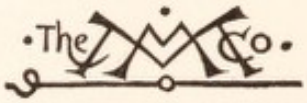
The action of the living cell : experimental researches in biology / by Fenton B. Turck.

THE ACTION OF THE LIVING CELL



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THE ACTION OF THE LIVING CELL

EXPERIMENTAL RESEARCHES IN BIOLOGY

BY

FENTON B. TURCK

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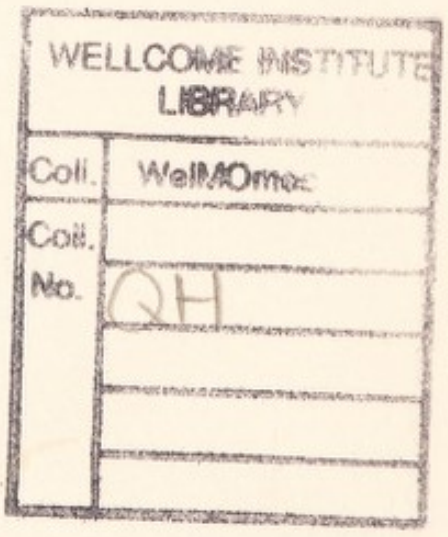

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To my son
FENTON B. TURCK, JR.,
my companion
in
spirit and in truth

"The intrusion of something which the flesh engenders—not entering from outside."

IMHOTEP, 4000 B.C.

PREFACE

WHILE working in the Lincoln Park Greenhouse in Chicago some forty years ago, the writer made some curious observations concerning the growth of plants. In attempting to find the reason for the failure of plants to grow for a succession of years in the same greenhouse soil, it was found that the addition of virgin soil from Wisconsin to the exhausted soil proved of little avail unless the amount of virgin soil mixed with that from the greenhouse was equal to, or greater than, the amount of the latter. Of itself, this constituted a matter of little interest, but in connection with this study the surprising observation was made that the addition of a small quantity of the exhausted soil to the virgin soil resulted in a better growth of the fronds than was obtained in the virgin soil alone. The reason for this remained obscure for a number of years, but the phenomenon excited the writer's interest to further investigations in the field of biology.

This interest was greatly intensified by a period spent in Europe, where, under the guidance of the great Virchow, the writer's interest was directed towards animal cells and the effect of environment upon them. At that time the majority of the students of biology were greatly influenced by the writings of Darwin and Huxley. In consequence, many investigators were enmeshed in the study of "the survival of the fittest," as Herbert Spencer tersely stated the doctrines of Darwin. While recognizing the impor-

tance of such studies, the writer's interest was drawn to the factors which rendered unfit those that could not survive the rigors of an enforced environment.

This aspect of experimental biology, which has been neglected by most biologists except in so far as it relates to medicine, has continued to occupy the writer's attention for many years, and the results of numerous experimental researches have been published from time to time over a period of four decades. Many of these investigations have been recorded in journals not readily accessible to biologists; hence at the instigation of my son, Fenton B. Turck, Jr., I have deemed it advisable to publish the main results of my investigations in monograph form. Such a decision has proved advantageous, as it has permitted a more logical sequence and correlation of related ideas than was possible in the original publications. Indeed, in reviewing some of the older data, we have been able to deduce relationships between what at first appeared to be unrelated facts; and in numerous instances it has been gratifying to find that other investigators have recorded observations which coincide nicely with some of our own.

In a large measure the author's work has been the outgrowth of his initial studies on the cause of shock, and death from this cause. Almost at the beginning of our experiments, the conclusion was reached that shock induced in any manner whatsoever is essentially the expression of the action of a toxic substance liberated from injured tissues. Subsequent investigation has completely substantiated this concept, and has shown that the same toxic substance may affect the behavior of animal cells in a variety of ways. We shall

therefore begin our discussion by reviewing the initial experiments concerning the toxic nature of shock.

A portion of the contents of the first few chapters may not appear to be of immediate interest to the general biologist. However, the investigations discussed therein really formed the basis of the author's concepts, which have been extended to some problems of distinct general interest. In essence, the experimental evidence appears to indicate that under appropriate stimulus, living cells appear to be able to yield an endocellular component which is capable of both exciting and depressing the activities of neighboring cells. For want of a better name, this substance has been termed *cytost,* and is so designated throughout the present discussion. The postulation of the existence of cytost and its rôle in the physiology of living systems was necessitated in order to explain and harmonize a diversified series of experimental observations.

All that one may ask of any scientific theory is that it satisfactorily explain known facts and if in the future new facts are found which are not in harmony with that theory, then the theory must be discarded. Hence if in the future the author's cytost concept is replaced by some other hypothesis, it matters little. For the present the cytost concept is of service in interpreting the meaning of performed experiments and in guiding the imagination of the investigator in the conduct of new experimental researches. Thus, as will be shown later, the cytost concept has enabled us to explain the curious stimulating effects of exhausted soil upon the growth of plants in virgin soil. To be sure, this appears to be a far cry from the cause

of shock in animals, but it is the correlation of such apparently remote topics which attests to the progress of science and permits of a unified concept of the activities of living systems.

It is sincerely hoped that the various aspects of the cytost concept discussed in the succeeding pages will be of value in this way to workers in the biological sciences. Primarily the material presented in this monograph has been brought together with this in mind.

The science of medicine as distinct from the art of medicine has depended upon advances in biological science, and it frequently happens that new biological concepts have contributed much to medical thought. Some of our experiments have given rise to concepts which the writer believes to be of distinct applicability to the problems of pathology and medicine. Since, however, this material is not of general interest to the professional biologist, we have segregated such discussion in a separate chapter at the end of the book.

During the course of his career the writer has been especially fortunate in numbering among his friends many who have greatly aided him in the prosecution of his experiments. It is a pleasure to acknowledge the helpful advice and assistance of Prof. W. S. Hall, Drs. Elsie Berwig, A. Weesner, W. A. Evans, E. L. Heintz, Isador Diamond, L. J. Tint, M. Lincoln, Anton Rose, Arthur Knudson, Orville McKim, the late Edwin Banshof, J. A. Behar, and Victor Carrabba, who at one time or another have assisted the writer in the laboratory.

FENTON B. TURCK

New York
June 5, 1932

CONTENTS

CHAPTER I

INTRODUCTION

ONE of the most striking properties of living systems is their ability to modify their activities, not in a continuous fashion, but in response to changes in their environment. While responsiveness to external variations is also shown by inorganic systems, the induced changes of state are by no means so complex and varied as those shown by living matter.

A further characteristic of living matter is that in many instances an extremely minute environmental variation brings about a response in an organism, which is out of all proportion to the magnitude of the change in the environmental conditions which has elicited the biological response.

For example, the presence of 0.0000000249% (about 1 part in 4,016,000,000) of copper sulphate in water was found by Kanda (1904) to have a definitely inhibitory effect upon the growth of seedlings of peas, broad beans, and buckwheat. Mast (1928), working with *amoeba proteus,* observed that when these protozoans are transferred from a hay infusion medium to freshly distilled water, a large proportion of them rapidly becomes inactive and markedly radiate in form. Furthermore, the number of individuals which attach themselves to the vessel walls is determined by

the nature of the latter. Many more attach themselves to common glass than to quartz, pyrex glass, or paraffin.

If on the other hand any one of fourteen salts is added to the water, the animals attach at once, and become lobose in a few minutes. These experiments show definitely that the environment determines the activity of this unicellular organism.

Every modification of an organism's activity may be regarded as a response to an external stimulus,—that is, to a change in the physical or chemical nature of the environment. While it is true that one portion of the organism may respond to some change in another portion of itself, in the last analysis this primary change in the latter is initiated by variations in the environment.

The response to external stimuli may be very slow indeed, or may take place with almost explosive rapidity. In the latter case the initial stimulus may be regarded as a sort of trigger which releases a rapid series of internal changes, in much the same fashion as the impact of a firing-pin on the primer cap of a high explosive shell initiates a destructive explosion at a considerable distance from the gunner. Indeed, this somewhat crude analogy bears a close resemblance to the chain of events which takes place in a biological system, wherein, as we shall see, a simple physical stimulus often initiates a chemical reaction which in turn propagates a series of vital changes.

It has been pointed out above that the application of a stimulus to one portion of an organism may produce a response in a distal portion of the same organism. In higher organisms, the conduction of the series

of events leading to the response of an effector at a distance from the point stimulated may take place by way of an elaborate nervous mechanism or by means of a humoral transmission. For example, when a tasty food is taken into the mouth, saliva is secreted by the salivary glands although the food stimulus is at a distance from the glands. As is well known, this effector response is brought about by the stimulation of sensory nerve endings in the mouth, the nervous impulse thus started passing by way of the brain to the salivary glands.

Bayliss and Starling's classical investigation of secretin offers perhaps the most convincing evidence of the humoral transmission of a stimulus. These workers found that acids acting upon the intestinal mucosa of a denervated loop of intestine caused the pancreas to secrete. Since the injection of acids into the portal vein did not induce a similar pancreatic activity, it was concluded that the acid acted upon some component of the intestinal mucosa to produce a substance, termed secretin, which was carried by way of the circulation to the pancreas and there caused the effector response.

The validity of this assumption was established by the observation that when intestinal mucosa is ground with dilute hydrochloric acid, and then boiled to coagulate the soluble proteins, an extract results which upon injection into an animal induces the pancreas to secrete. Such experiments yield irrefutable proof of the secretin hypothesis, although to date the chemical nature of secretin remains an open problem.

The type of reasoning employed in connection with the above mentioned experiments on secretin is com-

mon in the biological sciences. Frequently, as we shall see, we are unable definitely to isolate and characterize the nature of a physiologically active substance, although empirical experimental facts demonstrate the presence of such substances and the probable rôle which they play in the activity of the living organism. As the American physicist Millikan (1931) remarks, "The distinguishing feature of modern thought lies in the fact that it begins by discarding all *a priori* conceptions concerning the nature of reality—and takes as its starting point experimental facts whether they fit into any general philosophical scheme or not.—In a word, modern science is essentially empirical."

Galileo's simple experiment on the velocity of falling bodies of dissimilar mass sounded the death knell of an experimentally unsupported philosophical belief, and may be regarded as the birth of experimental science. In the biological sciences, both pure and applied, similar unsupported hypotheses exist even today, but these are one by one being subjected to the critical test of experiment. Indeed, at present experimental biology is far in advance of any adequate theoretical background.

In a colony of cells we must consider the presence of all members of the colony as environmental factors of any single cell under consideration. In such a colony the activities, growth and reproduction (which may conveniently be regarded as a special aspect of growth) of individual cells is conditioned by the presence of other cells, both homologous and heterogeneous. For example, the growth of yeast cells is more rapid in cultures heavily seeded with the inoculum than in cultures started from a few cells. In 1901

Wildiers advanced the hypothesis that a substance which he termed *bios* was necessary for the growth of the yeast cell. Fulmer, Nelson, and Sherwood found that yeast will grow in a synthetic medium prepared from inorganic salts and pure sugar without the addition of *bios* from another source. It follows therefore that the yeast is itself able to produce the growth-stimulating substance. In order to explain the difference in growth rates as a function of the amount of inoculum used, it is assumed that the growth of yeast is accelerated by the presence of a sufficient amount of *bios* of which each cell is capable of supplying a certain amount; if the amount of the inoculum is insufficient to contribute the necessary quantity of *bios* then the growth of the yeast is retarded.

Alternatively, a pure culture of cells may, by their metabolic activities, give rise to toxic substances which hinder and even completely repress the normal activities of the cells. As an illustration we may cite the fact that dextrose broth cultures of *B. typhosus* become sterile after a few days, due to the lethal effects of the end products of the bacterial metabolism. It is worthy of note, however, that the medium may still be utilized as a suitable environment for the growth of bacteria of another species.

It is well known that tissue cultures *in vitro* suffer death unless supplied with fresh medium from time to time. This fact is open to two interpretations: that death may be due to the exhaustion of some essential food factor; or that the cells, analogously to *B. typhosus,* produce a toxic product. That the latter hypothesis is the correct one is shown by the observa-

tion of Carrel, that a change of the medium surrounding the tissue is not sufficient to keep the culture in a healthy condition. However, he found that if the tissue is carefully washed and then supplied with fresh culture media a normal rate of growth ensues. Evidently, by such a process autotoxic substances are removed.

Many workers have observed that certain infusoria when left undisturbed do not remain scattered through their aqueous environment, but aggregate into more or less dense groups. Having observed that *Paramoecia* may aggregate about a drop of dilute acid or carbon dioxide solution, Jennings (1902) concluded that the carbon dioxide produced by the infusoria themselves was responsible for their aggregation in a culture medium. This may or may not be true, but at any rate it appears that some product produced by the cells themselves is responsible for the observed effect.

Let us now consider the effect of one type of cell upon the growth of another. Here two possibilities exist,—that of symbiosis, and that of antibiosis, numerous examples of each being recorded in the literature. For example, Holman (1923) found that in the presence of a minute anaerobe resembling *B. pneumosintes, B. influenzae* exhibits a good growth upon blood agar slants, whereas in the absence of the former little or no growth of the latter takes place. The opposite effect is recorded by Passini (1926), who observed that *B. putrificus verrucosus* is able to destroy *B. tuberculosis* when present in the same culture.

That one type of cell may profoundly affect the behavior of another type is shown by the remarkable

experiment of Drew (1923), who found that whereas in tissue cultures embryonic kidney cells grow as an undifferentiated mass, the insertion of a bit of connective tissue stimulates the kidney cells to differentiate.

Similar effects are to be observed in multicellular organisms; indeed, the humoral transmission of stimuli already referred to may be regarded as an example of the influence of a chemical substance produced by one group of cells upon another tissue. Such effects have also been observed in plants, particularly in their heliotropic responses. As is well known, green plants bend towards the light. Many years ago, Darwin (1880), experimenting with the seedlings of *Phalaris,* reached the conclusion that the effect of the light is not upon that part of the plant which actually bends, but is due to a modification of growth which is brought about by a stimulus transmitted from the tip of the plant. This he proved by preventing the access of light to the tip of the seedling, whereupon, although the stem was illuminated, no heliotropic bending took place.

It is a reasonable assumption that the stimulus is transmitted by means of some substance resulting from a photochemical change induced by the light in the cells at the surface of the tip. In agreement with this concept, Blaauw (1908) found that the heliotropic response of etiolated oat seedlings conformed to the Bunsen and Roscoe law—namely, that the chemical effect produced by light is proportional to the product of the intensity and the duration of the illumination.

Further proof of the chemical nature of the helio-

tropic response is to be found in the ingenious experiment of Paal (1918), who found that if the tip of a seedling was severed from its stalk and then replaced after the insertion of a sheet of collodion between the stalk and the tip, a more or less normal heliotropic reaction was obtained. When, however, tin foil was used in place of the collodion no such response could be obtained. Obviously the former is permeable and the latter impermeable to the photochemical product produced in the plant tip. According to Blaauw's measurements, an intensity of light equal to seventeen hundred thousands candle-meter is sufficient to elicit a heliotropic response in the oat seedlings examined by him. This is equivalent to saying that the seedlings in a darkened room would bend towards a lighted candle some two hundred and fifty feet distant. These surprising figures well illustrate the sensitivity of plants to changes in their physical environment.

A similar reaction is observable in the stereotropic response of the young roots of plants. The application of pressure to the apex of such a root causes it to grow away from the source of pressure. A unilateral trauma, bruise, or application of lunar caustic elicits a similar growth response. Hence it is highly probable that each of these agencies causes the elaboration of a substance similar to that formed in the illuminated plant tips.

Such responses to changes in the physical environment are by no means limited to plants. Jacques Loeb as well as numerous other investigators have observed similar phototropic and stereotropic responses in animals; and in consequence he was for a long time an

ardent advocate of the essentially chemical nature of such reactions.

The foregoing illustrations well illustrate the essentially chemical nature of the mechanisms for correlating the activities of living systems. Any marked change induced in an organism must of necessity interfere with such a correlating mechanism and hence give rise to abnormal responses. Hence a pathological state results. In the root tip experiments cited above, we have seen that simple mechanical pressure and the application of a caustic chemical elicit the same responses. Similarly Loeb (1924), in his experiments on the regeneration of *Bryophillum colycinum,* has shown that mutilation and illumination of the leaves both result in the liberation of various soluble substances which appear to stimulate the growth of the plant. Such experimental results as these offer evidence that divers stimulating agencies may be causative of the same response.

