cultivation *in vitro*, 126
JORES, on atypical epithelial growth, 31
JUNCKER, on homoplastic transplantation, 40

KAUFMANN, on autoplastic transplantation, 45; on the production of tumors, 21
KELLING, on the production of tumors, 23
KEYSSER, see v. WASSERMANN, KEYSSER, and WASSERMANN
KLEBS, on the conjugation of leucocytes with epithelial cells, 17; on autoplastic transplantation, 45; on mitosis, 14; on heteroplastic transplantation, 52
KLEMPERER, on treatment, 265
KOKUBO, on ætiology, 13
KRASKE, on autoplastic transplantation, 45
KRAUS, RANZI, and H. EHRLICH, on the distribution of immunity, 154

LACK, on autoplastic transplantation, 47; on the production of tumors, 21
LAMBERT, on immunity with autologous tissues, 150
LAMBERT and HANES, on the ameboid motion of cancer cells, 5, 125; on tissue cultivation *in vitro*, 125, 126
LANE-CLAYPON and STARLING, on hormones, 259
LANGENBECK, on heteroplastic transplantation, 50
LANZ, on homoplastic transplantation, 44
LAROCHE, see GOUGEROT and LAROCHE
LAZARUS-BARLOW, on the genesis of mouse tumors, 189
LEBERT, on heteroplastic transplantation, 51
LEBREDO, see COCA, DORRANCE, and LEBREDO
LEITCH, on hypersusceptibility, 181; on uterine carcinoma in the rabbit, 22
LENHART, see MARINE and LENHART
LEONIDES, on treatment, 2
LEOPOLD, on the production of tumors, 21
LEOPOLD, see LOEB and LEOPOLD
LEVIN, on immunity, 149, 150, 154; on the intense connective tissue reaction of rats, 33; on the production of tumors, 26
LEWIN, on age and susceptibility, 130; on athrepsia, 165; on immunity, 139, 142, 143, 146; on race and susceptibility, 135; on the resistance of the cancer cell, 82; on sarcoma development, 118; on sex and susceptibility, 137; on treatment, 258; on variations during transplantation, 107; see also MICHAELIS and LEWIN
LEWIN and MICHAELIS, on carcinoma mammæ in the rat, 188; on metastasis, 191 (footnote)
v. LEYDEN, on parasites and ætiology, 59; on treatment, 257
v. LEYDEN and BLUMENTHAL, on treatment, 257
LIEPMANN, on sarcoma development, 115
LIVINGOOD, on spontaneous tumors of the mouse, 189
LOEB, L., on ætiology, 25; on age and susceptibility, 129; on autoplastic transplantation, 223; on dosage, 73; on early stages, 59; on the importance of soil, 72; on metastasis, 190; on race and susceptibility, 131; on the resistance of the cancer cell, 81; on sarcoma development, 113, 114, 118; on sex and susceptibility, 137; on spontaneous absorption, 90; on a transplantable sarcoma of the rat, 57, 188; on the production of tumors, 24; on the transplantation of stationary or receding tumors, 78; see also WHITE and LOEB
LOEB and JOBSON, on carcinoma in cattle, 197
LOEB and LEOPOLD, on autoplastic transplantation, 223
LOEB and WHITE, on the effect of heat upon growth, latent period, and absorption, 83
LOEB, O., on the deposition of iodine, 267
LÖHE, see NEUBERG, CASPARI, and LÖHE
LUBARSCH, on amyloid degeneration, 95

MARIE and AUBERTIN, on uterine carcinoma in the rabbit, 22
MARIE and CLUNET, on the histology of tumors after exposure to X-rays, 260
MARINE and LENHART, on thyroid adeno-carcinoma in the trout, 242; on thyroid histology, 243
MARKS, see APOLANT and MARKS
MARTIN, on the production of tumors, 21
MAURER, on non-encapsulation of the thyroid in fish, 241
McCONNELL, on atypical epithelial growth, 31; on heteroplastic transplantation, 37
McCOY, on the zoölogical distribution of cancer, 184
MEDIGRECEANU, on the weights of organs in tumor-bearing animals, 93; on the amount of food ingested by tumor-bearing animals, 94
MERCIER, see CUÉNOT and MERCIER
METCHNIKOFF, on parasites and ætiology, 52; see also ROUX and METCHNIKOFF
MEYER, on atypical epithelial growth, 35