For some thirty years the writer has argued that the apparent stimulating and repressive activities of various physical and chemical agents, as well as the pathological changes which they induce, are due to a common cause,—the release by the cells themselves of a substance capable of modifying cellular activity. For purposes of convenience in discussion, the author has termed this important substance *cytost.*

Although the author developed his concept of *cytost* on the basis of experiments conducted in his laboratory, the underlying idea is by no means new. Indeed, a similar conception dates back to early antiquity, as recently shown by Professor Breasted (1930) in his study of the *Edwin Smith Surgical Papyrus;* for it is

recorded that the early Egyptian pyramid builder Imhotep (4,000 B.C.), who, it is believed, was deified as Esculapius, the Greek god of medicine, believed that injury, traumatic or otherwise, produced its effects by "not something entering from the outside," but by "the intrusion of something which the flesh engenders." Breasted remarks, "This rational distinction is unmistakable, and demonstrates the surgeon's ability to discriminate judiciously in the world of objective phenomena and natural causes."

It will be noted that Imhotep's intuitive conclusion is exactly the same as that which has been reached by Loeb and others in order to explain certain tropistic responses in plants and animals. At the present time we hear a good deal about the therapeutic effects of sunlight, and by analogy to the interpretation of the phototropic response in plants, we may imagine that in the animal external radiation stimulates the release of some substance from the tissues which is of benefit to the body as a whole. This conclusion was likewise known to the early Egyptians. Together with other aspects of Egyptian culture, this knowledge was passed on to the Greeks, who constructed the first celebrated solarium, which was conducted by the great physician Hippocrates on the island of Coz. This solarium was dedicated to the temple of Esculapius, and the observed beneficial effects of sunlight were supposed to be due to its power to release the "something which the flesh engenders."

Further, it was recognized by the early Egyptians that the process of wound healing must depend upon a stimulating substance released by the injured tissues. Consequently their physicians attempted to stimulate the healing of wounds by binding fresh flesh

over the approximated edges of the injury. After the juices from the flesh had started the healing process, the wound was stitched together and dressed with an antiseptic, stimulating honey.

Lamarck, in seeking to explain the influence of such environmental factors as light, temperature, pressure, motion and use, imagined that these physical factors set in motion "certain subtle existing fluids that ceaselessly flow," bathing the body cells in a vitalizing medium. While it is unlikely that Lamarck knew of Imhotep's speculations, it is of some interest that he assigned an important rôle to a subtle entity ever present in the tissues, in much the same fashion as did Imhotep.

Although at the outset such conclusions appear to savor strongly of the metaphysical, the author has been forced to assume a somewhat analogous hypothesis in order to effect a rational explanation of the results of his experiments conducted during the last forty years. These experiments, which have covered a variety of biological problems, have led to results which can be interpreted only on the assumption of the elaboration by tissue cells of some substance which is capable of modifying the activities of other cells. This substance, which has been termed cytost, appears to be capable, depending upon its concentration, of exerting either a stimulating or toxic action upon cells with which it comes in contact.

In the 1880's the writer observed that slightly mutilated plants outgrew controls. Some years later it was found that in animals a slight injury frequently produced a similar stimulating effect upon the animal, whereas a more extensive injury resulted in a depressing or morbid effect. As will be shown in the succeed-

ing chapters, many experiments conducted in divers ways have demonstrated that these results and many other biological phenomena may be explained by the hypothesis that when injured, cells liberate cytost into their surrounding fluids, and depending upon the amount of this substance liberated, other tissues in contact with these fluids are affected in one manner or another. Carefully controlled experiments have shown that when the latter are brought into contact with the cytost of the same species, the growth and activities of tissues are stimulated if the amount of cytost in the surrounding fluids is small. On the other hand, if the concentration of cytost in such fluids is too great, the activities of the cells are inhibited, often to such an extent that death ensues.

The theories of the writer are the logical outgrowth of certain observations which were made concerning the nature and cause of shock. In order that the reader may follow the development of the writer's concepts and thereby be prepared for the discussion of the very interesting experiments made in the writer's laboratory in recent years, we shall consider in more or less chronological sequence the various experimental investigations which have led to the development of those concepts.

At the outset it should be noted that although from time to time the author has enjoyed the collaboration of several competent chemists, all attempts to isolate and characterize cytost as a definite chemical entity have been disappointing. Its presence, like that of the enzymes and hormones, is made manifest only by its action upon the living cell.

Chapter II

SHOCK, AN INDICATION OF TISSUE DAMAGE

Our ignorance concerning the mechanism of living processes makes it exceedingly difficult to define life explicitly. We are accustomed, therefore, to differentiate animate systems from inanimate by contrasting their inherent properties, such as growth, reproduction, and response to stimuli. In such fashion alone may we discriminate between the two.

Our ignorance regarding the transformation of inorganic nature into living systems—that is, the origin of life—coincides with our knowledge of the reverse process, death. Owing to our continual consciousness of life, we are individually more interested in the causation of death than in the origin of life.

Nordenskiöld (1928) records that the aborigines considered natural death to be far more wonderful than life. While they regarded violent death at the hands of an armed enemy or a wild beast as a matter of course, the aborigines were so awed by so-called natural death that they importuned an armed demon as the causative factor. Although refusing to acknowledge the existence of such a demon, we are today almost as blissfully ignorant as was primitive man,

albeit numerous theories of death have been borrowed from physical sources, such as the spontaneous disintegration of the radioactive elements.

If one considers the energy changes accompanying such transformations as a property of the radioactive "life" of the element, then the "life" process ceases when the substance has been completely transformed to elements of lower atomic weights. Analogously, some biologists have reasoned that the death of an organism coincides with the exhaustion of some life-giving substance.

Other workers, notably Child, Loeb, and Pearl, have adopted the alternative view that in the course of life the organism suffers an accumulation of toxic metabolites which finally attain a concentration sufficiently great to interfere seriously with the normal metabolic processes, so that death ensues.

Nowadays it is generally accepted that the chemical transformations upon which the organism depends are determined by the presence of enzymes. In this connection, it is worthy of note that *in vitro* experiments with enzymes have frequently shown that the latter are inactivated by the end-products of the reactions which they catalyze. (Ter Muilen, 1905; Morgulis and Beeber, 1928.)

The careful investigations conducted by Woodruff and his students have demonstrated unicellular organisms to be immortal. The common ciliate, *Paramecium,* was found during a period of years to pass through several thousand generations without the intervention of natural death. Similarly, the *in vitro* culture of the somatic cells of various higher animals has indicated such cells to be potentially immortal.

That connective tissue cells from the chick heart may be kept alive in the laboratory for years exceeding the normal life span of the fowl, and presumably, if the tissue culturist so desires, for an indefinite period, was shown conclusively by Carrel and Ebeling. Natural death, it thus appears, is restricted to multicellular organisms.

During the evolution of such organisms, the cells of the various tissues have become dependent upon their neighbors for such necessities of life as food and oxygen. For example, if the blood-flow to an individual organ is interrupted, the cells of that organ die, owing to starvation and want of oxygen, while other tissues continue to live unless the death of the first organ in some fashion deprives the other tissues of their normal environment. If, for example, the cells of the glomerulus of the kidney are damaged, as in nephritis, the loss of proteins from the blood causes water to diffuse more easily into the other tissues of the body, which thus become waterlogged.

Coincidentally the elimination of nitrogenous waste products is impaired and the body cells suffer from their accumulation. In the brain, the accumulation of such waste products results in coma and eventually in the death of the organism. In similar fashion, the injury or death of any of the component tissues of a multicellular organism will result in disturbances and perhaps the death of other tissues in the body.

When an animal is decapitated, death ensues; yet for some time after the decapitation the individual tissues may be shown to be alive. The heart, if removed to a suitable environment and supplied with the necessary salts, will continue to beat for some

hours, and the muscles, upon stimulation, will contract. Indeed, the major part of our knowledge of neuro-muscular physiology is based upon experiments made upon the surviving organs of "dead" animals. Eventually, of course, such isolated tissues will die, owing to starvation or other change from the environment in which they normally find themselves in the intact animal.

One must, therefore, distinguish between the death of the organism and the death of the individual components, the tissue cells of the organism. From a practical standpoint, the former is simply a loss of the nicely balanced and integrated activities of the individual tissue cells, while the latter is an actual death which may result from the former.

On the other hand, as has been noted above, if the tissue cells are transplanted to a suitable environment, such as that employed in the technique of tissue culture, there is no reason to believe that they should not live forever.

The localized death of tissue cells—i.e., necrosis—may therefore be brought about in either of two fashions: by a localized injury—for example, the application of a caustic chemical or a destructive physical agent such as X-radiation; or by a change in the internal environment induced by the disfunctioning of some remote tissue or organ, such as that of the kidney, previously mentioned. This being the case, it is logical to assume that because of the reciprocal relations existing between the component tissues of an animal, a localized injury must inevitably result in induced changes, or perhaps even the death of other

organs and tissues at a distance from the site of the injury.

Thus, it is a matter of common experience that an animal may be killed by a rap on the head. Here, as in the case of decapitation, it is clear that the death of the individual tissues does not immediately ensue, but follows later as a result of the inability of those tissues to function in the correlated and reciprocal fashion necessary to keep one another alive. It is not so generally recognized that an apparently minor injury, such as a crushed extremity or a superficial burn, may lead to the same end. Unfortunately, however, this is a fact which is amply attested to by the results of experience both in practice and in the laboratory.

In such instances as those cited above, by appropriate experimental procedures we may determine the order in which the various body tissues die, and the histological changes which either accompany or result from their death. Little more, however, may be learned from such experiments. In order to determine the mechanism causing the death of animals under such conditions, we must resort to other modes of investigation which afford an insight into the dynamics of the principal physiological and structural changes which precede the end result of the experiment. Such methods and the findings which have resulted from their application must now be considered.

It is a common and disconcerting experience that patients who have suffered an injury involving a localized mutilation of tissue frequently die although the injury has been easily repaired by the usual procedures. Prior to death, such unfortunate individuals

exhibit a highly characteristic series of symptoms: generally diminished reflexes, and complete loss of the postural reflexes, together with dilated pupils. These are accompanied by extensive circulatory disturbances manifested by a lowered blood pressure, rapid but weak pulse, frequent and shallow respirations, and a subnormal temperature.

Taken collectively, these symptoms are termed the shock syndrome, and the animal exhibiting them is said to be in a state of shock. Since in such cases as that just considered the state of shock results from an injury, it is termed traumatic shock, to differentiate the condition from shock induced by other means.

Shock, or perhaps more properly the series of symptoms enumerated above, may be induced in various other ways. For instance, if a rabbit, particularly one with a large abdomen, is held by its head in an upright position for half an hour, it will exhibit the symptoms of shock, presumably because of the stagnation of blood in the abdomen, and an inadequate filling of the heart during diastole. If the rabbit is held in a binder during the course of such an experiment, shock does not ensue, because engorgement of abdominal vessels is prevented.

Any other condition which leads to the same circulatory difficulties, as for example extensive hemorrhage, produces a similar state of shock. The same symptoms may be elicited by the injection of histamine and various bacterial toxins, and an analogous state has been frequently observed in certain pathological conditions, such as intestinal obstruction. In such instances, the condition has been designated as toxic shock.

While the clinical aspects of shock are of vast importance, and in other publications the writer has devoted considerable attention to them, we shall here consider shock merely as an interesting physiological state which may be produced, as we shall show, in experimental animals. We shall utilize the shock syndrome merely as an indication of a definite physiological state which may be induced at will in a living animal.

Because, as has been shown above, this state may be elicited by a variety of means which obviously by their very nature have little in common, it appears highly probable that each of them initiates a common series of events which lead ultimately to the same physiological state. Apparently the simplest type of shock is the postural shock, which may be elicited in the intact animal as previously mentioned. The induction of shock by this means is especially interesting because it involves neither the introduction of any substance into the animal, nor any manipulation tending to injure its tissues. In this instance, the only abnormality involved is that of suspending an animal in an abnormal position such that its blood under the influence of gravity accumulates in the splanchnic area.

From this simple experiment we may infer that in all cases of shock, however induced, the physiological changes observed are in great part due to engorgement of the splanchnic vessels. That this does actually occur has been demonstrated in many experiments, performed by the writer and by others.

The fact that animals may be thrown into shock by traumatic injuries, burns, and the injection of various

toxic substances must mean that by all of these means one may cause a marked stasis of blood in the splanchnic area. It was the investigation of this point which occupied the author's attention some thirty years ago and led to the various experimental investigations to be considered in this book.

Before further discussing the phenomena of shock and considering the underlying mechanism in detail, let us briefly recount the results of a few experiments conducted by the author. (Turck, 1918a.)

Cats were etherized, and after the application of an elastic ligature to a leg in such a fashion as to shut off the circulation, the thigh muscles below the ligature were bruised and the tissues mangled. After the lapse of an hour and a half, the constriction was released and the limb occasionally massaged towards the body in order to reëstablish the circulation.

Shortly afterwards the animals began to show symptoms of shock, culminating in death, although other animals which had been treated in precisely the same fashion except that the ligature was not released, did not exhibit signs of shock during the same interval of time. Bits of muscle were removed from the mangled limbs of the latter animals and the juice expressed was injected intravenously into the same animal, whereupon shock promptly ensued, although the ligatures on the limbs had not been loosened.

A similar result was obtained by transplanting the bruised muscle tissue from below the ligature to the uninjured muscles above it. An even more striking result was obtained by transplantation of the injured tissue into the thigh of an uninjured homologous animal, the latter invariably suffering shock and death

while the injured animal was frequently saved by amputation of its injured limb above the site of injury.

Such experiments as these show unquestionably that so-called traumatic shock results from the absorption of a toxic substance released by the damaged tissues. If it were otherwise, then we should not expect that the mere presence of the ligature above the site of the injury would prevent the onset of shock. Further, as is well known, tissues may be transplanted from one animal to another of the same species without the onset of shock. Hence, in the transplantation experiments, such as those cited above, which result in shock, another factor must be present: i.e., a toxic moiety derived from the injured tissues which were transplanted to the uninjured animal. The experiments above referred to are of relatively recent date, but the essentially toxic nature of traumatic shock has long been stressed by the author. Indeed, as long ago as 1895 the writer pointed out that in cases of carcinoma of the stomach the carcinoma early produced a toxic product capable of altering the gastric, secretory and motor activities of the organ, and when brought to operation such cases frequently pass into severe shock. A year later (Turck, 1896), the conclusion was reached that such unfortunate individuals were in such a state of autointoxication that the additional tissue products released by the operative trauma sufficed to precipitate shock.

In a series of papers published since these initial observations were made, the author has contributed a considerable amount of experimental evidence which shows definitely that the shock following injury is due to the release of an endocellular component

which under normal conditions is held innocuously within the cell.

Without at the moment going into details, we must note that since this substance is endocellular, cellular disintegration or autolysis is a necessary prerequisite for its release into the surrounding environment. With this in mind, we may clearly understand the above-quoted experimental results concerning traumatic shock. Since, as is well recognized, tissue autolysis, in common with other protolytic digestions, is a relatively slow process, we are enabled to account for the fact that the shock syndrome does not immediately follow an injury, but, as a rule, becomes apparent only after a lapse of some hours.

The validity of the preceding argument is strikingly proved by the following experiment. (Turck, 1918a.) Fresh muscle taken from a normal animal was permitted to undergo aseptic autolysis until microscopic examination revealed a loss of nuclear staining. The autolyzed tissue was then ground in a mortar with normal saline and the supernatant fluid was injected intravenously into homologous animals. Shortly after the injection of a small quantity of the autolysate the animal passed into shock and eventually died. Increasing quantities of the autolysate hastened the onset of shock and death. Indeed, a single injection of a lethal quantity resulted in death within three minutes. On the other hand when a saline extract of fresh tissue—i.e., before autolysis—is injected in similar fashion, shock does not follow. These very simple experiments show conclusively that autolysis is a necessary prerequisite for the formation of the toxic entity responsible for traumatic shock.

If the arguments advanced above are valid, then shock should be induced by any other means capable of producing a localized death of cells and the subsequent release of the shock toxin by *in vivo* autolysis. Further to test this hypothesis, animals under anesthesia were superficially burned, whereupon shock ensued. This result, of course, was to be expected from our knowledge of the effect of severe burns on humans. When, however, the burned area was transplanted to the back of a second animal, and the normal skin of the latter grafted onto the area of the first where the burned tissue had been removed, the second animal passed into shock and died, whereas the donor which had been burned recovered with but slight symptoms of shock. In one such experiment, in which the extent of the burn was slight, the host did not develop symptoms for four days. On the other hand, a third animal upon receiving an injection of a sterile autolysate of the burned tissue of the first animal suffered immediate shock.

Such experiments as these demonstrate that *in vivo* some time is necessary for the autolysis of the injured tissue, and consequent production of a concentration of the toxic substance sufficiently high to elicit the response which we term shock. It is because of this that the prompt removal of damaged tissues following an injury is efficacious in preventing the onset of shock, whereas cases in which surgical intervention is unduly delayed often show some degree of it, all too frequently resulting in death. From the experimental results cited above, it is clear that in such instances in order to be efficacious in preventing shock,

removal of the damaged tissues must be accomplished before autolysis has progressed to any appreciable extent.

Objectively, but without understanding the underlying reason for the procedure, this fact was recognized by Duboys, who served with the French troops in this country during the Revolution. He states that "American surgeons amputated at once and lost but few, but the French delayed and lost many." The recognition of the value of such treatment was perhaps due to Benjamin Rush, the surgeon-general of our army during that period. Similarly, Larrey, a French surgeon who early advocated the "debridement" of wounds and thereby saved numerous lives during the Napoleonic Wars, remarked, "The effects of commotion (shock), far from being aggravated, diminish and disappear insensibly after the operation."

The question may arise in the reader's mind: Why does not the tissue damage which inevitably results from simple cuts, burns, and bruises cause shock? Simply because such injuries are not sufficiently extensive. Obviously when the area of tissue damaged is but small, the toxins liberated are inadequate in quantity to elicit serious consequences. Nevertheless since the state of shock is but the culmination of a series of physiological changes, we may well expect the liberation of the shock toxin even in small amounts to manifest some physiological activity.

This indeed is the case, and the writer's experiments have, he feels, placed this substance in a unique position in the internal environment of the animal: that is, as a substance capable of modifying to a considerable

degree the activities of the component body cells. For this reason, the name shock-toxin has been abandoned, and the name *cytost* applied to the substance liberated by cells which initiates the series of physiological changes which *may* result in shock. It must be remembered, however, that for such a state to ensue, a relatively large amount of cytost must be liberated.

From the standpoint of general biology, the effects of small quantities of cytost upon the organism are of far more importance than the effects of quantities sufficiently great to cause shock. Indeed, as the reader will see as our discussion progresses, cytost apparently plays a unique rôle in the physiological behavior of the animal. As the investigations concerning this aspect of our subject have been carried out almost exclusively in the writer's laboratory, the primary object of the succeeding chapters will be to disclose the methods used in our experiments, the results obtained, and the conclusions which may logically be deduced from them.

However, before passing to this admittedly more interesting portion of the author's work, we shall further discuss the earlier experiments relative to shock. Such discussion is necessary for two reasons: first that the reader may gain an insight into the finer details of the action of cytost; and second that he may follow without difficulty the gradual development of the writer's thesis as it has taken place over a period of some years.

Let us now return to our discussion of shock particularly as regards the modes of experimentation other than those previously considered.

Years ago (Turck, 1897), the writer found that

shock could be induced in animals by exposing the viscera to a draught of cold air. Further, the onset of symptoms was found to be accelerated by frequent manipulation of the exposed viscera. After dogs had been caused to pass into a profound state of shock by such means, they were bled and their separated blood-serum was injected into normal dogs. The effects so elicited in the latter were found to be governed by the amount of serum injected. For example, 5 cc. of such serum produced little effect; 50 cc. caused the animal to show decided evidence of fatigue; while larger quantities brought about collapse. (Turck, 1901.) At that time the analogy was drawn between these results and those obtained by Mosso with *fatigue-stuff*. The latter author found that if the blood of a fatigued animal were injected into a normal animal, the unfatigued one showed signs of fatigue. In other words, the production of shock in an animal brings about the presence in the blood of some substance capable of causing the onset of the signs of fatigue in normal animals. As will be shown later, it appears that this substance is cytost, the substance released from injured tissues which, as we have seen, is the primary cause of shock.

Further, the analogy between the shock-toxin and the fatigue stuff of Mosso is especially interesting for, as will be shown in a later chapter, there exists a close correlation between an animal's resistance to fatigue and to cytost.

The evidence so far presented refers only to the primary cause of shock,—the release, by injury, of the endocellular substance cytost, which is capable, if present in sufficient concentration, of causing the

unique train of physiological events which finally lead to the condition termed shock. While recognizing that an adequate understanding of the cause of such a condition is of paramount importance to the surgeon, our interest in the subject is to be rather closely confined to the underlying physiology of the reaction and the relationship of such physiological behavior to the problems of general physiology.

That the reader may properly understand the underlying mechanism by which such reactions are produced, we must revert to some experiments which antedate by some years the majority of those presented above.

Since our problem is to determine the effects of a localized tissue damage upon the organism as a whole, it is obvious that development of a technique for producing such a localized injury in various parts of the body is highly desirable. By means of an apparatus termed the gyromele, the author (Turck, 1896b, 1903a) has been able both to apply various reagents to specific areas of the stomach mucosa and to withdraw at will material from any portion of the organ. The instrument consists essentially of a hollow rotating sound which may be introduced into the stomach by way of the esophagus. By means of the rotating mechanism, the distal portion of the sound may be approximated to any desired area of the gastric mucosa, the exact position of the tip being easily located by palpatation or fluoroscopy. Once the tip of the instrument has been placed in the desired position, a small sponge heretofore sheathed in the hollow sound may be extruded, thus permitting the localized application of any desired reagent to the mucosa. Similarly

the sponge may be utilized for the extraction of mucus, bacteria, etc., from such foci. After the sponge has been utilized for the desired purpose, it is resheathed, and the instrument withdrawn from the stomach.

The course of action of reagents applied in this manner may be followed cytologically by the microscopic examination of samples of the stomach mucosa removed by means of intragastric nippers designed for this purpose. In some instances the course of action of gastric irritants was observed by means of a permanent fistula established by making a valvular opening in the anterior wall of the stomach. This was accomplished by folding the mucous membrane in such a fashion as to make a valve similar to the ileocecal. (Turck, 1896a, 1898.) Upon introducing a speculum and a small electric lamp through such a fistula, the entire interior of the stomach could be viewed; at other times, the valvular fold prevented the escape of the stomach contents.

Let us now consider the results obtained by the application of such experimental methods. By means of a needle douche introduced through the esophagus, a 0.2% emulsion of mustard in water was douched over the stomach mucosa of dogs in which gastric fistulae had been established. The changes so induced were observed through the fistula as described above. Within five minutes a local hyperemia of the mucous membrane was observed; and after twenty minutes the tissues were markedly congested. A similar localized hyperemia of the mucosa was induced by the introduction of water at a temperature of 40° to 45° C., and by the mechanical stimulation of the membranes

by means of the gyromele, which was revolved within the stomach for three minutes.

The important point which we must here stress is that a similar localized response—namely hyperemia—is elicited by means of three decidedly different agents: a chemical irritant, a temperature rise, and mechanical irritation. (Turck, 1900.) Repetition of these experiments in animals whose abdomens had been opened in such fashion as to permit observation of the splanchnic circulation showed that a moderate stimulation of the gastric mucosa by any of the three agents discussed above did not produce any marked changes in the splanchnic circulation. When, however, the chemical or mechanical irritation was prolonged, or the temperature of the water introduced into the stomach was sufficiently high, there was evidenced a marked change in the splanchnic circulation. There first appears a marked hyperemia, which is followed by an intense venous congestion, the veins becoming distended, and as a result the intestines assume a dark purplish shade. Finally such splanchnic congestion causes a pronounced peripheral anemia. It is worthy of note that this picture is identical with that observed in shock produced by any other means. (Turck, 1897.)

A similar state of affairs was induced by inflating the stomach with air after ligation of the pylorus. Within five minutes, venous engorgement of the gastric vessels ensued, followed in another five minutes by splanchnic congestion, and subsequently by cardiac dyspnea, respiratory failure, and finally complete collapse of the animal. Comparable results were obtained

by inflating the intestines with air. When a stream of compressed air was allowed to impinge upon the exposed viscera of rabbits, the viscera became bluish-black, due to engorgement, and the animals developed the general symptoms of shock.

Lastly the writer has induced shock symptoms in intact animals by placing them for a minute or less close to the arm of a centrifuge revolving at ten thousand revolutions per minute. (Turck, 1918a, 1918b.) The same result was also obtained by discharging a 38-calibre blank cartridge in a box containing the experimental animals. In the case of aquatic animals such as fishes, turtles, and frogs, a similar state ensued when the cartridge was fired just beneath the surface of the water in the aquarium. (Turck, 1918b.) At autopsy, the animals used in such experiments were found to have suffered a splanchnic congestion identical with that observed in experimental shock produced by other means.

Although these experiments were originally designed to duplicate in the laboratory a condition analogous to the "shell-shock" often experienced by soldiers in combat, they are here presented merely as an illustration of a means of producing a profound state of shock in intact animals. While by such experimental means no externally obvious injury is produced, it seems clear that the pressure waves engendered alike by the centrifuge and the explosion cause an injury which leads to a series of *in vivo* changes finally resulting in a state of shock.

Since the same final state may be induced by such a variety of markedly different experimental methods, the question arises: What is the common factor which

eventually elicits the same end? It is quite apparent that the experimental methods, such as the application of caustic chemicals, burns, trauma, and air pressure, have, from a purely physical standpoint, nothing in common. We must therefore seek the common factor within the animals themselves, or more properly within the living cells whose individual activities, as we have seen, determine to a large extent the behavior of one another.

In order to understand the cellular changes which follow the application of such agents as those enumerated above, let us return to the author's experiments on the stomach mucosa. In these experiments, by the methods previously mentioned, we were enabled to establish a localized injury by mechanical means, or by the application of irritating chemicals. After experimentation with various irritants, mustard, because of its relatively mild, yet prolonged, irritating qualities, was utilized for the production of an acute gastritis. (Turck, 1902.) That we may follow the course of the physiological changes induced by the mustard, we shall first consider the local effects.

After the washing out of the stomachs of healthy dogs which had been without food for twenty-four hours, a ten per cent aqueous suspension of ground mustard was introduced into the organ by means of a stomach tube. After the mustard had remained in the stomach for an allotted time, the abdomen of the animal was opened under anesthesia and specimens of tissue were excised from the oardia, fundus, body pylorus, duodenum, and gall ducts, and prepared by fixation and staining for cytological study. Such material was obtained at various times up to forty-eight

hours after the introduction of the mustard. (Turck, 1902.) The salient cytological changes observed may be summed up as follows:

First an exudate, consisting of cells, granular débris, fibrillar masses, leucocytes, and erythrocytes, appeared on the surface of the mucosa within an hour or two after introduction of the mustard emulsion. At this time the cells, including the nuclei, stained readily. The capillaries within the interglandular connective tissue beneath the epithelium were markedly engorged, and the blood vessels in the submucosa were likewise distended. After about two hours, the mucosa was found to be covered with a thick layer of broken down, desquamated cylindrical epithelial cells. Some cells in the ducts appeared to be degenerated acid cells, while some proliferation of the interstitial tissue was apparent. The blood vessels of the outer coats, at this time, were found to be somewhat distended and filled with blood.

At the expiration of six hours, mitosis was visible everywhere, the nuclear figures being plainly discernible. The interglandular connective tissue, especially at the upper portions of the mucosa and beneath the surface lining, was markedly infiltrated with leucocytes and round cells. The blood vessels of the submucosa and serous coats were enormously distended and filled with blood. The cardia fundus showed the most marked inflammation, the epithelial cells being disarranged and exhibiting various degrees of degeneration ranging from a simple granular stage to complete necrosis. The cytoplasm of such cells stained comparatively easily with eosin, the intensity of the staining being conditioned by the degree of degenera-

tion, while the nuclei took the nuclear stain lightly or not at all.

Stomach tissue which had been exposed to the action of the mustard for longer periods exhibited much the same picture, but with further separative changes and extension of the inflammatory process to the duodenum and bile ducts. The surface epithelium, curiously enough, was not destroyed in proportion to the very marked changes occurring in the deeper tissues of the mucosa. Apparently, then, the mustard emulsion produced a localized tissue injury, which in turn was responsible for the more extensive damage observed in the deeper tissues. This observation is of especial importance because it shows clearly that when one tissue is damaged by experimental means, adjacent tissues not directly in contact with the injurious agent may suffer from the primary injury. This argues for the transmission of an injury from one tissue to another and presents us with the problem of finding the mechanism of such a transmission.

Previously, we have called attention to the fact that the introduction into the stomach of a dilute mustard emulsion brings about a congestion of the visceral circulation analogous to that caused by mechanical irritation of the stomach lining. When more concentrated mustard emulsions are utilized for this purpose, the general symptoms of shock are elicited. In such an experiment, within five minutes after the introduction of the irritant, the splanchnic vessels were found to be hyperemic; within ten minutes the splanchnic veins evidenced marked congestion and distention; and after the lapse of twenty minutes, the splanchnic congestion had become so extensive as to

cause peripheral anemia. (Turck, 1900.) Clearly, a condition of shock had been induced by the localized action of mustard in the stomach.

That the action of the irritant *per se* was limited to the stomach and not due to its absorption was easily demonstrated as follows: After the mustard had remained *in situ* long enough to induce hyperemia of the splanchnic vessels, it was removed by lavage and the administration of slippery elm water, whereupon the splanchnic congestion was speedily reduced.

If, as these experiments indicate, the action of the mustard is purely local, then the general physiological changes which are induced by its application to the gastric mucosa must be brought about by some substance liberated by the tissue cells under the action of the mustard. Such an hypothesis must necessarily be advanced to explain the production of shock by the mechanical irritation of the stomach, for obviously the mechanical instrument utilized to effect the primary injury can by no stretch of the imagination be endowed with any other than a purely local action.

We are now in a position to understand why shock may be induced by such diverse means as trauma, mechanical irritation, burns, and chemical irritants. Each of these means results in cell destruction with the liberation of the endocellular substance, *cytost,* which is absorbed, and causes a splanchnic congestion, in turn giving rise to the external symptoms collectively termed shock.

If this hypothesis is correct, then any agent capable of effecting the liberation of *cytost* in sufficient quantity will cause splanchnic congestion, stagnation, and shock. For example, toxins formed in the stomach

as a result of bacterial growth may act as local irritants and thus initiate a series of events similar to those observed in the experiments with mustard. This was pointed out by the author thirty-five years ago. (Turck, 1896.)

The accumulation of such toxins in the stomach may result in atony, splanchnic congestion, and eventually in collapse. This was shown experimentally as follows:

The stomach contents were withdrawn from a patient suffering from gastritis with marked distention, who had fasted for fourteen hours. The fluid was filtered through paper and then passed through a Pasteur filter, the sterility of the filtrate being checked by a control culture. A portion of the filtrate was then injected subcutaneously into rabbits. Within half an hour the animals showed paralysis of the hind legs, the toxic effect resembling that observed in rabbits bitten by venomous snakes. Injection of a further quantity of the filtrate caused the death of the animal within three hours. Injection of the stomach contents of healthy individuals does not elicit such marked symptoms. (Turck, 1896.)

The normal healthy stomach is not a satisfactory environment for the growth of microörganisms. However, any factor which causes a marked irritation of the stomach mucosa results in an exudation of a sticky material which accumulates upon the stomach wall. The author found this to be an excellent culture medium for various bacteria known to occur in the stomach. Apparently *in situ* the mucin deposit protects the organisms from the action of the hydrochloric acid of the gastric juice. Cultures from such

material are readily obtained by means of the gyromele.

The introduction of mild irritants into the stomach of an animal results in pathological changes which render the mucosa a satisfactory culture medium for the growth of microörganisms. In his early experiments the author found that if a dog's stomach is washed out daily with tannic acid for a period of two weeks and then inoculated with material taken from the stomachs of patients suffering with gastritis, the bacteria grow readily and by their growth induce a series of tissue changes resulting in a pathological picture identical with that found in cases of gastritis in humans. (Turck, 1896.)

First the gland cells become enlarged and cloudy, numerous leucocytes appear on the surface and marked hyperemia is evident. After the lapse of a few months the cellular changes appear more marked, cystic degeneration of some glands occurring while others undergo a metamorphosis into mucoid glands. Eventually there is an extensive round cell infiltration and connective tissue formation, while the surface becomes coated with leucocytes. This pathological picture is distinctly analogous to that obtained by the action of mustard upon the stomach mucosa. Hence again we may reasonably postulate the intervention of a common factor resulting from tissue injury.

It was easily supposed that the general toxic effect which accompanies gastritis was due to the absorption of toxins generated by the bacteria growing in the stomach. This was of course a natural supposition. Since, however, much the same state of affairs may

be induced by the action of mustard in the stomach, and, as the author has shown (Turck, 1902) that in such experiments the exudate which first forms is not a good medium for the growth of bacteria, it seems more reasonable to postulate that in gastritis the apparent general toxemia is due to endocellular substances released from the cells injured by the metabolic activities of the microörganisms.