MICHAELIS, on athrepsia, 166; on cancer cages, 197; on classification, 213; on the distinction between tumors and inflammatory swellings, 78; on the genesis of mouse tumors, 186; on immunity, 143; on the stimulation of growth energy, 68; on infiltrative growth, 191, 192; on the interval after which growth becomes apparent, 77; on metastasis, 191; on passive resistance, 156; on race and susceptibility, 132; on the resistance of the cancer cell, 81; on selection, 141; on spontaneous absorption, 90; see also EHRENREICH and MICHAELIS, and LEWIN and MICHAELIS
MICHAELIS FLEISCHMANN, and PINCUSSOHN, on immunity, 140, 142, 146
MICHAELIS and LEWIN, on variations during transplantation, 107
MICHAUD, on the deposition of iodine, 267
MILLER, on Altmann's granules, 194
MILNER, on implantation cancer, 49
MININGHAM, see ILL and MININGHAM
MINOT, on growth rate, 84; on pregnancy and growth, 98
MOORE, see FARMER, MOORE, and WALKER
MOORE and WALKER, on the resistance of the cancer cell, 81
MORAU, on the hereditary transmission of susceptibility, 157; on pregnancy and susceptibility, 137; on a transplantable carcinoma of the mouse, 54
MORESCHI, on hypersusceptibility, 180; on immunity with alien tissues, 148; on immunity with normal tissues, 146; on tumor growth and nourishment, 92
DE MORGÁN, on ætiology, 18
MÜLLER, on the cellular structure of tumors, 3
MURPHY, see ROUS and MURPHY
MURRAY, on adaptation, 65, 208; on the clinical course of spontaneous tumors, 221; on the frequency of cancer in mice, 199; on hepatic carcinoma in the cow, 207; on heredity and ætiology, 202, 204; on the histology of carcinoma mammæ, 219; on immunity and histology, 108; on the interval after which growth becomes apparent, 77 (footnote); on the malignancy of mouse tumors, 192; on metastasis, 191; on spontaneous absorption, 220; on sweat glands in the mouse, 185; on the technic of inoculation, 70; on thyroid adeno-carcinoma of the trout, 236; on a transplantable keratinizing adeno-carcinoma of the mouse, 100; on a transplantable osteo-chondro-sarcoma, 100; on the transplantation of metastases, 79; on tumors of the mouse, 188; on the zoölogical distribution of cancer, 184; see also BASHFORD and MURRAY, BASHFORD, MURRAY, and BOWEN, BASHFORD, MURRAY, and CRAMER, BASHFORD, MURRAY, and HAALAND, and BASHFORD, MURRAY, HAALAND, and BOWEN
NEUBERG and CASPARI, on treatment, 265
NEUBERG, CASPARI, and LÖHE, on treatment, 266
NEVE, on the Kangri basket, 6
NEWSHOLME, on cancer houses, 43; on heredity and ætiology, 201
NICHOLS, on the production of tumors, 25
NOVINSKY, on the transmissible lympho-sarcoma of the dog, 227

OEFELE, on Egyptian medicine, 1
OERTEL, on cancerous degeneration, 12
ORDWAY, see TYZZER and ORDWAY
ORTH, on sarcoma development, 118
OSWALD, on diffusion, 267

PARACELSUS, on *Galen's* hypothesis, 3
PARÉ, on treatment, 3
PERRY, see CHALMERS and PERRY
PETERSEN, on ætiology, 13; on retrograde metastasis, 44
PETIT, see BORREL and PETIT
PEYRILHE, on heteroplastic transplantation, 50
PFEIFFER, on a transplantable melano-carcinoma of the mouse, 53; on anaphylaxis, 182
PFEIFFER and FINSTERER, on anaphylaxis, 182
PFÖRRINGER, on ætiology, 13
PICK, on thyroid adeno-carcinoma of the trout, 238
PICK and POLL, on the genesis of mouse tumors, 185; on thyroid adeno-carcinoma of the trout, 238
PINCUSSOHN, see MICHAELIS, FLEISCHMANN, and PINCUSSOHN
PLEHN, on thyroid adeno-carcinoma of the trout, 237, 238 (footnote), 239; on the zoölogical distribution of cancer, 184
PLICQUE, on irritation and ætiology, 6
PODWYSSOZKI, on asymmetrical mitosis, 15
POLL, see HERTWIG and POLL, and PICK and POLL
PRICE-JONES, on the blood of tumor-bearing mice, 96
PRINGLE, see CRAMER and PRINGLE
PURVIS, on thyroid adeno-carcinoma of the trout, 236

QUINCKE, on autoplastic transplantation, 47

RANZI, on anaphylaxis, 182; see also KRAUS, RANZI, and H. EHRLICH
RAUM, on Altmann's granules, 194
REGAUD, on parasites and ætiology, 196
REICHER, on treatment, 262
REINKE, on atypical epithelial growth, 26; see also HERXHEIMER and REINKE
REMAK, on cell division, 4
RIBBERT, on ætiology, 10; on anaplasia, 16; on asymmetrical mitosis, 15; on autoplastic transplantation, 224; on heteroplastic trans-

plantation, 52, 224; on the production of tumors, 23
RICHARDSON, on autoplastic transplantation, 47
RICHET, see HERICOURT and RICHET
ROUS, on hypersensibility, 182; on parabiosis, 259; on pregnancy and transplantation, 28; on the specificity of immunity, 147; on a transmissible sarcoma of the fowl, 244; on the production of tumors, 29; on tumor growth and nourishment, 93
ROUS and MURPHY, on a transmissible sarcoma of the fowl, 244
ROUS, MURPHY, and TYTLER, on a transmissible sarcoma of the fowl, 244
ROUX and METCHNIKOFF, on heteroplastic transplantation, 52
ROVSING, on treatment, 268
RUSS, see BECKTON and RUSS
RUSSELL, on extirpation and immunity, 163; on the stroma reaction in immunity, 174; on the immunization of tumor-bearing animals, 151; on the duration of immunity, 155; on antibodies in immunity, 157; on sarcoma development, 110, 122; see also BASHFORD and RUSSELL