Such a concept is in agreement with the well known fact that bacterial toxins, with the exception of that formed by *B. botulinus,* are not absorbed through the gastrointestinal tract.

Early in the history of anesthesia, it was recognized that a state of profound shock may be induced by the anesthetic. In 1866, Sabaarth reported 119 cases of death during chloroform anesthesia, less than half of which appeared to be due to a direct toxic effect of the anesthetic. In the same year Nothnagel reported that inhalation, or injection of chloroform and ether, produced a fatty degeneration of various organs. So far as we know, the experiments of the latter author were the first to demonstrate the specific toxic action of these anesthetics upon the body cells.

Early in the author's career, he was impressed by the fact that some experimental animals showed evidence of shock more readily than others of the same species, even comparatively trivial manipulation under anesthesia resulting in shock and subsequent death a day or two later. As it seemed likely that such results were due largely to the effect of the anesthetic *per se,* the latter were investigated in the absence of experimental surgery. (Turck, 1903b.)

A series of healthy dogs were kept under deep

chloroform or ether anesthesia for periods of from three to six hours. From twelve to twenty-four hours after the removal of the anesthetic, the animals uniformly exhibited the symptoms of shock, such as subnormal temperature and blood pressure, and pale tongue and cold surface; frequently death ensued. At the time these experiments were published the author commented that: "The conclusions that can be drawn from the details of the experiments made on animals under chloroform or ether are that where shock is produced it does not materially differ from the shock that results from trauma. The most constant pathologic factor is failure of circulation, and this is especially expressed in splanchnic congestion." (Turck, 1903b.)

When blood serum obtained from dogs which had been subjected to anesthesia the day previous was injected intravenously into normal dogs, the latter developed symptoms of shock. This result shows that in shock produced by anesthesia cytost is liberated in the blood in much the same fashion as in shock induced by other means.

The following protocols taken from the paper above referred to clearly show the nature of this reaction.

Experiment 16. Mongrel, placed under ether anesthesia; pressure 140; kymographic tracings. Ether. Temperature, 41. Injected 90 cc. serum from dog which had been under anesthesia for 5 hrs. The serum had been removed the following day. Blood pressure fell 80–70; forty-five minutes, temperature 36° C. *Coagulation* marked, interfering with further blood pressure records. Anesthesia removed; animal placed in warm location; temperature following day 37° C. After three days, animal still showed symptoms

of shock. Died on third day. Postmortem revealed no evidence of infection. Cultures negative.

Experiment 17. Small black and tan under chloroform. Blood pressure, 130. Serum used from a dog which had been under anesthesia five hours, and bled the following day; 25 cc. of serum injected. Fifteen minutes, pressure fell to 115; twenty minutes, 110; thirty minutes, 95; 25 cc. serum injected. Blood pressure, 15 minutes following the last injection, 65–70. Wide excursion. Anesthesia removed. Animal died the following day.

Experiment 18. Mongrel bull. Ether anesthesia. Blood pressure 150. Serum from an animal that had been under ether for four hours. The animal had been bled immediately after removal of the anesthetic. Injection of 22 cc. followed by another injection of 22 cc. No effect on blood pressure, 20 cc. more no effect on blood pressure; 20 cc. pressure 100. In one hour pressure 85. Anesthetic withdrawn and animal recovered.

Experiment 22. Dog, yellow; temperature 38.8°. Injected serum from animal six hours under anesthesia. Temperature, 38.3°. Amount injected, 25 cc. and 20 cc. Temperature went down to 37.2°. The following day rectal temperature 38.3°. Animal listless; cannot be around, and lips appear pale; marked loss of sensation. Second day, temperature 39.5°. Animal depressed; marked loss of appetite. Fourth day, animal bled; shows rapid *coagulation* and *laking.*

At this point it should be noted that when animals are anesthetized for a period sufficiently long to induce shock, invariably it is found that the coagulation time of the blood is shortened. We shall reserve discussion of the significance of this observation for a later chapter, but it may not be amiss here to note that an increased ease of coagulation of the blood will facilitate clogging of the capillaries and hence congestion in the larger vessels—a characteristic phenomenon in all cases of shock regardless of the means by which this unique physiological state is induced.

For a long time it has been common knowledge that

when subjected to surgical procedures, animals or patients are more apt to pass into a state of shock if the anesthesia is unduly prolonged. This is readily understood when we recall that our experiments have shown that both anesthetic and trauma are able to effect a liberation of the shock-toxin, cytost. Hence, when operated upon, an anesthetized animal is subjected to the action of a higher concentration of cytost than in the case of subjection to either of the experimental methods alone. This state of affairs, then, is closely analogous to that previously postulated to explain the marked susceptibility to surgical shock of patients with carcinoma of the stomach.

In this connection it is of interest to note that such cases when subjected to operation are subjected to the action of cytost liberated in three ways: by the carcinoma, by the anesthetic, and by the operative procedure—a truly formidable combination of factors calculated to liberate cytost.

At this point it is perhaps proper to stress the fact that in general the subjection of animals to two or more harmful agencies, as in the examples just considered, may lead to a synergism, or apparent potentiation of one another. Thus, in 1900, the writer found that animals which had been in shock were easily susceptible alike to the action of *Staphylococcus albus, Staphylococcus pyogenes aureus,* and *Bacillus coli,* whereas injection of cultures of the first and third of these organisms into normal animals yielded only negative results.

It follows therefore that in the presence of a high concentration of cytost resulting from the action of the agency utilized to produce shock, the virulence of

these saprophytic organisms was apparently increased. When, however, we remember that by the virulence of a microörganism we mean little more than a quantity which is inversely proportional to the resistance of the host, it is seen that in the presence of a relatively high concentration of cytost the ability of the host to overcome a comparatively weak invader is nullified. This interesting aspect of the subject will be treated more fully in a subsequent chapter.

Let us now return again to the effects of anesthesia, for we must consider yet another source of toxemia resulting from the action of the anesthetic upon the gastrointestinal tract. (Turck, 1903b.) Animals in which gastric fistulae had been previously established were anesthetized with chloroform or ether in such a fashion as to preclude swallowing of these substances. This was accomplished either by tracheotomy or effective plugging of the esophagus. After brief periods of anesthesia, the stomach and its contents were examined by means of the fistula.

An initial hyperchlorhydria was observed, this being followed by a diminished secretion of acid and ferments. Subsequently large quantities of mucus were formed. These results harmonize with those previously found when other irritants such as tannin and mustard were applied to the gastric mucosa. (Turck, 1895, 1896.) Incidentally, these results were subsequently confirmed by Pawlow (1902).

The presence of anesthetics in the stomach was proved by distilling washings and applying the usual chemical tests to the distillate. Early in this chapter we have summarized the author's work concerning the production of shock by gastric irritants. From

what has been said, it should be apparent to the reader that since, following inhalation, the anesthetics actually reach the stomach and elicit therein effects analogous to those produced by other gastric irritants, we may well expect shock to result from the action of ether and chloroform on the stomach mucosa. This is in agreement with the observations of Brunton (1895), who found that the ingestion of chloroform produced vomiting and irritation of the gastrointestinal tract, leading to circulatory depression similar to that observed in shock.

For many years, clinical observers have noticed that following anesthesia there may be a marked atony of the stomach and intestines. In animals after ligation of the pylorus and cardia the writer has observed through a fistula that such atony results in an accumulation of gases which distend the stomach. When the gases are removed, gas formation is found to continue at a rate more rapid than could be accounted for by fermentation. The gas, therefore, must have resulted from diffusion of the blood gases. As a result of the distention of the organ by these gases, the splanchnic circulation must be affected in much the same manner as was found to be the case when the stomach was inflated with air.

That toxins are actually produced in the stomach as a consequence of atony was easily demonstrated by the injection of filtered stomach contents into normal animals. When stomach contents withdrawn from normal animals was injected into dogs, quantities as great as 40 cc. did not produce toxic effects, whereas smaller quantities of such material taken from patients with atony of the stomach—particularly that result-

ing from chloroform and ether anesthesia—upon injection caused marked symptoms of toxemia.

As a result of the experimental research presented in this chapter we may safely conclude that the primary cause of shock, regardless of the technique utilized to elicit the reaction, is the liberation of an endocellular toxin which, by its action upon the circulatory system, results in an engorgement of the vessels in the splanchnic area.

In subsequent chapters we shall consider the conditions under which cytost is liberated, its probable nature, and the mechanism of its action on tissues. We shall have little more to say regarding the various aspects of shock; nevertheless it may interest the reader to note that the writer's early experiments upon the toxic nature of shock laid the groundwork for more general investigations which are to be considered in this book.

In the interests of historical accuracy, the following may prove worthy of note:

In 1896 and subsequently the writer published a series of papers in which it was maintained that traumatic shock was essentially a "shock by toxin" from damaged tissues. In discussing the results of experiments with anesthetics it was stated (1903b): "The explanations that are usually given for the complex symptoms found in shock from anesthesia cannot be explained by the simple failure of blood pressure and respiration, but there appear to be some direct toxic bodies formed that are found in the blood serum. The injection of serum from animals that have shown evidence of shock produces many of the symptoms manifested in shock, which include loss of sensation,

general malaise, marked fall in blood pressure, stertorous respiration, and, what is generally more significant, rapid fall in blood pressure."

And, "The conclusions that can be drawn from the details of the experiments made on animals under chloroform or ether are that where shock is produced (by the anaesthetic) it does not materially differ from the shock that results from trauma."

While in papers published previously to that from which the foregoing is taken, the author stressed the concept that shock was induced by toxins liberated from damaged tissues, the above statements show clearly that the author was cognizant of the very general nature of the underlying mechanism of shock brought about by divers experimental means. This was early recognized by Munch, who published a review of the writer's early experiments in 1903.

In general, however, it must be admitted that the writer's long-standing thesis did not until recently receive either general recognition or experimental confirmation by others. However, with the advent of the Great War, and the appalling incidence of shock amongst the wounded, a concentrated effort was made by many workers to discover the underlying physiology of shock. Until almost the end of the War, nevertheless, a number of prominent research workers were committed to the theory that shock is of nervous origin. In 1918, Quenu, on the basis of his experience with the wounded, was led to postulate the existence of a shock toxin. Shortly afterward, Dale and Laidlaw (1919) found that the intravenous injection of histamine would produce symptoms identical with those of shock, and in consequence it has been postu-

lated that this substance, or a similar substance, is liberated when tissues are injured. As will be shown in our subsequent discussion, it is improbable that cytost and histamine are identical.

Bayliss, Cannon and their associates have shown in a fashion analogous to that employed by the author that crushing the thigh muscles of anesthetized cats will induce shock in the animals. Cannon (1923) showed further that if circulation in the animal's legs is arrested by clamping the abdominal aorta, no symptoms of shock appear until the release of the aortic clamp.

These results are in agreement with those of the author's experiments, wherein he utilized a tourniquet to prevent circulation in the animal's limbs. Again, by means of a parabiotic union of the circulation of two animals, Cannon has shown that the production of an injury in one animal leads to a state of shock in both animals.

This type of experiment has also been conducted by the writer (unpublished), but in view of the fact that much the same result is achieved by the injection of the blood serum or tissue juices of the injured animal into a normal animal, the writer has felt it unnecessary to adopt the elaborate technique of parabiotic experiments. Furthermore, in such an experiment, the "normal" animal may well be rendered hypersensitive to the shock reaction because of the tissue damage incident to the establishing of the parabiosis.

The validity of the writer's contention concerning the toxic nature of shock has been recognized by Jeanneney (1921), Hartmann (1922), and Limousin (1923).

The reader interested in the history of the author's views on the toxic nature of shock is referred to these authors, and especially to two papers by the writer published in 1922 and 1929, entitled respectively "Shock and Fatigue" and "Researches on the Shock Reaction before the Great War, Confirmed by Actual Experience in War." The latter paper contains a review of our investigations from the time of their inception, and the former contains a number of detailed protocols of the experiments on shock.

In a recent publication, Zschau (1931) has pointed out that the so-called electrosurgery has an advantage over the ordinary methods of surgical procedure, in that the incidence of operative shock is unusually slight. This he attributes to the fact that the resorption of the products of tissue disintegration is considerably lessened by the lymph coagulation which attends the use of the electric knife, but does not take place when ordinary surgical methods are employed.

CHAPTER III

THE RELEASE OF CYTOST FROM CELLS

IN the previous chapters we have called attention to the fact that under the proper conditions, living cells appear to be potentially immortal. The "proper conditions" consist in an adequate food supply and a satisfactory mechanism for the removal of non-utilizable and toxic metabolites. By its growth and ever-changing activities the cell expends energy, and, not being endowed with an inexhaustible supply of the latter, obtains it by the oxidative degradation of foodstuffs.

While the greater part of ingested foods is actually utilized as a source of energy a smaller quantity is transformed by suitable chemical reactions into the proteins, carbohydrates and fats which constitute the basis of the morphological structure. As a consequence of the chemical changes taking place within the cells, numerous end products which are not utilizable either as a source of energy or for structural purposes are excreted. Many of these end products, if not removed from the immediate environment of the cell, exert a toxic action which ultimately results in the death of the cell.

It is now generally accepted that the chemical

changes incident to the life process are conditioned as regards both their velocity and specificity by biochemical catalysts or enzymes. The action of these remarkable substances is as yet but imperfectly understood; nevertheless the evidence for their existence is irrefutable. For example, starches may be hydrolyzed to glucose by means of strong mineral acid or by sprouted barley. In 1832 Payen and Persoz observed that an extract of barley which was free from living cells would, when added to starch, effect its hydrolysis with great ease.

For many years it was believed that the action of such extracts differed materially from the enzymatic activities of living cells. It remained for Buchner in 1897 to establish beyond a doubt that the chemical activities of cells were actually controlled by endocellular enzymes. This worker, in his now classic experiment, subjected ground yeast cells to a tremendous pressure, thus obtaining a yeast juice free of living cells. This expressed fluid was found to possess all the fermentative properties of the original yeast cells.

Since Buchner's time the presence of a great many enzymes has been demonstrated in somewhat analogous fashion. Indeed enzyme preparations have been obtained from various cells, thus enabling us to account for the majority of the chemical transformations effected by living matter. Nevertheless the chemical structure of the individual enzymes still remains one of Nature's secrets.

Now these enzymes for the most part exist within the cells and if an insufficient quantity of proper foodstuffs is available to the cell, they readily attack the

structural elements; hence, if such action is unduly prolonged the cell structure becomes disorganized and death ultimately results. This process of self-digestion was first pointed out by Hoppe-Seyler, who noted that the tissues of dead animals underwent liquefaction even though there was no apparent evidence of bacterial action. Some twenty years later Salkowski (1890) found the end products resulting from such liquefaction to be nearly identical with the end products produced by the action of pancreatic trypsin on proteins. While today it is known that the intracellular proteases differ in their properties from the intestinal proteases, their actions are nevertheless analogous as first surmised by Salkowski. The essential difference between these two classes of enzymes appears principally in the optimal hydrogen ion concentration for their activities—the intracellullar proteases exhibiting their maximal activity in faintly acid media whereas trypsin is inactivated in the presence of acids.

In 1900 Jacoby observed that when a portion of liver was ligated *in situ* it underwent changes analogous to those previously found by Hofmeister and Salkowski in dead tissues. Jacoby coined the word autolysis to describe the self-digestion of tissues both *in vivo* and *in vitro*. It has been pointed out above that starving tissues undergo autolysis. In this connection it is worthy of note that Cusa-Bianche states that tissues taken from starving animals and tissues of normal animals which have undergone *in vitro* autolysis present much the same histological picture.

Analogous conditions are found in unicellular organisms; for Condradi (1903), Rettger (1904)

and Effront (1905) have drawn attention to the fact that while many bacteria and yeast do not suffer self-digestion in culture medium, autolysis becomes rapid when such cells are transferred to either distilled water or saline solutions. Obviously under such conditions the microörganisms are deprived of adequate nutriment and hence are placed in a position comparable in some respects to the tissues of a starving animal.

Since these early investigations, a very considerable literature has accumulated concerning the nature of autolysis. In general it has been found, in agreement with the fact that tissue proteases are active only in acid media that the addition of acid substances increases the rate of autolysis of excised tissues, whereas the converse is true of alkalis. Indeed if tissues are made sufficiently alkaline, autolysis ceases. In the case of mammalian tissue no evidence of autolysis either *in vivo* or *in vitro* can be detected as long as the tissues are held at a hydrogen ion concentration equal to or less than that characteristic of the animal's blood. It has long been known that excised tissues develop a post mortem acidity; and potentiometric measurements by Dernby (1918) and by Kochler, Sevinghaus and Bradley (1921) have shown the velocity of this acid production to be extremely rapid. While the mechanism by which injured or dying tissues produce the acid is not known, it is nevertheless real, as various workers have actually isolated lactic acid from such tissues. As a consequence any pathological change which causes an increase in the acidity of a cell will result in an autolytic process, and diffusible substances are produced which diffuse out of the cells; hence the

mass of the latter decreases. The mechanism by which the intracellular acidity is raised is immaterial for atrophic changes will always follow. Halogenated organic compounds such as phosgene, mustard gas and chloropicrin, readily penetrate cells undergoing hydrolysis with the liberation of hydrochloric acid. As a result the cells die and autolyze rapidly.