SAILER, on the inoculability of carcinoma, 57
SAUERBRUCH and HEYDE, on parabiosis, 258
SAUL, on parasites and ætiology, 196
SCHLEIDEN, on the cellular structure of plant tissues, 3
SCHMIDT, on pulmonary tumor emboli in man, 115
SCHMINCKE, on atypical epithelial growth, 35; see also WACKER and SCHMINCKE
SCHÖNE, on athrepsia, 161; on immunity with embryo, 145; on the specificity of immunity, 147; on immunity in tumor-bearing animals, 150
SCHWANN, on the cellular structure of animal tissues, 3
SCOTT, on thyroid adeno-carcinoma of the trout, 236
SELIGMANN, see SHATTOCK, SELIGMANN, and DUDGEON
SENN, on homoplastic transplantation, 43; on autoplastic transplantation, 49
SEYBERTH, on bladder tumors in aniline dye workers, 33
SHATTOCK, on uterine carcinoma in the rabbit, 22
SHATTOCK, SELIGMANN, and DUDGEON, on pregnancy and growth, 28
SMITH, on homoplastic transplantation, 42
SMITH and WASHBOURN, on the transmissible lympho-sarcoma of the dog, 228
SNOW, on atypical epithelial growth, 32
SPIESS, on treatment, 262
SPUDE, see EBERTH and SPUDE
STAHR, on atypical epithelial growth, 33; on the interval after which growth becomes apparent, 77; on the production of tumors, 23, 188; on race and susceptibility, 135; on sarcoma development, 123; on the technic of inoculation, 71
STARLING, see LANE-CLAYPON and STARLING
STEFFENHAGEN, see UHLENHUTH, HAENDEL, and STEFFENHAGEN
STICKER, on the transmissible lympho-sarcoma of the dog, 228; on the premetastatic stage, 152; on sarcoma development, 118; on treatment, 235; on the zoölogical distribution of cancer, 184; see also BERGELL and STICKER
STOCKARD, on non-encapsulation of the trout thyroid, 240
STOEBER, on atypical epithelial growth, 34
STOEBER and WACKER, on atypical epithelial growth, 34
STROEBE, on asymmetrical mitosis, 16

TAKEMURA, on the deposition of iodine, 267
THIERSCH, on ætiology, 5, 6; on metastasis, 5
THOREL, on the development of spontaneous tumors during immunity, 128
THORN, on autoplastic transplantation, 46, 47, 49
v. TIESENHAUSEN, on the production of tumors, 27
TRACY, see BEEBE and TRACY
TULPIUS, on homoplastic transplantation, 40
TYTLER, see ROUS, MURPHY, and TYTLER
TYZZER, on the hereditary transmission of immunity and susceptibility, 157; on heredity and ætiology, 204; on race and susceptibility, 136
TYZZER and ORDWAY, on the zoölogical distribution of cancer, 184

UHLENHUTH, HAENDEL, and STEFFENHAGEN, on athrepsia, 162; on the distribution of immunity, 154; on dosage, 75; on the duration of immunity, 155; on hypersusceptibility, 181; on passive resistance, 157; on treatment, 259, 263
UHLENHUTH and WEIDANZ, on immunity, 147, 149; on pregnancy and susceptibility, 137; on race and susceptibility, 136; on the resistance of the cancer cell, 83; on the transplantation of metastases, 79; on treatment, 263
UNGER, see FRANK and UNGER

VON DEN VELDEN, on treatment, 266
VELICH, on a transplantable sarcoma of the rat, 55
VERSÉ, on ætiology, 13
VESALIUS, on *Galen's* hypothesis, 3
VIDAL, on treatment, 260, 269
VIEL-HAUTMESNIL, on homoplastic transplantation, 41, 50
VIRCHOW, on ætiology, 4; on the analogy between tumor cell and ovum, 17; on autoplastic

transplantation, 48; on heteroplastic transplantation, 51; on metastasis, 4

WACKER, see STOEBER and WACKER
WACKER and SCHMINCKE, on atypical epithelial growth, 36
WADE, on the transmissible lympho-sarcoma of the dog, 234
WAKASUGI, on autoplastic transplantation, 47
WALDEYER, on ætiology, 5, 7
WALKER, on treatment, 258, 265; see also FARMER, MOORE, and WALKER, and MOORE and WALKER
WALKER and WHITTINGHAM, on heterotypical mitosis, 18
WASHBOURN, see SMITH and WASHBOURN
WASSERMANN, see v. WASSERMANN, KEYSSER, and WASSERMANN
v. WASSERMANN, KEYSSER, and WASSERMANN, on treatment, 263
WEHR, on the transmissible lympho-sarcoma of the dog, 227
WEIDANZ, see UHLENHUTH and WEIDANZ
WEIGERT, on ætiology, 11
WERNER, on SCHARLACH R, 37
WHITE, on atypical epithelial growth, 35; on the transmissible lympho-sarcoma of the dog, 230
WHITE and LOEB, on the transplantation of stationary or receding tumors, 78
WHITEHEAD, on homoplastic transplantation, 41
WHITTINGHAM, see WALKER and WHITTINGHAM
WILKIE, on thyroid adeno-carcinoma of the trout, 236
WILLIAMS, on the malignancy of mouse tumors, 189
WILMS, on the production of tumors, 25
WÖLFLER, on medicine in India, 1
WOGLOM, on immunity, 150, 154, 155, 175
WOLFF, on *Celsus*, 1
WYSS, on X-ray carcinoma, 33

YAMANOUCHI, on hypersensibility, 181

ZACUTUS, on homoplastic transplantation, 39
ZAHN, on the production of tumors, 21