However, the mechanism utilized to produce the primary acidity need not in itself be a chemical capable of liberating an acid, for as we have seen excised tissues themselves produce sufficient acid to initiate autolysis. We now know as a result of Warburg's (1928) experiments that the majority of tissues are capable of producing lactic acid, particularly if deprived of an adequate oxygen supply. In other words, when the metabolism of the tissues is altered, acids accumulate.

That such an altered metabolism may be induced by relatively slight changes in the physical environment is shown by the experiments of Kubowitz (1929). Utilizing Warburg's technique, this worker found that upon exposure to temperatures slightly higher than 25° C. the frog's retina suffered an inhibition of the Pasteur reaction with a consequent production of lactic acid by glycolysis. While at present evidence is lacking that other types of injury may cause a similar alteration in metabolism in other tissues of higher animals, it is a reasonable supposition that such is the case, because various types of injury are known to cause the onset of autolysis. Further, it has long been recognized that trauma results in an increased acidity of tissues; and Chambers and Pollack (1927) have recently observed this in single cells—the starfish egg.

By means of a micro-pipette these authors injected the indicator, brom thymol blue, into the eggs. Upon injury of the egg by repeated thrusts of a micro-needle the indicator underwent an immediate change in color from blue to yellow, indicating an increase in the hydrogen ion concentration. Previously Chambers (1924) had noted that tearing of an egg by a micro-needle leads to cytolysis which spreads from the site of injury; and the experimental results of Chambers and Pollack show that the increase in acidity apparently precedes the cytolysis, for they state that upon injury of an egg suspended in sea water colored with brom thymol blue, the water adjacent to the egg transiently became yellow prior to cytolysis.

These beautiful experiments are especially pertinent to the present discussion because they unquestionably demonstrate the production of an acid by a single cell when injured. As yet the precise mechanism underlying this curious behavior is unknown. However, it is clear that once the acid is formed, the intracellular proteolytic enzymes will be enabled to hydrolyze the cell proteins and hence the degradation of morphological structure will ensue. Incident to this, various substances of endocellular origin will be released into the surrounding medium—the sea water in the case of the starfish egg and the body fluids in the case of the cells of higher animals and plants. If the foregoing is accepted, we have a rational explanation of the mechanism by which cytost is released from the injured cells.

Various observations of the author have suggested that a tissue toxin or cytost may be liberated under

a variety of conditions which we may briefly consider. In 1893 the writer had under his care a patient suffering from alcoholism. Curiously enough, although the use of alcohol had been discontinued for some months, a marked toxemia persisted. Hence the conclusion was reached that the toxic symptoms evident in the individual in question arose from the absorption of toxic products resulting from the necrosed cells which had been produced by the injuries inflicted by the previous prolonged ingestion of alcohol (Turck, 1893). In view of the preceding discussion, we may now assume that in this instance the prolonged use of alcohol had resulted in an autolysis of the tissue cells of the gastrointestinal tract and that the observed symptoms of toxemia were due to the continued absorption of cytost from the injured tissues.

Two years later, while investigating the bacteriology of the stomachs of patients with gastric carcinoma, it was found that in such cases the carcinoma created a favorable soil for the microörganisms, particularly lactic acid-producing organisms. Fresh sections of the gastric mucosa which were examined immediately after removal showed typical pathological changes incident to inflammation: cells loosened from the membrane, cystic degeneration, mucoid metamorphosis, marked interstitial changes, leucocytic infiltration, and engorgement of the blood vessels. The writer stated, "If it is not alone the product formed in the growth of carcinoma of the stomach that causes this inflammatory and rapid destructive process, then the infection from growing pathologic and nonpathologic microörganisms colonizing upon the sur-

face of the mucous membrane offers a possible explanation of these changes." (Turck, 1895.) When cultures of microörganisms obtained from patients' stomachs were introduced into the stomachs of normal dogs, the microörganisms rapidly disappeared. This result indicates that the bacteria alone were incapable of causing the degenerative changes observed in the stomach, and that the real cause of the alterations in the stomach cells was the liberation of cellular toxins as a result of cellular autolysis, primarily caused by the carcinoma.

In agreement with this it was found that if prior to the introduction of the bacteria the animal's stomach was daily swabbed with tannic acid for a week, the organisms grew rapidly and the animal showed loss of appetite, refused food and grew thin. Apparently the tannic acid had in a fashion similar to that of carcinoma so altered the gastric mucosa that the microörganisms could easily multiply.

Shortly after these experiments, other caustic irritants, such as mustard, were applied to the gastric mucosa, whereby a similar result was obtained, the sticky exudate resulting from the action of the irritant forming an excellent culture medium for the rapid proliferation of bacteria. (Turck, 1902.) Furthermore the histological picture of the stomach tissues in cases of carcinoma was found to be closely akin to that obtained by the prolonged action of mustard emulsions on the gastric mucosa.

As a result of his observations of toxemias in patients suffering from carcinoma, alcohol poisoning and various gastric disturbances, the writer came to the conclusion that cell necrosis played an important rôle

in the various pathological processes. In the case of stomach ailments, various experiments were devised to ascertain the presence and the nature of toxins formed in the stomach. In the previous chapter we have referred to experiments which showed that the injection of the gastric contents of patients suffering from gastritis produced signs of toxemia and shock.

Clinical observers have repeatedly noted symptoms of so-called auto-intoxication in patients with gastric atony and dilation. This suggested a study of the fatigue of gastric muscle. Experimentally it was found that the gastric muscle could conveniently be fatigued in either of two ways: by rapidly revolving the gyromele with the stomach; and by alternately inflating and collapsing a rubber bag inserted into the stomach. (Turck, 1903a.) By either of these means the muscles may be so completely fatigued that they fail to respond to stimuli, and show marked elongation and lack of tone. After such treatment portions of the stomach tissues were subjected to histological examination whereby the muscle fibers of the pylorus were found to have suffered the most marked changes, the nuclei staining poorly with methylene blue while the cytoplasm appeared to be less granular than normally. These latter observations are indicative of autolysis and show that injury of the stomach tissues is followed by morphological changes analogous to those found in other tissues under similar conditions. Further congestion and dilation of the capillaries was evident. These histological findings are in agreement with Gilman's (1903) observations on the effect of fatigue on the nuclei of voluntary muscle cells.

In order to determine the presence of "fatigue toxins" in the muscles, the muscularis was removed, minced and extracted with saline. Upon injection of such extracts into the muscle walls of the unfatigued stomachs of other animals, the abdomen being opened, it was found that a contraction occurred, followed in half an hour by a marked dilation, the stomach ultimately becoming distended by gas to three or four times its normal size. Rhythmical contractions vanished and the musculature became flabby and would not respond to stimuli. As a control, a volume of saline equal to that of the extract employed was injected into the stomach walls of other animals. In such control experiments the organ showed spontaneous movements within twenty minutes after injection and the muscles readily responded to stimulation.

The extract of fatigued muscle was injected into the peritoneal cavity of a dog. Some eighteen hours later, the animal showed an intense thirst but immediately vomited water after drinking. This behavior is noteworthy since it is identical with that frequently seen in shock. Twenty-seven hours after the injection the animal's abdomen was opened and the stomach found to be dilated and flabby, and to contain fluid. Rhythmical movements were scarcely discernible and the response to stimulation was poor. A similar effect was obtained by the injection of the fatigue extract into the jugular vein. A normal dog was fed a mixture of bread, milk and bismuth. Radiographic examination showed peristalsis to be proceeding normally. Injection into the jugular vein of the extract of fatigued gastric muscle completely inhibited the peristaltic movements.

These experiments show that distention of the stomach results in the production of a toxic substance and this is in agreement with the clinical findings. It will be remembered that in the preceding chapter experiments were presented which showed that a state of shock could be induced by distending the stomach with air. In the latter case a sufficiently high concentration of toxins was produced to yield generalized effects. In both instances, however, the liberation of the toxic substance is due to the same cause—dilation of the stomach—but in the shock experiments the forced dilation was of considerably greater duration.

How, we may now ask, is the toxin produced? When the stomach is dilated by any means blood is forced out of the vessels in the wall of the organ. In using the method of intermittent dilation which has been discussed above, it was noted that "at the rest pause, the peritoneal walls became bluish-purple, and the vessels engorged. As each distention occurred, the outer wall showed an anemic appearance from stretching, when upon releasing the air it would become rosy in tint and hyperemic. After a repetition of this dilation, the engorgement of the vessels disappeared. (Turck, 1903a.) Further, in an experiment in which the femoral vein was exposed it was noted that distention of the stomach by air caused a distention of the vein, which reverted to its normal size when the air was permitted to escape from the stomach.

Such circulatory disturbances must of necessity interfere with the normal metabolic processes of the cells of the stomach tissues; hence, as has been dis-

cussed at the beginning of this chapter, autolysis must result. In the case of the simple distention experiments this autolysis in all likelihood does not proceed very far; nevertheless protein decomposition products are liberated in sufficient quantity to affect the muscle cells locally, resulting in a state of fatigue. On the other hand when the stomach is distended by air for a considerable period, as in the shock experiments, the blood supply to the tissues is interfered with for a sufficiently long time to permit marked autolysis to progress; hence a much larger amount of protein split products is liberated into the general circulation and shock results. These inferences are in agreement with the histological evidence which, as previously stated, showed a decrease in the cell granules and the affinity of the nucleus for stains—a result always encountered in autolyzing cells. Further, it is now common knowledge that impairment of the circulation leads to autolysis and atrophy.

In 1897 the author reported the results of various ligation experiments. It was found that ligation of the portal vein resulted in the death of the animals within three days, and the liver tissues whose blood supply had been thus interfered with showed evidence of extensive autolysis. After ligation of the veins along the lesser curvature of the stomach, microscopic examination revealed evidence of local autolysis and prominent erosions in the gastric mucosa. Similar results obtained by the local ligation of various portions of the stomach lead to the conclusion then expressed that "Erosions of the stomach occurred wherever the venous outlet was obstructed."

In 1914, incidental to a study of the diffusion of bacteria into the intestinal wall, various portions of

the intestine were ligated. In a series of one hundred and fifty-five animals including monkeys, rats, rabbits and guinea pigs, it was observed that "the higher the ligation the more quickly was the issue fatal." In some experiments, bits of intestine were removed some six hours after the operation for ligation. Microscopic examination revealed necrosis of the entire coat, including the muscular coat. This was evidenced by the poor staining ability of the cytoplasm and nuclei of the cells. The animals all showed the shock syndrome a few hours after the tying off of the intestinal loops; in conformity with the conclusions expressed in the previous chapter, it seemed likely that the observed symptoms and death were due to a toxic proteose fraction resulting from the tissue autolysis. Further experiments proved this to be the case, for it was found (Turck, 1922) that when the submucous juice obtained from such animals was diluted with an equal volume of normal saline and injected either into the same animal or into another of the same species, a fatality always resulted.

Some of the results of the experiments just mentioned are summarized in the tables on pages 60 and 61.

Reference to the results shown in the tables shows that in general the higher the ligation of the intestine, the more rapid was the onset of symptoms and subsequent death. This is readily understood when it is remembered that the autolytic process is an enzymatic one. In general, tissue autolysis, as we have seen, is the result of changes in the internal environment of the cell which in turn so alters conditions that the endocellular protolytic enzymes are able to effect hydrolysis of the cell proteins. Consequently when

TABLE I

Animal	Position of Ligature	Time of Appearance of Shock	Time of Death	Time of Examination	Findings
No. 2	Duodenum	4 hrs.	24 hrs.		Erosions, Mucosa } fluid
No. 3	Jejunum	5 hrs.		24 hrs.	Hemorrhage, Peritoneal coat
No. 4	Jejunum (lower)	5 hrs.	48 hrs.		
No. 5	Ileum	8 hrs.		24 hrs.	Effusion of blood in coats
No. 6	Ileum	7 hrs.		24 hrs.	Effusion of blood in coats
No. 7	Ileum	9 hrs.		24 hrs.	Effusion of blood in coats
No. 8	Ileum	5 hrs.		24 hrs.	
No. 9	Section tied (small and large intestines)	12 hrs.		24 hrs.	Hemorrhagic points near ligature
No. 10	5 loops at different levels	2 hrs.		4 hrs.	
No. 11	8 loops	1 hr.		3½ hrs.	Shock syndrome
No. 12	Colon and ileum loops	5 hrs.		24 hrs.	

The intestines were tied off in positions indicated in the second column. The figures in the third column indicate the time which elapsed before the symptoms of shock became apparent, while the figures in the fifth column indicate the time which elapsed between the ligation and examination under anesthesia. The principal findings of interest are indicated in the sixth column.

cellular necrosis is permitted in organs which themselves secrete proteolytic enzymes, the digestion of tissue proteins is markedly facilitated since the latter are subjected to attack not only by the endocellular

TABLE II

Dogs

Dog	Loops	Time	Intravenous Inj.	Death
No. 1	Jejunum	3½ hrs.	5 cc.	3 minutes.
No. 2	Upper ileum	5 hrs.	1 cc.	2 hours.
No. 3	Lower ileum	6 hrs.	2 cc.	24 hours.
No. 4	Duodenal jejunum loops	3½ hrs.	0.5 cc.	3 minutes.
No. 5	Middle jejunum loops	4 hrs.	1 cc.	2 hours.
No. 6	Upper ileum	8 hrs.	2 cc.	24 hours.
No. 7	Colon	8 hrs.	3 cc.	24 hours.
No. 8	Colon	10 hrs.	5 cc.	24 hours.

In these experiments loops of intestine were tied off as indicated in the second column. Submucous juice was withdrawn from the loops after the periods noted in the third column. This was diluted with an equal volume of normal salt solution and the quantities of the extract noted in the fourth column were injected intravenously into the animals. This always caused death within from three minutes to twenty-four hours, as shown in the fifth column.

enzymes but by those exterior to the cells as well. Now enzymatic action is known to be greater in the upper alimentary tract than at lower levels. It follows that for a given injury, the cells lining the upper portion of the tract must be more rapidly autolyzed than those at lower levels. As a net result the concentration of cytost necessary to cause generalized symptoms is more rapidly released than is the case in lower levels of the intestine. Thus we arrive at an adequate theoretical basis for the results observed in these experiments.

The results of these experiments are in harmony with the author's early contention that a tissue toxemia frequently caused death from shock during abdominal operations. (Turck, 1901.) Further, they offer a rational explanation for the statement that "in

operations in the upper abdominal area there is a greater danger from shock than in operations on the lower abdominal area. Even traction on the stomach (i.e, interference with circulation) greatly increases the liability to shock, while exposure to air adds greatly to the danger. If death does not follow, still the toxins formed are detrimental to convalescence, and the future welfare of the patients." (Turck, 1902b.) This conclusion, expressed some thirty years ago, has since been confirmed by both clinical observation and laboratory experiments. Indeed the last sentence of the above quotation suggests the investigation of the action of small quantities of cytost upon the well-being of the animal. During the last decade this aspect of the problem has been carefully investigated in our laboratory and the results obtained are of very considerable interest and will constitute the major portion of the discussion in previous chapters.

Individuals suffering from intestinal obstruction frequently suffer from an intense toxemia. At one time it was assumed that this was due to the absorption of bacterial toxins produced by the activity of microörganisms in the stagnant contents of the intestinal tract. More recently such toxemias have been ascribed to the liberation of toxic products from the cells of the intestine. Roger was forced to this conclusion by his observation that extracts of the intestinal mucosa were more toxic to animals than extracts of the intestinal contents. This, it should be noted, is in perfect harmony with the results of the writer's experiments—in which the presence of bacteria could not have played any significant rôle. These conclusions have furthermore been substantiated in recent

years by the experiments of Draper and his co-workers. (Draper and Johnson, 1930.)

In the first chapter attention has been called to the fact that local application to the gastric mucosa of caustic chemicals leads to cell necrosis and subsequent shock. The experiments upon shock induced by general anesthesia suggested the mechanism of the reaction to be the same as in other cases of experimental shock; i.e., an autolysis of tissue giving rise to the shock toxin. If this hypothesis is correct, then one should be able to induce a localized tissue autolysis at any desired site by the injection of a substance such as chloroform. If the extent of the autolysis induced in this manner is sufficiently great, then the experimental animals should exhibit symptoms of shock.