INDEX OF SUBJECTS

Absorption, spontaneous, **90**; connective tissue in, 91, 92, 221, 234; in foreign race, 136; giant cells in, 91, 92; heated tumor, 83; hemorrhage in, 91, 92; histology, **91**, 221, 234; during hypersusceptibility, 178; during lactation, 138; lymphocytes in, 92, **177**, 221; mitosis, 234; phagocytes, 92, 221; during pregnancy, 138; re-inoculation after, 139; of spontaneous tumor, 87, **220**, 228, 241, 243; of transplanted tumor, 83, 87, **90**, 120, 122, 136, 138, 177, 178, 221, 228, 229, 232, 244, 246
Absorption of graft in immunity, 175
Acquired resistance, **138**, 228, 230, 234, 241, 243, 245
Active resistance, 138
Adaptation, **61**, 208, 226
Adrenalin, 91, 260, 262, 263
Ætiology, **195**; age, 6, 7, 9, 11, 129, 130, 184, **199**, 202, 231, 238; alien cells, 23; anaplasia, **14**, 17; asexual phase of development, 19; avidity for food-stuffs, 7, 33, 64, **208**; bile, 1, 2; cancer cages, 195; cancerous degeneration, **12**, 14; conjugation of cells, 17; embryonal rests, **8**, 9, 10, 11, 13; function, 200; heredity, **200**, 230, 238, 239; heterotypical mitosis, 17, 18; in-breeding, 198; infection, **195**, 229, 230, 235, 237, 238, 239, 241, 242, 244, 252; insects, 76, **195**, 196; irritation, inflammation, and trauma, 4, **5**, 6, 7, 8, 10, 11, 13, 14, 18, 21, 23, 24, 34, 200, **205**, 250; lactation, 200; lipoids, 38; parasites, 195, **207**, 238; sensitization and stimulation, 25; sex, 199; side chain theory, 208; slumber cells, 18; soil, 11; stimulus, 5, 11, 13, 17, 18, 25, 34; trauma, **6**, 8, 250; twin pregnancy, 18; X-rays, 30, 33
Age, in ætiology, 6, 7, 9, 11, 129, 130, 184, **199**, 202, 231, 238; limit of, in mouse, 201; as affecting transplantation, 76, **129**, 212, 228, 245, 255; of tumor and differentiation, 99, **102**; of tumor and metastasis, 190
Allergie, 172
Altmann's granules, 193
Alveolar carcinoma, 105
Ameboid motion of cancer cell, 5, **125**
Amitosis, 59, 60, 246, 247
Amyloid degeneration, 95
Anaphylaxis, 182
Anaplasia, **14**, 16, 103
Angioblasts, chemotaxis of cancer cell for, 109
Angioplastic stroma reaction, 61
Aniline dye workers, tumors of the bladder in, 33
Animals, frequency of tumors among, 183
Antibodies, 63, 125, **155**, 159, 163, 165, 166, 171, 182, 257, 259, 267
Appetite in tumor-bearing animals, 94
Arsenic, 1, 3, 263
Athrepsia, **159**, 208
Atoxyl, 235, 263
Attraxin, 31
Autoplastic transplantation, 27, **44**, 151, 209, **212**, **223**, 245
Avidity for food-stuffs, 7, 9, 29, 64, 99, **161**, **208**

Bed bugs, attempted transmission by, 76
Beet, transplantable tumor of, 184
Betel-nut, **6**, 10
Bile as an ætiological factor, 1, 2
Biological qualities of tumor cells, alternations in, 75
Bladder, tumors of, in aniline dye workers, 33
Blastosis, 38
Blood in tumor-bearing animals, **96**, 229, 234
Blood stream, metastasis by, 190, 229, 246
Body, growth during pregnancy, 98

Cachexia, 92
Cancer à deux and *cancer à trois*, 42
Cancer cages, 195
Cancer cell, alternations in biological qualities, 75; ameboid motion, 5, **125**; chemotaxis, 61, 109, 173, 175; growth energy, 83; resistance of, 56, **80**, 163, 171, 229, 248, 252, 261; serum proof, **163**, 170
Cancer houses, 42; see also "Cancer cages"
Cancer, ovarian, production of, 21; and twin births, 18; zoölogical distribution of, 184
Carcinoma, alveolar, 105; sarcomatodes, 80; X-ray, 33; in the young, 7
Cattle, carcinoma inner canthus, 197
Cell as a tissue unit, 3
Cell genesis and intercellular substance, 4
Chemotaxis, by cancer cell, 61, 109, 173, 175; by fat stains, **31**, 32, 33, 35
Chemotherapy, 263
Chromosomes, in asymmetrical mitosis, 15
Classification of mouse tumors, 213
Clinical course of transplanted tumors, 92; of spontaneous tumors, 220
Collagen, 60

Comparative growth rate of the malignant cell, 83
Conditions, of growth, **129**, 130, 147; of origin, **129**, 130
Conjugation of cells, 17
Connective tissue, growth energy of, **6**, 7, 10, 11, 13; intense reaction in rats, 33; in irradiated tumors, 261; mitosis in, 60; removal of from tumor emulsions, 71; in spontaneous absorption, 91, 92, 221, 234
Contact transplantation, 44
Contagion, **39**, **195**, 230, 242
Cow, hepatic carcinoma in, 207
Cultivation of cells *in vitro*, **124**, 233
Cure, see "Absorption, spontaneous," and "Treatment"
Cysts, in mamma, 205; production of, 21, 26

Deciduoma, production of, 24
Degeneration, amyloid, 95; cancerous, **12**, 14
Dermoid cyst, production of, 26
Diet, amount in tumor-bearing animals, 94; and metastasis, **93**, 134; and recurrence, 134; and susceptibility, 133, 135; and tumor growth, 92
Differentiation, age of tumor, 99, **102**; cyclical variability of, 108; dual, 101, **104**; in embryo and tumor, 9; and growth energy, 14, 105, 220; latent, 104; lost, 11, 104; and metaplasia, 103; in metastases, 103; in thyroid tumors, 108; in transplanted tissues, 22, 24, 25
Distribution, of active resistance, 153; of mamma in the mouse, 186
Dog, transmissible lympho-sarcoma, 227
Dosage, and athrepsia, 165, 166, 167, 168, 170; and growth energy, 65; and hypersusceptibility, 180, 181; and immunity, 75, 140, 141, 145, 146, 149, 150, 151; importance of accurate, 65, 69, **73**, 75, 86; small and large, 67, 72, 88
Dose, minimal tumor-forming, 75, 76, **145**
Duration of active resistance, 154
Dye workers, tumors of bladder in, 33

Early stages, see "Stroma reaction"
Edematous changes in the stroma, 219
Emaciation as a terminal event, 92
Embryo, growth energy of, 84; transplantation into, 253
Embryonal rests, 8, 9, 10, 11, 13
Emulsion, transplantation by, 68, 71, 165
Encapsulation, of trout thyroid, 240; of tumors, 192
Endothelioma, 185, 186, 189, 217, 219
Epithelioma, production of papillary, 23
Epithelium, conjugation of leucocytes with, 17; growth energy of, **6**, 7, 10, 11, 13; spindle shaped, 106
Equilibrium between tissues, **6**, 7, 10, 11, 12, **13**
Ereption, 170

Ferments, in metabolism, 98; treatment with, 235, 263
Fetal cells, immunity against, 148; immunity with, 145
Fetus, nitrogen value of, 99; and tumor compared, 98
Fibroblasts, penetration of graft by, 58, **60**, 174, 246
Fibroplastic stroma reaction, 61
First appearance of active resistance, 154
Flies, attempted transmission by, 76
Fluctuations in growth energy, 67, **85**, 220
Food-stuffs, avidity for, 7, 9, 29, 64, 99, **161**, **208**; for mouse tumors in the rat, 160; specific, 138, **160**, 164; specific, in pregnancy, 138
Fowl, transmissible sarcoma of, 244
Fox, transmission of dog tumor to, 229, 234, 235
Frequency of tumors among animals, 183
Function, and growth energy, 16; and ætiology, 200

Gametoid neoplasms, 17
Gastric contents, 97
Generation stages, 15
Gestation, see "Pregnancy"
Giant cells in spontaneous absorption, 91, 92
Gland, hibernating, 185
Graft, absorption during immunity, 175; and soil, relative importance of, 71; transplantation by, 68, 71; vascularization of, 58, **60**, 168, **174**, 246, 247; see also "Stroma reaction"
Growth, of body during pregnancy, 98; cytotypic and organotypic, 9; expansive, 192; infiltrative, 120, 123, **191**, 229, 230, 237, 238, 240, 241, 247; interval after which it becomes apparent, **77**, 83, 225; necessary for immunity, 144, 149; negative phase, 67, 135; of normal tissues during pregnancy, 28; and origin, conditions of, 129, 130; positive phase, 67; production of atypical epithelial, 21, 22, 26, **30**; its rapidity in hypersusceptibility, 178; retarded in immunity, 145, 146, 147, 172; rhythms of, 88; specific conditions for, 147; substance, 64; of transplanted tissues, 20; of tumors during pregnancy, 55, **137**; of tumor, relation of nutrition to, 92
Growth energy, in athrepsia, **161**, 164, 169, 171; in autoplastic transplantation, 225; and avidity, 7, **208**; of the cancer cell, 83; comparative in same mouse, 225; decreased, 55; and differentiation, 14, 105, 220; and dosage, 65; effect of cold upon, 80; effect of heat upon, 80; effect of X-rays upon, 261; of embryo, 84; of embryonic rests, 8, 9, 10, 11; of epithelium and connective tissue, **6**, 7, 10, 11, 13; fluctuations in, 67, **85**, 220; and function, 16; in grafts from stationary or

receding tumors, 78; and histology, 105, 220; inhibition of, 68; in lympho-sarcoma of the dog, 230; of ovum, 17, 84; in recurrences, 162, 164, **209**; in sarcoma development, 120, 122, 123, 124; of spontaneous tumor, 220; stimulation of, 68; in transmissible sarcoma of the fowl, 246; transplantability and virulence, 169

Hare, tumors of, 155, **172**
Health as affecting transplantation, **137**, 167
Hemolysis, 38
Hemorrhage, in irradiated tumors, 262; in spontaneous absorption, 91, 92; as a terminal event, 92
Hemorrhagic, changes in the stroma, 219; tumors, stroma of, 109; tumors, transplantation of, **72**, 212
Heredity, in ætiology, **200**, 230, 238, 239; transmission of immunity and susceptibility by, 55, **157**
Heteroplastic transplantation, 37, **50**, 229, 234, 235, 255
Hibernating gland, 185
Histological, expression of growth energy, 105, 220; variations during transplantation, 99
Histology, and immunity, 107; of irradiated tumors, 261; and malignancy, **104**, 107, 217, 238; of mammary tumors, 186, 213; of receding tumors, **91**, 221, 234; of spontaneous tumors, 184, **213**; and stroma reaction, 109
Historical review, 1
Homoplastic transplantation, 27, **39**, 53
Hormones, 259
Horn core, 6
Host and tumor, relation between, 223
Hybrids, susceptibility and immunity, **158**, 240
Hyperemia, 8, 10
Hypersensibility, 181
Hypersusceptibility, **178**; and dosage, 180, 181; and immunity, 179, 181; local, 173; optimum of, 181; produced by normal tissues, 146, 151, 156, 157, 179, 180, 181; produced by tumor, 151, 178, 179, 180, 181; rapidity of growth in, 178; specificity of, 180; spontaneous absorption in, 178; and time interval, 178, 180, 181
Hypertrophy, nodular, 205; of organs in tumor-bearing animals, 93