Experiments of this type have been reported by the writer (Turck, 1922b). Cats were injected intramuscularly twice weekly with 0.25 cc. of absolute alcohol, ether or chloroform. The animals died after five or six months of such treatment. At autopsy these animals all showed a chronic interstitial nephritis with stagnation of blood and autolysis of the tubules. In these experiments it was of course recognized that the observed pathology might have been due to the direct action of the drugs upon the tissues and not caused by cytost released from cells at the site of the injection. In order to settle this point experiments of the following nature were conducted. Chloroform was injected deeply into the tissues of cats. After a lapse of time sufficient to permit a complete local necrosis, the tissues were removed from the site of the injection. A sterile saline extract was prepared and injected two or three times a week into normal animals. At autopsy,

the kidneys of the latter were found to have suffered degenerative changes similar to that produced in animals by the direct injection of chloroform. Only one conclusion may be drawn from these results; that the observed pathology was due not to the direct action of the drug on the kidney but was the result of endocellular substances released from those tissues directly injured by the chloroform,—i.e., those tissues at the site of injection.

Further evidence substantiating these conclusions is to be found in the following experimental results:

An isotonic saline solution was injected into the muscles of the thigh. After a brief period the fluid was aspirated from the site of injection and injected intravenously into another animal of the same species. No evidence of discomfort of physiological change was observed in the second animal. When however either chloroform, ether or alcohol was substituted for the saline solution quite different results were obtained. The drugs were allowed to remain *in situ* for one or two hours; the fluid was then aspirated from the resulting sterile abscess and injected intravenously into homologous animals, whereupon the latter quickly showed the symptoms of shock and subsequently died.

The results of such experiments are summarized in Table III, page 65.

As shown in the table, the rapidity with which death may follow the injection of tissue break-down products is very striking. At autopsy, all of the animals were found to have suffered a marked congestion of the splanchnic viscera, identical with that observed in shock induced by other means. Kidney lesions were

TABLE III

Animal	Irritant	Quantity Injected in First Animal	Quantity Aspirated and Injected into Second Animal	Time of Death after Injection
1. Rabbit	Chloroform	2 cc.	1.0 cc.	5 Min.
2. Cat	Chloroform	2 cc.	1.5 cc.	3 Min.
3. Dog	Chloroform	4 cc.	2.5 cc.	10 Days
4. Cat	Chloroform	4 cc.	2.5 cc.	1 Day
5. Rabbit	Ether	4 cc.	1.5 cc.	1 Min.
6. Cat	Ether	4 cc.	1.5 cc.	2 Min.
7. Dog	Ether	6 cc.	2.0 cc.	4 Min.
8. Cat	Ether	4 cc.	1.0 cc.	2 Hours
9. Cat	Ether	6 cc.	1.5 cc.	10 Hours
10. Rabbit	Alcohol	10 cc.	1.0 cc.	2 Min.
11. Rabbit	Alcohol	15 cc.	2.0 cc.	8 Hours
12. Cat	Alcohol	15 cc.	2.0 cc.	24 Hours
13. Dog	Alcohol	20 cc.	1.5 cc.	15 Hours
14. Cat	Alcohol	15 cc.	1.5 cc.	5 Min.
15. Cat	Alcohol	15 cc.	2.0 cc.	18 Hours

found in those animals which lived for some hours after the injection of the aspirate. It is of interest to note that this is a common finding in animals which have suffered extensive tissue destruction by burns or various infections.

These experiments demonstrate conclusively that the anesthetics so far examined are actually able to effect tissue destruction with the consequent liberation of the toxic cytost—that is they cause an *in vivo* autolysis.

A second series of experiments were made with cats in order to compare the ease with which chloroform, ether and alcohol effected tissue autolysis. For this purpose the animals received two intramuscular injections of the chemicals, the second injection being administered two hours after the first. A few hours

after the second injection all of the animals developed the typical symptoms of shock and later died. The results are summarized in Table IV.

TABLE IV

Reagent	Quantity		Onset of Shock after Injection	Time of Death after Injection
	1st. Injection	2nd. Injection		
1. Chloroform	1 cc.	1.5 cc.	4 Hours	24 Hours
2. Chloroform	2 cc.	1 cc.	3 Hours	36 Hours
3. Chloroform	1.5 cc.	1.5 cc.	5 Hours	48 Hours
4. Chloroform	2 cc.	2 cc.	4 Hours	72 Hours
5. Ether	2 cc.	2 cc.	6 Hours	24 Hours
6. Ether	2 cc.	2 cc.	3.5 Hours	36 Hours
7. Ether	3 cc.	3 cc.	4 Hours	40 Hours
8. Alcohol	5 cc.	10 cc.	6 Hours	72 Hours
9. Alcohol	5 cc.	10 cc.	5 Hours	112 Hours
10. Alcohol	2 cc.	10 cc.	4.5 Hours	80 Hours
11. Alcohol	10 cc.	10 cc.	4 Hours	24 Hours

From these results it is apparent that chloroform is the most active reagent of the three. Since shock is produced by cytost liberated from the tissues it follows that chloroform is able to liberate the cell contents more easily than does ether or alcohol. This result is of interest in view of the fact that animals anesthetized for long periods with chloroform pass into a state of shock more frequently than is the case with the other narcotics. The results of the experiments just discussed offer ample proof of the statement previously made, that shock produced by general anesthesia is due to the same causative factor as is shock caused by other injuries. (Turck, 1903b.)

Under general anesthesia the excretory functions of the organism is impaired, hence the animal is unable to rid itself of toxins as easily as is the case when

other means are employed to induce shock. Thus, although the concentration of anesthetic in the tissues may be insufficient to cause an extensive necrosis, the added factor of diminished excretory power perhaps permits a greater accumulation of cytost in the tissues than might be the case in unanesthetized animals injured by other means.

The foregoing may be summarized as follows: injuries regardless of their nature stimulate the autolysis of cells. Such autolysis results from the activity of endocellular enzymes. Now it should be obvious that cellular autolysis may be complete or partial, for it is conceivable that if the injury be slight, the damage may be easily repaired and consequently the autolytic process will not proceed far enough to seriously disorganize the cell.

This fact is attested to by the observations of Chambers and other micro-dissectionists who have frequently injured cells of various sorts by their manipulations. If the injury be slight and non-extensive, the cells show no apparent ill effects and continue to live normally. On the other hand, as has been stated previously, if the injury is marked, as for example in Chambers' experiments with the starfish egg, then cytolysis, death and presumably autolysis ensue.

For some years it has been recognized that whereas the normal living cell exhibits a selective permeability, the latter ceases upon death (Jacobs, 1924). So far as is known today, all normal cells are impermeable to colloids. Hence they are probably impermeable to enzymes since all enzymes which have been carefully studied appear to be colloidal (Waldschmidt-Leitz, 1926; Gortner, 1929). It follows

therefore that any endocellular enzymes found in the medium surrounding cells must have been liberated by necrosis. This concept finds confirmation in the fact that active cytolytic agents or mechanical disintegration may be employed to achieve that extraction of enzymes from suspensions of cells.

When, as a result of natural processes or injury, autolysis of cells takes place, the contents of the cell including the endocellular proteases responsible for the autolysis may diffuse out into the surrounding fluids. In the case of higher animals these substances find their way into the blood and are eventually excreted in the urine. This has been proven by Pfeiffer (1914) who was able to detect the presence of proteolytic enzymes in the blood and urine of individuals suffering from extensive burns. Similarly in infectuous diseases accompanied by tissue destruction abnormal quantities of such enzymes have frequently been found in the blood and urine.

Just as beginning students of physiology frequently ask "Why do not the stomach and intestines digest themselves?" we may ask "Why do not the autolytic enzymes destroy normal cells?" While the first question has never been satisfactorily answered, it was pointed out at the beginning of this chapter that the hydrogen ion concentration of the normal cell is not sufficiently great to permit activity of the endocellular proteases.

Prior to the recognition of the importance of the intracellular hydrogen ion concentration in controlling the activities of the endocellular enzymes, it was believed that autolysis was inhibited solely by the presence of specific antienzymes. Some years ago

Hildebrandt (1893) found that blood serum inhibited the action of trypsin. Since that time many other workers have studied this effect and it is now known with certainty that some substance present in the serum inactivates all proteolytic enzymes which may find their way into the blood. For our purpose it is not necessary to consider the various hypotheses which have been advanced to explain this fact.

As we have seen endocellular proteases may diffuse from injured cells into the blood. Under normal circumstances, the inhibitory substances present in the serum may diffuse into injured cells and perhaps there inhibit the activities of the proteolytic enzymes. Any factor which prevents such an inhibition will therefore enhance the rapidity of the autolytic process. Simniezki (1902) found that depending upon the concentration used, alcohol either weakened or completely inhibited the antienzymatic activity of blood. In connection with the experiments on shock produced by general anesthesia the author (Turck, 1903) compared the antiferment action of serum from normal animals with that obtained from animals that had shown symptoms of shock induced by anesthesia. For this purpose the well known Mett method of measuring proteolysis was adapted as follows: egg albumin was aspirated into glass capillary tubes of 1 to 2 mm. diameter and coagulated by heating to 95° C. for a short time. The tube containing the coagulated protein was broken into short lengths and these were added along with proteolytic enzymes to the sera under investigation and to normal serum. These preparations were then held at 37° C. for ten hours, after which the proteolytic activity of the mixtures

was ascertained by measuring the difference between the length of the capillaries and the length of the column of undigested egg white. The differences so determined are summarized in Table V. The weights reported in the table are the amounts of dried enzyme preparation added to the tabulated volumes of serum. In the case of anesthetized animals similar quantities of serum were utilized in each case.

TABLE V

ANTIFERMENT ACTIVITY OF BLOOD SERUM AS MEASURED BY THE METT TECHNIQUE

		+PANCREATIN	+CARICA PAPAYA
Serum from normal dogs		3.2 mm.	2.4 mm.
		3.1 mm.	1.5 mm.
Serum from anesthetized dogs			
Anesthesia lasted for	6 hr.	9.0 mm.	8.8 mm.
	6 hr.	8.4 mm.	8.6 mm.
	4 hr.	7.4 mm.	8.7 mm.
	1 hr.	3.4 mm.	4.7 mm.
	1 hr.	4.3 mm.	4.1 mm.

The figures in this table represent the lengths of egg albumin in the Mett tubes which was digested by the various preparations during an interval of 12 hours at 37° C. In each instance 1.5 gram of the dry enzyme was added to 5 cc. of serum. Note that the serum from the anesthetized animals permitted digestion to take place much more rapidly than was the case with normal serum. (Turck, 1903.)

From the tabulated results it is apparent that normal serum inhibits the action of the added enzymes to a greater extent than does serum from anesthetized animals. Further, the longer the period of anesthesia, the less is the extent of the inhibition.

In order to ascertain whether or not the anesthetics employed were capable of lowering the antiferment activity of normal serum the following procedure was

followed. Chloroform or ether was added to serum and after the lapse of two or three hours, the chemicals were removed by warming the serum to 45° C. *in vacuo*. When sera treated in this fashion were examined by the Mett method it was found that they had lost their ability to inhibit the action of proteolytic enzymes.

From these results it follows that by lowering antiferment action of serum, anesthetics may aid the autolytic process. Confirmation of this hypothesis is found in the experiments of Yamakawa (1918) who found that in the presence of various surface active substances such as alcohol and chloroform, the serum proteins undergo auto digestion. The experiments of this investigator are of importance for they demonstrate the fact that autolysis may take place in the serum providing that the activities of the ever present antienzymes are inhibited. It follows therefore that during anesthesia toxic protein fractions may possibly be derived from serum proteins as well as from intracellular proteins.

It therefore seems reasonable to postulate that the shock observed during anesthesia may in part be due to the action of such hydrolytic products of the serum proteins.

While on the subject of anesthetics it may be well to mention some other observations made at the time (1903) these experiments were conducted. It was noted that after prolonged narcosis the animal's blood coagulated more rapidly than is normally the case. This may be interpreted as indicating the presence within the serum of some substance which hastened coagulation. The various theories of blood coagula-

tion uniformly assume that incident to an injury some substance is liberated from the tissues which initiates the series of changes which finally lead to the formation of the fibrin clot. While the nature of this tissue substance and its mode of action are still in dispute, its importance in initiating the clotting process is quite generally accepted. For our present purposes, however, the exact details of the clotting process are immaterial. The fact that the blood of anesthetized animals coagulates more rapidly than that of normal animals is indicative of the fact that prolonged anesthesia causes the liberation of tissue components in much the same fashion as does a traumatic injury.

When serum drawn from an animal under anesthesia for three hours was added to a drop of normal blood, agglutinization of the corpuscles occurred immediately. Serum from animals anesthetized for longer periods exhibited this agglutination action even after a fivefold dilution.

Frequently the immediate coagulation of blood has been noted after prolonged anesthesia. However, it is not generally realized that this is due to some substance formed in the circulating blood as a result of the action of the anesthetic. The author in 1902–3 observed that the addition of "shock serum" to an equal quantity of normal blood resulted in the immediate coagulation of the latter. In general it was found that the intensity of the agglutinizing and clot forming reactions depended upon the duration of the anesthesia. Little such action was noted with the sera of animals anesthetized for an hour or two, whereas longer periods of narcosis invariably led to the formation of such agglutinins and precititins. For the

moment we wish merely to note that such bodies appear to be formed in shock produced by other means. Their formation may be assumed to arise as a result of tissue autolysis; hence they may be identical with cytost, may be formed concomitantly or they may result from the action of the liberated cytost. These concepts will be discussed in a later chapter.

Chapter IV

THE PHYSIOLOGY OF CYTOST ACTION

It will be remembered that our purpose in introducing the subject of shock was not to present an exhaustive account of the various theories and their relation to surgical practice, but rather to present evidence of the physiological relations between various cells. We shall not therefore offer an extensive review of this interesting subject, but shall limit the following to the author's own experiments. The reader desirous of pursuing further the clinical aspects of the subject is referred to the reviews of Cannon (1923), MacLeod (1930), and Turck (1922, 1929).

In previous chapters, experiments have been presented which show the primary cause of shock to be the liberation of cytost from injured tissue cells. So far, however, the discussion has been restricted to the evidence concerning the release of cytost and the terminal results of its action. We may now briefly consider the manner in which the released cytost affects the animal as a whole—that is, the physiological changes which ultimately lead to a state of shock.

At the time the author began his investigation it was generally held that shock was of purely nervous

origin. In support of this contention were the experiments of Goltz (1872), who found that, upon percussing the mesentery of the frog, there followed a reflex vagal inhibition of the heart and subsequent dilation of the splanchnic vessels. Beginning in 1899, Crile recorded observations showing that long continued stimulation of the sensory nerves elicited a fall in blood pressure. Since in severe operations or injury to the peritoneum a large number of sensory nerves are subjected to excessive stimulation, Crile concluded that shock under such conditions was due to exhaustion of the vasomotor center. It was therefore natural that at the outset the author's contention that shock was the result of tissue toxins should meet with disapproval, as, for example, the statement of Dr. James G. Kiernan (1897) : "I do not want unduly to swell the neurologic trend of the discussion at the present time, yet I cannot but take issue with the position that local tissue change irrespective of nerve action underlies the results as Dr. Turck has claimed. This seems to be turning back the page of pathology to the days of Broussaid. . . ." Curiously enough, however, this attitude persisted up till the time of the World War, despite the fact that in the interim the author had published the results of numerous experiments, showing the toxic nature of shock. The high incidence of shock in wounded soldiers stimulated others to investigate the underlying physiology. As a consequence, various investigators, notably Bayliss, Cannon, and their associates (1919), arrived at conclusions essentially the same as those expressed by the author some twenty years earlier.

In 1897 (Turck, 1897), the writer published ob-

servations made upon dogs which had been brought into a state of shock by various experimental means, such as exposure of the abdominal viscera to a draught of air, and the injection of stomach contents. In these experiments it was noted that coincident with the onset of shock, the stomach and intestines assumed a dark blue color due to stagnation of blood in the vessels. In experiments in which both arterial and venous pressures were recorded, it was noted that during shock the veins and viscera showed an increased pressure, whereas the arterial pressure dropped below the normal value. As the depth of shock increased, stagnation in the visceral vessels was found to increase, the intestinal vessels becoming engorged more rapidly than those of the stomach. When means were taken to revive the animals, the reverse of the above changes took place, the congestion being reduced and the arterial circulation reëstablished. On the basis of such observations the conclusion was drawn "That we cannot apparently experimentally produce profound shock without the occurrence of *congestion* and that the reduction of such shock does not occur until we reduce the congestion and distribute the blood over the surface." In all subsequent experiments in which shock was produced, this stasis of blood in the visceral vessels was always found. It is this visceral congestion which is responsible for the various external symptoms of shock, such as weakened pulse, diminished respiration, and lowered temperature.