Immune zone, 230
Immunity, **128**; abrogation by operation, 162; absorption of graft in, 175; acquired, **138**, 228, 230, 234, 241, 243, 245; active, 138; and age, 76, **129**, 228, 245, 255; and allergie, 172; athreptic, **159**, 208; with autologous tissue, 150; with autolyzed tissue, 137, **149**, 150; to autoplastic transplantation, 151; and chemotaxis, 173, 175; common and specific, 142; concomitant, 67, 70, **74**, 87, 162, 165, 167; development of spontaneous tumors during, 128; distribution of, 153; and dosage, 75, 140, 141, 145, 146, 149, 150, 151; duration of, 154; against fetal cells, 148; with fetal cells, 145; first appearance of, 154; growth necessary for, 144, 149; growth retarded in, 145, 146, 147, 172; as affected by health, **137**, 167; hereditary transmission of, 55, 157; and histology, 107; in hybrids, **158**, 240; and hypersusceptibility, 179, 181; with intact homologous normal cells, 148; with intact homologous tumor cells, 143; leucocyte in, 236; lymphocyte in, **176**, 234, 247, 248; lymphoid cells in, 173; macrophage reaction in, 173; mitosis in, 174; natural, **128**, 228, 229, 241, 247; nature of, 159; with normal tissue, 144; and operation, 162; and parabiosis, 258; survival of parenchyma in, 174, 175; partial, 107, 172; passive, 155; plasma cell in, 173, **177**; and pregnancy, 137; premetastatic stage of, **152**, 230; and race, 55, 76, 79, **130**, 231, 245, 255; resistance of tumor cell to, 163; and selection, 141; and sex, 137; specificity of, 122, **141**, 147; stroma reaction in, 168, **174**, 247; with tumor, 122, **138**; in tumor-bearing animals, **150**, 152, 161; tumor mixtures in, 133; X-stuff in, 160
In-breeding, 198
Incitant, specific, 160
Individuality, 212, **225**
Infection, in ætiology, **195**, 229, 230, 235, 237, 238, 239, 241, 242, 244, 252; as a terminal event, 92; or transplantation? **58**, 229, 230, 231, 232, 234
Infectiveness, variations in, 89
Infectivity, 195
Infiltrative growth, 120, 123, **191**, 229, 230, 237, 238, 240, 241, 247
Inflammation, in ætiology, 7, 8, 10, 13, 24, **205**; chronic, of mamma, 205
Inheritance, Mendelian, and susceptibility, 158
Inoculation, site, 76; of stationary or receding tumors, 78; of tumor mixtures, 79; see also "Transplantation"
Insects in ætiology, 76, **195**, 196
Intercellular substance and cell genesis, 4
Interval after which growth becomes apparent, **77**, 83, 225
Intervals between successive inoculations, 71
Inunction, transplantation by, 75
Iodine, 242, 243
Irritation in ætiology, 4, 5, 6, 7, 10, 18, 21, 23

Kangri basket, 6, 10
Karyokinesis, see "Mitosis"
Kataplasia, 16
Keloid, production of, 29
Keratin, 83, 100, 107

Lactation, as an ætiological factor, 200; regression of tumors during, 138

Latent period, effect of heat upon, 83
Leeches, attempted transmission by, 76
Leucocyte, conjugation with epithelium, 17; in immunity, 236
Lipoids, significance of, 38, 266
Lymph nodes, enlargement recognized by Celsus, 2
Lymphocyte, in immunity, **176**, 234, 247, 248; in spontaneous absorption, 92, **177**, 221
Lymphoid cells in immunity, 173
Lympho-sarcoma of the dog, 227
Lymph stream, metastasis by, 53, 57, 190, 192, 227, 229, 246

Macrophage reaction in immunity, 173
Malignancy, **189**; and histology, **104**, 107, 217, 238; and metastasis, 161; and mitosis, 215, 218; of mouse tumors, 189
Malignin, 260
Mamma, chronic inflammation of, 205; cyst formation in, 205; distribution in the mouse, 186; histology of tumors, 186, 213; nodular hypertrophy of, 205; normal, 205; in old mice, 207; origin of tumors in, **184**, 193, 200; sclerosis of, 205
Marrow in tumor-bearing animals, 96
Membrane in relation to therapeutics, 267
Mendelian inheritance and susceptibility, 158
Mercury, 243
Metabolism in tumor-bearing animals, 97
Metaplasia and differentiation, 103
Metastases, transplantation of, 79
Metastasis, **189**; age of tumor, 190; and athrepsia, 161; by blood stream, 190, 229, 246; and diet, **93**, 134; differentiation in, 103; in the dog, 227, 228, 229, 230; early stages in, **114**, 250; in the eyelid, 244; in the fowl, 244, 246, 250; in the heart, 244, 246; immunity against, 134, 151; in the intestinal wall, 244; in the kidney, 190, 244; in the liver, 190, 191, 244, 246; in the lungs, 92, 114, 189, 192, 222, 244; by lymph stream, 53, 57, 190, 192, 227, 229, 246; and malignancy, 161; in the mediastinum, 190; metastatic stage, 152, **230**; mode of origin, 4, 5, 114, 250; in the ovary, 190; on the peritoneum, 190; premetastatic stage, 152, **230**; prevention of, 93, 134, 151, 258; retrograde, 44; in the retroperitoneal tissue, 190; in sarcoma development, 112, 114, 120, 123; in the skin, 244; in the spleen, **190**, 227, 246; in spontaneous tumors, 53, 57, **189**, 192, 193, 226; stroma reaction in, **114**, 250; in transplantable tumors, 190, 192; in the trout, 238, 239, 240, 241, 242
Metastatic stage, 152, **230**
Mincing machine, 69
Mitosis, asymmetrical, **15**, 68; in connective tissue surrounding graft, 60; in young grafts, 60, 174, 233, 247; in the fowl, 247; heterotypical, **17**, 18; hyperchromatic, **14**, 217; hypochromatic, 14; in immunity, 174; and malignancy, 215, 218; in spontaneous absorption, 234; stimulation of, 26; *in vitro*, 125, 126, 233
Mixed tumors, **109**; purification of, 82, 117, 120; see also "Tumor mixtures" and "Sarcoma development"
Mixtures, see "Tumor mixtures"
Morphology, see "Histology"
Mouse, age limit in, 201; distribution of mamma in, 186; tumors of, 184; carcinoma, growth of, in rat, 159
Multiple, spontaneous tumors, **188**, 199, 200; transplantation, 72; transplanted tumors, 72, 150, 152, 161, 208

Natural resistance, **128**, 228, 229, 241, 247
Nature of the resistant state, 159
Needle for inoculation, 70 (footnote)
Nipple, retraction of, 2
Nitrogen balance, 98
Nitrogen value of fetus, 99
Normal tissues, growth of, 21; growth during pregnancy, 28; hypersusceptibility produced by, 146, 151, 156, 157, 179, 180, 181; resistance produced by, 144
Nucleus, discovery of, 3
Nutriceptors, **64**, 208
Nutrition and tumor growth, 92
Nutritive capacity, limit of, 167

Operation, and immunity, 162; recurrence after, 189, 192, **222**, 229; re-inoculation after, 161; results of, 221; transplantation during, **47**, 226
Organs, transplantation into, **77**, 154; hypertrophy of, in tumor-bearing animals, 93
Origin, conditions of, 129, 130; of metastases, 4, 5, 114, 250; multicentric, **205**, 217; site of, **184**, 193, 200
Osteo-chondro-sarcoma, transplantable, 100
Ovum, growth energy of, 17, 84

Pan-immunity, 141
Papilloma, production of, 23
Parabiosis, 258
Parasites in ætiology, 195, **207**
Parenchyma, histological variations during transplantation, 99; secondary changes in, 215; survival in immune animals, 174, 175; survival after transplantation, 58, **59**, 168, 174; variations during transplantation, 99
Partial immunity, 107, 172
Passage, rapid, 71
Passive immunity, 155
Phagocytes, in spontaneous absorption, 92, 221; *in vitro*, 125
Pin prick, transplantation by, 76
Plasma cell in immunity, 173, **177**
Pluri-immunity, 142
Pregnancy, and athrepsia, 172; growth of body during, 98; growth of normal tissues during,

28; growth of tumors during, 55, **137**; as affecting transplantation, 137; specific food-stuffs in, 138; spontaneous absorption during, 138; weight of organs during, 93, 94
Premetastatic stage, 152, **230**
Prevention, of metastasis, 93, 134, **151**, 258; of recurrence, 1, 134, **151**, 164, 192
Proliferative energy, 169; see also "Growth energy"
Purification of mixed tumors, 82, 117, 120

Rabbit, uterine carcinoma in, 22
Race as affecting transplantation, 55, 76, 79, **130**, 231, 245, 255
Radium, 83, 91, 149, 181, **261**
Rat, food-stuffs for mouse tumors in, 160; growth of mouse carcinoma in, 159; intense connective tissue reaction in, 33; tumors of, 188
Receptors, **64**, 171, 208
Recession, see "Absorption, spontaneous," and "Treatment"
Recovery, see "Absorption, spontaneous" and "Treatment"
Recurrence, and diet, 134; growth energy in, 162, 164, **209**; prevention of, 1, 134, 151, 164, 192; after operation, 189, 192, **222**, 229; in relation to re-inoculation, 164; after sarcoma development, 113, 123; increase in weight during, 222
Refractory condition, see "Immunity"
Regression, see "Absorption, spontaneous" and "Treatment"
Reinoculation, after operation, 161; in relation to recurrence, 164; after spontaneous absorption, 139; of tumor-bearing animals, 150, 152, 161, 164, 166, 167, 168, 212, 223, 230, 232; and virulence, 140, 143, 171
Relation between tumor and host, 223
Resistance, 128; see also "Immunity"
Resistance of cancer cell, 56, **80**, 163, 171, 229, 248, 252, 261
Retraction of nipple, 2
Retrograde metastasis, 44
Rhythms of growth, 88

Salvarsan, 235
Sarcoma, development, 79, 106, **109**; production of, 23, 30; stroma reaction in, 59, 116; X-ray, 30
Scharlach R, 27, **30**
Sclerosis of mamma, 205
Selection and immunity, 141
Selenium, 263
Senility, see "Age"
Sensitization, 25
Serum-proof cancer cells, **163**, 170
Sex, in ætiology, 199; as affecting transplantation, 137
Side chain theory in ætiology, 208
Size attained by tumors, 57, 62, 192
Slumber cell hypothesis, 18
Soil, in ætiology, 11; and graft, relative importance of, 71; and sarcoma development, 110, 116
Spontaneous, absorption, 90; tumors, 183
Stimulation, of growth power, 68; of mitosis, 26
Stimulus in ætiology, 5, 11, 13, 17, 18, 25, 34
Stroma, death following transplantation, 58, 59, 168, 174; edematous changes in, 219; hemorrhagic changes in, 219; in hemorrhagic tumors, 109; histological changes during transplantation, 109; relation to sarcoma development, 111; sarcoma development in, 109; secondary changes in, 215
Stroma reaction, **58**; amitosis in, 60, 246, 247; angioplastic and fibroplastic, 61; in athrepsia, 168; in the dog, 229, 231, 232, 234; in the fowl, 246; and histological structure, 109; in immunity, 168, **174**, 247; in metastases, 114, 250; mitosis in, 60, 174, 233, 247; in the mouse, **58**, 168, 174, 176; in the rat, 59, **175**; in sarcoma, 59, 116; specificity of, 60
Structure of origin in the mouse, **184** 193, 200
Structure, see "Histology"
Sudan III, 30
Summary, 270
Susceptibility, and age, 76, **129**, 212, 228, 245, 255; and diet, 133, 135; and health, **137**, 167; hereditary transmission of, 55, **157**; of hybrids, **158**, 240; increased, 165, 166, 168, **178**, 223; and pregnancy, 137; and race, 55, 76, 79, **130**, 231, 245, 255; and sex, 137; in tumor-bearing animals, 165, 166, 168, **223**; for tumor mixtures, 79, **133**
Syringe for inoculation, 69