The splanchnic circulation, physiologically considered, is distinct from the somatic or peripheral circulation, there being an inverse relation between the volume of blood circulating in the vessels of the

somatic and splanchnic areas. Because of this, dilation of the peripheral vessels is normally accompanied by contraction of the vessels of the splanchnic area. The latter, in fact, may be regarded as a reservoir or regulatory apparatus for the entire circulation, blood passing from this area to the peripheral vessels when these are adequately stimulated. Conversely, stimulation of the abdominal vessels, by functional activity or any other means, results in an increased flow of blood through the splanchnic vessels. We know that upon passing from a cool into a warm atmosphere, the peripheral vessels dilate; upon returning to the warm atmosphere, the peripheral vessels rapidly contract, forcing the blood back to the splanchnic area. In consequence, the vessels of the latter dilate to accommodate the increased influx of blood. Such facts as these illustrate the extreme delicacy of circulatory regulation.

In a previous chapter, there has been presented experimental evidence showing that direct stimulation of the gastrointestinal mucosa promptly causes a local hyperemia. If the stimulation be excessive, as by the prolonged action of mustard in the stomach, or the air blast, then this hyperemia becomes pathological and eventually results in a marked venous congestion.

The pronounced sensitiveness of the splanchnic vessels to stimuli is a necessary function, the mild hyperemia following such stimulation being necessary for the secretory and absorptive processes of the stomach and intestines. Such mild dilation of the capillaries of this area does not result in any obvious outward symptoms. When, however, such dilation becomes excessive, the diminished blood flow in the peripheral

vessels results in a lowered blood pressure, weak pulse and lowered surface temperature, the magnitude of such changes being conditioned by the extent of the splanchnic dilation.

The author's (Turck, 1900) observations that splanchnic congestion might be brought about by changes in temperature or irritation of the gastric mucosa are not surprising when viewed from our present knowledge of the physiology of the splanchnic circulation. Very striking, however, was the observation that upon subcutaneous injection, into rabbits, the gastric contents from a patient with a dilated stomach caused dilation of the splanchnic vessels, and collapse. A more rapid response was obtained by injecting such material into the mesenteric vein of a rabbit. When a similar quantity of water was injected in like manner, the animal exhibited no change other than a slight hyperemia, which always follows exposure of the viscera. At the time these experiments were published (1900), attention was called to the essential similarity between these results and the effects produced by the entry of peptones, primary and secondary albumoses, into the circulation. Further, the analogy was drawn between splanchnic congestion and severe hemorrhage, which produces much the same outward signs. This was summed up in the statement "that splanchnic congestion simulates hemorrhage; it might be expressed as a bleeding within oneself." (Turck, 1900.)

We are thus in a position to explain in part the occurrence of so-called postural shock, which was mentioned in the first chapter. When a rabbit is held by the head in a vertical position, the force of gravity

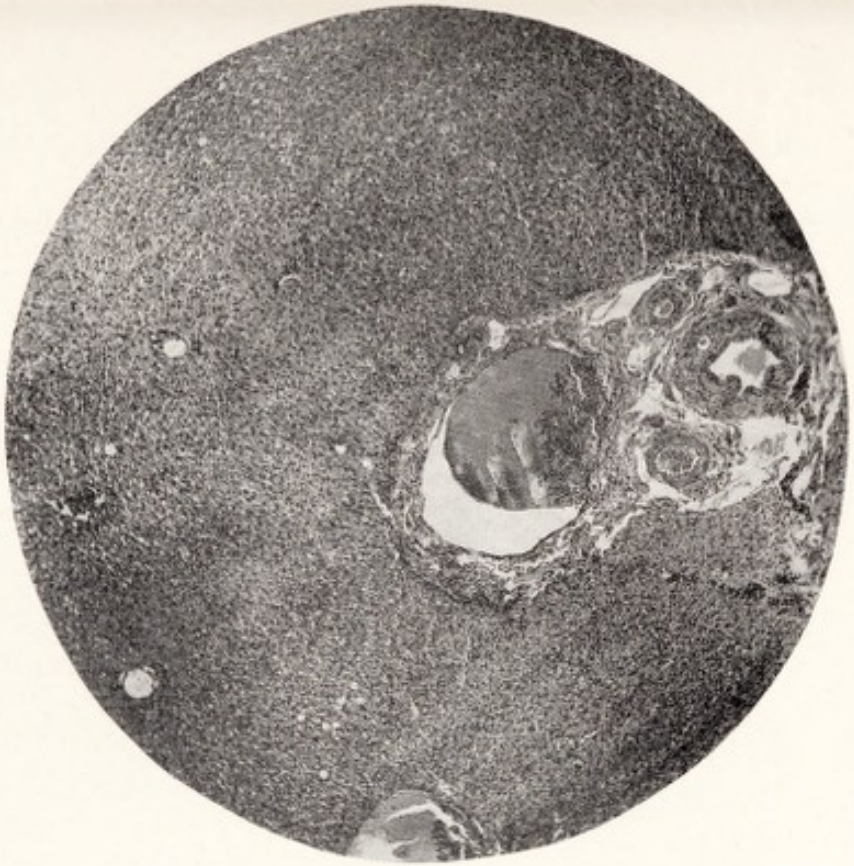

Figure 1.

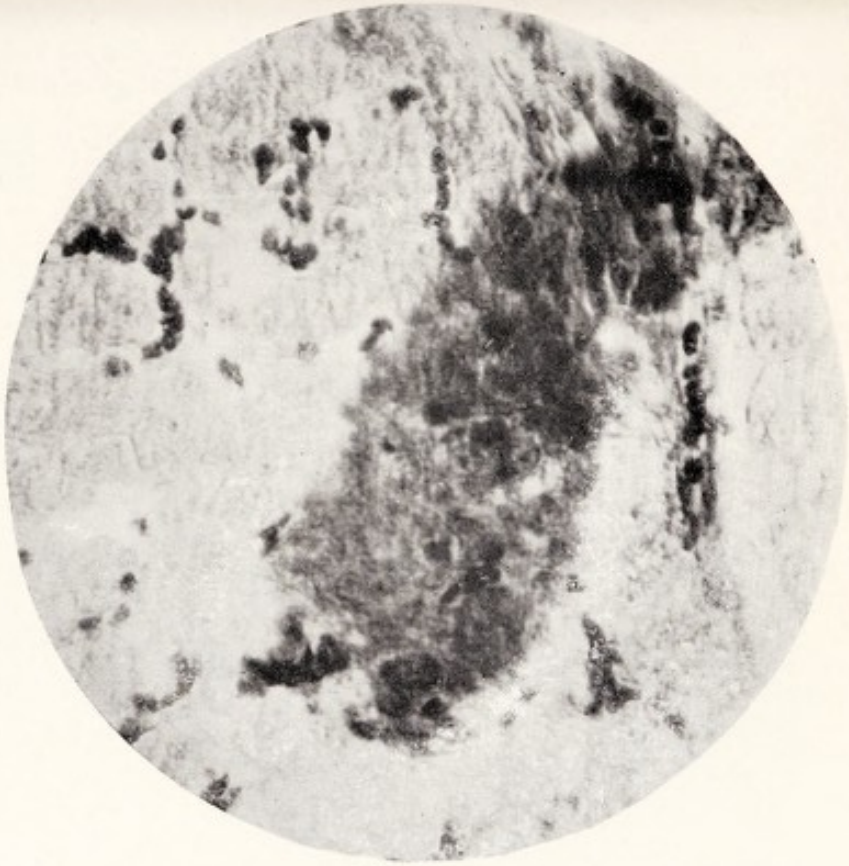

Figure 2.

Figure 1. Section of liver taken from a rabbit in severe shock caused by absorption of the products of injured tissue. This is typical of the portal zone reaction which always accompanies shock. Note the marked "clumping" of the corpuscles and that the central veins are completely empty, this evidencing the circulatory interference.

Figure 2. Section of liver taken from cat in fatal shock. This preparation clearly shows that the congestion of the capillaries is confined to the portal zone. This has caused stagnation extending back into the larger vein (in middle of photograph). It is interesting to note that the cell nuclei of this preparation did not stain well. This indicates that necrosis and autolysis of the cells had begun.

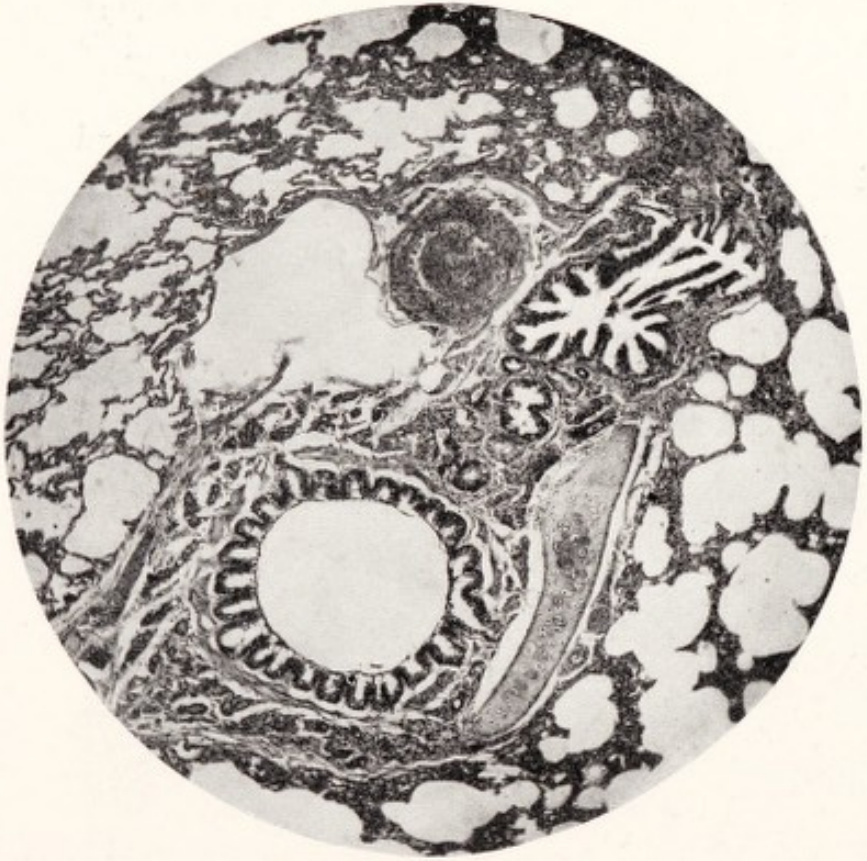

Figure 3.

Figure 4.

Figure 3. Section of a lung taken from a cat which died in shock. Clearly shows transverse section of pulmonary artery engorged with congealed blood while the pulmonary venous capillaries below are practically empty, indicating that the blood flow was arrested and retained in the pulmonary arterial zone.

Figure 4. Section of duodenum from cat in shock. Shows congestion beginning in the endodermal tissue and extending into the submucosa.

suffices to cause an accumulation of blood in the splanchnic area and consequently the animal manifests the outward symptoms of shock. Such an animal, however, will usually recover when returned to its natural position. In this respect, postural shock differs from the profound state of shock induced by tissue damage. It follows, therefore, that the production of true shock entails something more than a mere reversible accumulation of blood in the splanchnic area, as is the case in postural shock.

At this point we may recall the author's observations, mentioned previously, that in experimental shock the blood was found to clot more readily than is normally the case, and the serum from animals in profound shock contained substances capable of effecting hemagglutination and coagulation of blood drawn from intact animals. Such being the case, it follows that such processes may take place within the blood vessels. Thus, the blood which accumulates in dilated vessels may suffer a partial or complete coagulation, the extent of this process depending upon the amount of coagulins present in the blood. In any case, even though the extent of agglutination and coagulation be slight, the net effect will be to raise the viscosity of the blood and thus hinder its passage through the vessels and thence mechanically to distend the vessels even more.

Leo Loeb (1904) has shown that coagulins may be extracted from tissues and this fact is in harmony with the author's concept that shock is produced primarily by the products of cellular disintegration.

As a result of the agglutination and coagulation of the blood, the capillaries may be easily blocked, thus

causing a stagnation of blood in the smaller and then in larger vessels. In order to follow such changes it is necessary to resort to histological methods. For this purpose, bits of tissue were removed from animals in a state of shock and examined by the usual histological procedures. Before passing to a discussion of the actual observations, we may summarize the essential results of such an examination. In all cases of acute shock, regardless of the experimental means used to produce the reaction, general examination disclosed that marked stasis originated in the capillary bed of the visceral organs, the stomach, intestines, liver and lungs. "This stasis occurs in the venules and backs down into the larger veins in the portal zone of the liver, pulmonary and arterial zone (venous) of the lung, and submucous zone of the stomach and intestines." These conclusions are based upon a study of many reactions such as those shown in the following photomicrographs. The principal points of interest are noted in the legends accompanying the photographs.

It is noteworthy, as shown in figure 6, that in shock the brain tissues exhibit no evidences of congestion, the cells appearing normal. In all experiments with shock, wherein the brain has been examined, the same result has always been found. This constitutes additional evidence that shock is not the result of a central nervous disfunction.

A study of the accompanying photomicrographs, which are representative of hundreds prepared by the author, has shown conclusively that the first reaction which occurs in the liver appears to be confined to the finer capillaries, which are in direct contact with

the columnar liver cells. Apparently it is here that coagulation of the blood takes place, for, as is clearly shown in the pictures, the central vein (hepatic) is entirely empty. The coagulation, or gelation, in these fine capillaries prevents the free flow of blood. In consequence, as more blood enters from the portal vessels, a considerable pressure is exerted upon the capillary walls.

This pressure it is which brings about the distention of the capillaries, and their subsequent engorgement with blood. This series of events is the *primary* change induced in the liver and is always found in fresh specimens taken from animals in shock. A similar state of affairs is found in the lung (Fig. 3). In cases of profound shock or in animals whose death has been induced by the action of cytost, further changes are to be noted. For example, in the section shown in figure 5, a definite clear area containing blood corpuscles is observed surrounding the distended capillary. This clear area, which has not taken the stain, is due to exeresis of fluid from the capillaries. This outflow of fluid, as well as the presence of blood corpuscles in the clear area, is evidence of the increased permeability of the capillaries.

Such increased permeability has been recognized by many workers in this field. Following the initial work of Dale and Laidlaw (1919), who observed that the administration of histamine to cats caused a rapid concentration of the blood cells, due to increased permeability of the capillaries, it has been assumed by some that traumatic shock is due to the liberation of histamine or a histamine-like substance. This point will not now be elaborated upon, for as will be shown

in the next chapter, the properties of cytost are quite at variance with those of histamine. For the present, the point we desire to stress is that in shock the increased diffusion of fluid from the blood occurs secondarily to the congestion of the capillaries.

With the increased permeability of the capillaries, cytost present in the blood may be expected to diffuse along with other blood constituents into the fluid (lymph) bathing the tissue cells. This consideration is of importance since, as will be shown subsequently, cytost in sufficiently high concentration may exert a distinctly deleterious action upon the various cells with which it comes in contact.

In the experiments so far considered, cytost has appeared to act selectively upon the abdominal viscera and lungs. As will be seen presently, however, this selectivity of action is more apparent than real, for other tissues, such as muscle and brain, are affected by cytost. Such effects, nevertheless, become evident in such tissues only when they are exposed to the direct action of cytost in a concentration considerably greater than is necessary to bring about similar changes in the viscera.

The extreme sensitivity of the viscera to such a tissue toxin is perhaps analogous to the fact that among humans, at any rate, these organs are the most susceptible to disease. Statistical studies of the causes of death have shown that, in the great majority of cases, death is actually due to various derangements of these particular tissues. (Pearl, 1922.)

This is perhaps not surprising, for these organs really represent the actual metabolizing center of the mammal. The skeletal tissues are merely a support-

ing structure endowed with facilities for locomotion, while the nervous system serves mainly as a coördinating mechanism. The latter tissues are, of course, dependent upon the normal functioning of the viscera for their continued existence.

What have the various viscera in common that can account for their ready susceptibility to the action of cytost? While this question cannot be completely answered, a valuable suggestion is found in their common histogenesis. As the reader is aware, in the early stages of embryonic development three distinct germ layers are formed. These are termed respectively the ectoderm, the mesoderm, and the endoderm (entoderm).

Embryologists have been able to follow the subsequent development of these primitive layers into the various tissues of the body. During the course of embryonic development, the innermost layer, or endoderm of the vertebrate, has been found largely to retain its original epithelial character. From it are formed the epithelial lining of the enteron, its auxiliary organs and glands—the stomach, liver, pancreas, lungs, and urinary bladder.