Technic of inoculation, 68
Tellurium, 263
Teratoma, production of, 25, 26
Therapeutics, see "Absorption, spontaneous," and "Treatment"
Thyroid, adeno-carcinoma of the trout, 236; gland, non-encapsulation in the trout, 240
Toxins, in treatment, 235; in metabolism, 98
Transfer, of tumors from one person to another, 39; of human tumors to animals, 50
Transmissibility, earlier observations on, 39
Transmissible sarcoma of the fowl, 244
Transplantability, 62; diminished, 78 (see also "Resistance of cancer cell"); growth energy, and virulence, 169; of thyroid adeno-carcinoma of the trout, 240, 242
Transplantation, as affected by age, 76, **129**, 212, 228, 245, 255; autoplastic, 27, **44**, 151, 209, **212**, **223**, 245; and avidity, 208; into bearer, 44; behavior of tumor cell during, 101; by contact, 44; by contagion, **39**, **195**, 230, 242; into embryo, 253; emulsion and graft methods, 68, 71; first appearance of tumor after, **77**, 83, 225; as affected by health, **137**, 167; of hemorrhagic tumors, **72**, 212; heteroplastic, 37, **50**, 229, 234, 235, 255; his-

tological variations during, 99; homoplastic, 27, **39**, 53; or infection? **58**, 229, 230, 231, 232, 234; of injured cells, 80 (see also "Resistance of cancer cell"); through insects, etc., 76, **195**, 196; by inunction, 75; of metastases, 79; multiple, 72; needle for, 70 (footnote); during operation, **47**, 226; optimum conditions for, 70, 88; into organs, **77**, 154; survival of parenchyma after, 58, **59**, 168, 174; by pin prick, 76; as affected by pregnancy, 137; as affected by race, 55, 76, 79, **130**, 231, 245, 255; as affected by sex, 137; site of election for, 76; within the same species, 27, **39**, 53; of spontaneous tumors, 65, 71, 72, 75, 212, 223, **226**; of stationary or receding tumors, 78, 225; death of stroma after, 58, 59, 168, 174; increased susceptibility to, 165, 166, 168, **178**, 223; syringe for, 69; technic of, 68; as affected by trauma, 249, 251; into tumor-bearing animals, 150, 152, 161, 164, 166, 167, 168, 212, 223, 230, 232; of tumor mixtures, 79; of warts, 44; zigzag, 159

Transplantations, intervals between successive, 71

Transplanted tissues, differentiation in, 22, 24, 25 growth of, 20; growth during pregnancy, 28

Transplanted tumors, 58

Trauma, in relation to ætiology, **6**, 8, 250; in relation to transplantation, 249, 251

Treatment, 1, 2, 3, 19, 230, 235, 236, **256**

Trout, thyroid adeno-carcinoma in, 236; non-encapsulation of thyroid gland, 240

Tumor, first appearance after transplantation, **77**, 83, 225; clinical course of, 92, 220; and fetus compared, 98; and host, relation between, 223; hypersusceptibility produced by, 151, 178, 179, 180, 181; immunity produced by, 122, **138**; mixtures and athrepsia, 171; mixtures, susceptibility and immunity for, 79, **133**; mixtures, transplantation of, 79; receding, transplantation of, 78; size attained by, 57, 62, 192; the spontaneous, 183; spontaneous, development during immunity, 128; spontaneous, distinguished from transplantable, 97, 212; spontaneous, fluctuations in growth energy, 87; spontaneous, frequency of occurrence, 183, **199**; spontaneous, growth energy, 220; spontaneous, histology, 184, **213**; spontaneous, metastasis, 53, 57, **189**, 192, 193, 226; spontaneous, multiple, **188**, 199, 200; spontaneous, recurrence, 189, **222**; spontaneous, sarcoma development in, 113; spontaneous, spontaneous absorption of, 87, **220**, 228, 241, 243; spontaneous, temporary arrest, 220; spontaneous, transplantation of, 65, 71, 72, 75, 212, 223, **226**; stationary, transplantation of, 78, 225; transplantable, distinguished from spontaneous, 97, 212; transplantable, metastasis, 190, 192; transplantable, spontaneous absorption of, 83, 87, **90**, 120, 122, 136, 138, 177, 178, 221, 228, 229, 232, 244, 246; the transplanted, 58; zone, 230

Tumors, attempts to produce, 20; of the dog, 227; of the fowl, 244; of a nature still undecided, 227; of bladder in aniline dye workers, 33; growth energy in stationary or receding, 78; of the hare, 155, **172**; mixed, 109; of the mouse, 184; origin of mammary, **184**, 193, 200; of the rabbit, 22; of the rat, 188; transplantable, multiple, 72, 150, 152, 161, 208; of the trout, 236

Twin births in relation to cancer, 18

Variations, in infectiveness, 89; in parenchyma during transplantation, 99

Vascularization of graft, 58, **60**, 168, **174**, 246, 247

Virulence, and adaptation, 61; and athrepsia, 161, 164, 165, 166, 167, 169, 170, 171; increased, 79, 210; maximal, 170; and reinoculation, 140, 141, 143, 171; and sarcoma development, 112, 117, 118, 120, 124; transplantability, and growth energy, 169

Warts, transplantation of, 44

Weight, increase during recurrence, 222; of organs in tumor-bearing animals, 93; of tumor-bearing animals, **94**, 97, 221, 222

X-ray, carcinoma, 33; sarcoma, 30

X-rays, 30, 33, 260, 261

X-stuff, 160

Young, carcinoma in the, 7

Zigzag inoculation, 159

Zoölogical distribution of cancer, 184

REPLACEMENT
RC261
W82
1913
c.1

Woglom

... The study of experimental cancer.