Those organs which are particularly sensitive to cytost have then a common origin, and it is perhaps justifiable to conclude that their peculiar sensitivity is simply that of a primitive germ layer, the endoderm. This conclusion seems further justified when it is remembered that of the three germ layers, the endoderm suffers the least differentiation during development.

The marked sensitiveness of the tissues of endodermal origin was first stressed by the author in con-

What sensitiveness?

nection with his experiments on inflammation of the stomach. At that time, while considering the pathological changes induced by the introduction of caustics and bacteria into the stomach, it was concluded that "it seems that the entoderm is far more sensitive to irritation than the ectoderm."

In the foregoing discussion it has been shown that cytost appears to act selectively on the endodermal cells and thus affects the adjacent blood in the capillaries of the various viscera. When autolyzed tissue is injected into an animal, the pathological lesions produced in the lungs resemble those of other visceral tissues (Fig. 3). Since the capillary network of the air sacs is the most dense capillary plexus of the body, and material may be introduced into the lung without recourse to experimental surgery, the lung appeared to offer an excellent site for the investigation of the localized stasis induced by cytost. To this end, autolyzed tissue was introduced into cats' lungs. The results of these experiments, which are summarized in Table VI (Turck, 1919), were far more striking than could possibly have been imagined.

The experiments were conducted as follows: Fresh lung tissue from healthy cats was autolyzed in a sterile chamber of chloroform vapor for forty-eight hours, when the mixture was found to yield an intense biuret reaction. By means of a tube attached to a respiratory indicator, 0.5 to 1 cc. of such autolyzed tissue suspension was insufflated into the tracheae of normal cats. The animals' increased inspiration aspirated a portion of the material into the finer bronchii, and in many instances, as shown in Table VI, caused the animal's death within four or

Cytost from autolysis.

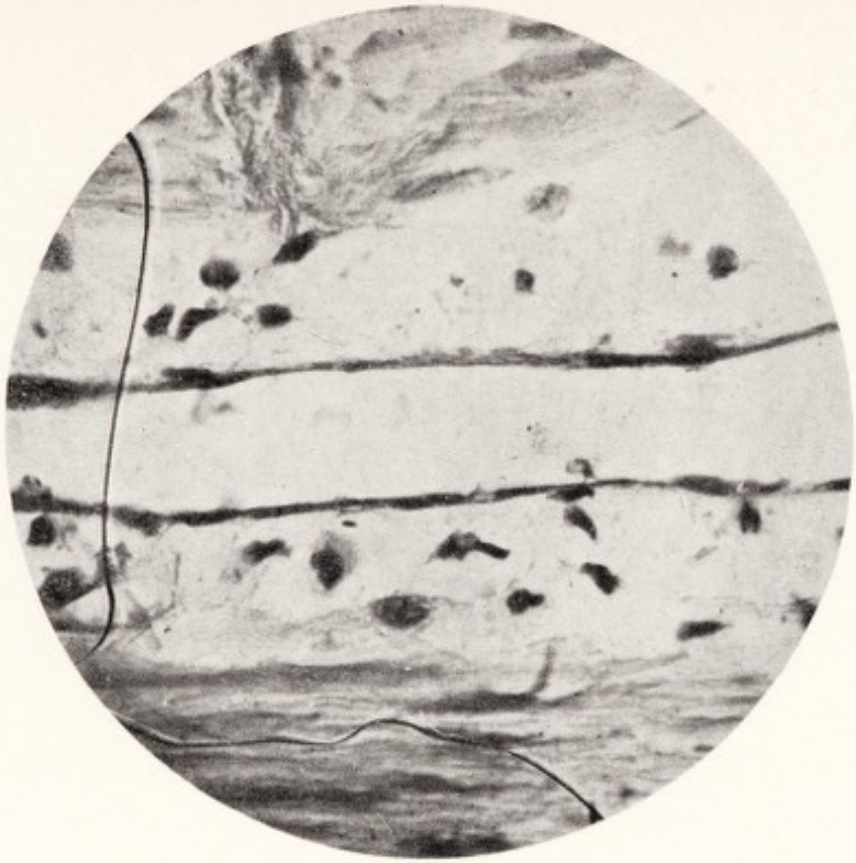

Figure 5.

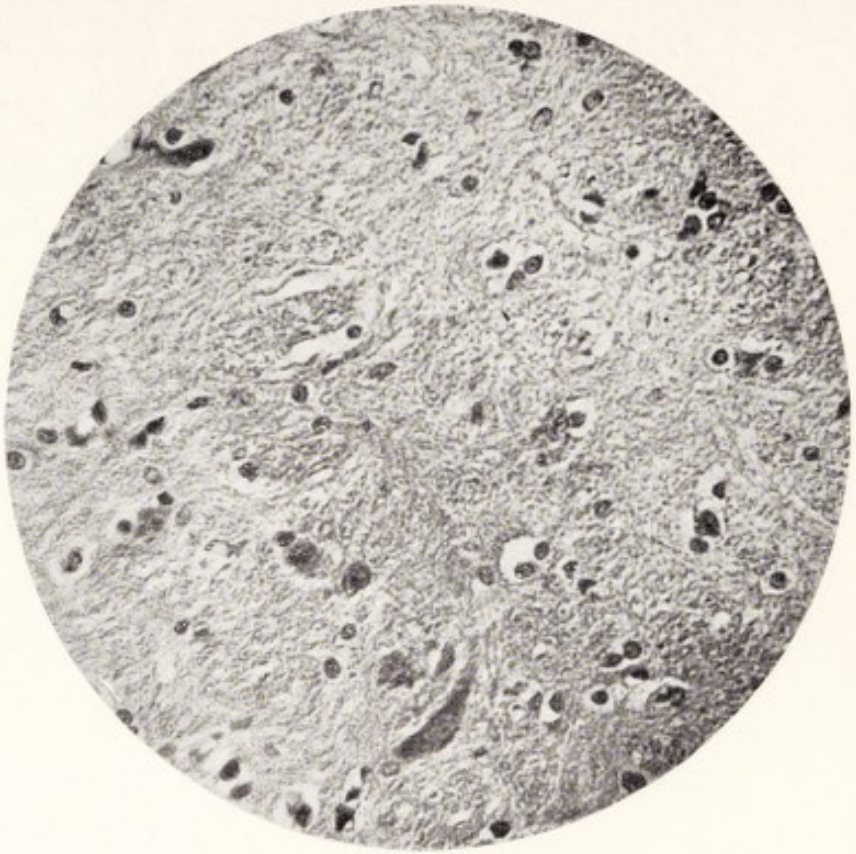

Figure 6.

Figure 5. Section of intestine near pylorus, under high power. Clearly shows exeresis from capillary into perivascular tissue. The latter has been pushed away from the vessel by the outflow of fluid as shown by the clear zone. Some blood cells have extravasated and some may be seen passing through the vessel walls.

Figure 6. Section of brain of cat which died from fatal traumatic shock. Note the absence of any congestion and the normal appearance of the cells.

TABLE VI

Cats Insufflated with Autolyzed Cat Lung Tissue

Cat No.	Time of Death after Insufflation	Time of Examination Made under Anaesthesia	Pneumonic Findings on Immediate Postmortem		Remarks
			Bronchial Type	Lobar Type	
P 1	4 min.	———	———	+	Intense congestion of both lungs.
P 2	3 min.	———	+	——	Lungs appeared soaked in blood.
P 3	5 min.	———	+	——	Liver lobes involved and lungs congested.
P 4	10 hours	———	———	+	Sneezing. Râles
P 5	———	45 hours	+	——	Râles.
P 6	24 hours	———	+	——	Coughing.
P 7	Shock	25 min.	+	——	Resection of lungs.
P 8	15 min.	———	+	——	
P 9	5 hrs.	———	+	——	Profound symptoms from the beginning.
P 10	10 days	———	———	+	Pleuritic effusion.
P 11	8 days.	———	———	+	Coughing and sneezing.
P 12	———	3 days.	+	——	Coughing. Râles.
P 13	3 min.	———	+	——	
P 14	2 hrs.	———	+	——	
P 15	36 hrs.	———	+	——	
P 16	4 min.	———	———	+	Intense engorgement.
P 17	5 min.	———	+	——	Reaction in both lungs.
P 18	Shock	5 min.	+	——	Reaction in both lungs, with hemorrhages.

five minutes. In such instances an immediate autopsy showed the tissue changes to be focal in type, with disseminated hemorrhagic areas, while the entire lung was hyperemic, occasionally being soaked with blood.

In those experimental animals which survived for several hours or longer, there was found classical pneumonitis of the bronchial type, with numerous focal lesions, becoming hard and resistant.

The rapidity with which small quantities of cytost insufflated in the lung cause death is most probably due to the fact that the thin respiratory epithelium allows the toxin to come into immediate contact with the endodermal cells without the necessity of passing through the capillaries. This results in a prompt stasis of blood in the vessels, and consequent death, due to stopping of the heart. That autolyzed lung tissue may actually effect such a stasis was shown by the fact that the intravenous injection of such material caused an instantaneous engorgement of all the lung capillaries.

This is in agreement with the observations previously reported, that autolyzed tissue products formed either *in vitro* or *in vivo,* when introduced into the circulation, cause capillary stasis in the lungs and other viscera. It is of considerable interest that, as revealed at necropsy, the insufflated autolysate caused no such reaction in the trachea or bronchioles, the reaction always appearing to take place in the distal portion of the bronchial alveolar system connected with the surrounding dense capillary plexus. Only when death is delayed does the reaction extend up to

Figure 7.

Lung removed from a cat which died immediately following tracheal insufflation of homologous cytost. This photograph presents a picture typical of that always found in such experiments. The lung is virtually soaked in blood and assumes the blood-red color in place of the normal gray color.

the bronchial mucosa, giving rise to classical pneumonitis of the bronchial type. It follows, therefore, that such an extension is secondary to the primary reaction in the capillary plexus. It is interesting to compare this action with that in the abdominal viscera, where, it will be remembered, the primary stasis was found to occur first in the capillaries, then extending to the larger vessels. These observations lead one to believe that the primary reaction to "shock-toxin," or cytost, is always in the endodermal cells and then extends to the capillary endothelium.

As, conceivably, one might consider the insufflation technique to involve the complicating factor of introducing a liquid into the lung, experiments of another type were conducted. (Turck, 1919.) "A group of ten cats was selected and examined to exclude any respiratory disturbance and then placed in a large cage. The front paws of five of these animals were coated with a thin paste of autolyzed cat lung tissue. The animals were tagged, and placed back in the cage with the other five cats that had not had the application of 'lung paste.' Within two hours, several of the cats began to sniffle and sneeze. Within twelve hours all the cats, those that had had their paws coated and the others in the same cage that had not had the application of paste, had symptoms of involvement of the lungs. Eight of these cats died from pneumonia within 48 hours to one week after the application of the lung paste. Some of the animals showed extensive respiratory pathological conditions. They also showed nasal discharge, eyes reddened, conjunctive discharging and extensive râles over both

lungs. The temperature at first dropped and later rose, but the temperature records were no guide to the degree of lung involvement."

At autopsy, the findings were of a nature similar to those observed in the preceding series of experiments, wherein the autolysate was introduced by insufflation. In agreement with the observations on shock produced by various means, the blood was early found to have a high viscosity and diminished clotting time. This, as we have seen, may be an important factor in the development of capillary stasis.

Results identical with those discussed above were obtained many times with different series of animals and were not therefore due to a chance infection in one particular group. As a control upon these observations, pneumococcus cultures, types I, II, III, and IV, were insufflated into the trachea of cats. No pneumonia was produced, provided "flooding" of the lung was prevented.

In the "cats' paw" series of experiments, the amount of cytost which found its way to the animals' lungs must have been exceedingly small. Indeed, that the amount necessary to elicit a typical lung involvement is minute to a degree was found in another series of experiments wherein the autolyzed tissue was not applied directly to the animals, but was merely sprayed about their cages by means of a nebulizer. Cats kept in such cages were found to develop respiratory involvements in much the same manner as the animals whose paws had been smeared with a paste of autolyzed lung tissue. At autopsy the findings were identical with those recorded in the latter case. (Turck, 1919.)

Incidental to the experiments just discussed, an observation of considerable interest was made. When autolyzed lung tissue from dogs, sheep, beef, rabbits and horses was applied to cats' paws or administered by insufflation, the cats usually failed to show any signs of respiratory involvement. Similarly, intravenous injection of such autolysates failed to provoke the symptoms of shock such as result when a tissue autolysate is injected into an animal of the same species.

These results make it appear that cytost possesses a certain species specificity. This aspect of the problem will be discussed further in a subsequent chapter.

As a result of these experiments we may conclude that the zone of attack of cytost lies in the endodermal cells of the lungs, liver and upper alimentary tract which are in intimate contact with the endothelium of the capillaries. The injection of cytost extracts in such concentration that immediate shock and death do not ensue leads to a more generalized involvement of the body tissues—particularly of the kidney. It has been known for years that a severe nephritis may follow a superficial burn. As has been shown in our previous discussion, burned tissues liberate cytost. If the burned area is extensive, then shock and death may follow promptly. On the other hand, if the tissue damage is restricted the animal may suffer a slow absorption of the toxic cytost. Under such conditions the concentration of cytost in the circulating blood is usually too small to cause coagulation and consequent marked interference with the circulation. Under such conditions, therefore, the cytost is enabled to exert its toxic action on more remote tissues. The kidney dam-

age which becomes evident following severe burns belongs in this category.

In order to study such conditions, areas on the backs or buttocks of animals were anesthetized by injections of novocaine. The muscle and skin were then grasped by clamps and burned to char by the application of a white-hot branding iron for a few moments. A few hours after such treatment the animals passed into shock (Turck, 1918). In Table VII are recorded the time of the onset of shock following such burns.

TABLE VII

No.	Animal	Onset of Shock after Burn	Death, Time after Burn
1	cat	2 hrs.	8 hrs.
2	cat	1½	10 hrs.
3	cat	4	24 hrs.
4	cat	3	63 hrs.
5	cat	5	recovered
6	dog	4	24 hrs.
7	dog	2	10 hrs.
8	dog	2	22 hrs.
9	dog	1½	8 days
10	rabbit	2	6 hrs.
11	rabbit	4	7 hrs.
12	rabbit	2	5 days
13	rabbit	3	recovered
14	rabbit		5 days

The crisp charred tissue was dissected from the burned areas of such animals and pulverized. One-gram portions of this material were then extracted with 10 cc. of water and filtered. This extract was then injected intravenously into normal homologous animals, 1 cc. portions being used for cats and rabbits, and 2 cc. portions for the dogs. By this means a much

higher concentration of cytost was introduced into the circulation than is the case when natural absorption of the burned tissue is permitted to take place. Consequently the onset of symptoms in this series of animals is much more rapid, as shown in Table VIII.

TABLE VIII

No.	Animal	Onset of Symptoms Time after Injection	Death in
15	cat	immediately	———
16	cat	immediately	———
17	cat	5 minutes	2 hours
18	cat	10 minutes	recovery
19	cat	30 minutes	24 hours
20	dog	immediately	———
21	dog	10 minutes	5 hours
22	dog	30 minutes	———
23	dog	immediately	30 minutes
24	rabbit	immediately	———
25	rabbit	5 minutes	6 hours
26	rabbit	4 minutes	recovery
27	rabbit	5 minutes	12 hours
28	rabbit	10 minutes	recovery

At autopsy, all the animals listed in Tables VII and VIII showed the same general pathology as that previously described in the earlier experiments with cytost. However, in this series of experiments special attention was directed to the involvement of the kidneys. Quite striking was the observation that within five minutes after the intravenous injection of the extract of charred tissue, kidney lesions, usually in the form of a glomerulitis, could be detected. This was determined by removal and sectioning of the organ.

It was further observed, as might be expected, that in general the type of kidney involvement varied

with the degree of the burn and the interval of time between the burning and removal of this kidney for examination. While for our present purposes it is not desirable to stress the pathology of kidney lesions, a few additional facts are pertinent to the general problem of the mode of action of cytost on the kidney.

Animals which had been burned and apparently had recovered from such maltreatment were found upon subsequent examination to be suffering from kidney lesions.

The kidneys of such animals showed changes of both the degenerative and inflammatory proliferative type in the glomeruli and approximal tubules.

Since glomerulitis may be observed within five minutes following the injection of cytost, it seems that this is the primary change in the kidney induced by stasis of the blood in the glomerular capillaries. Those animals which survived the injection of the burned tissue extract likewise were found to have suffered degenerative changes in the kidney similar to those induced by direct burns. It seems likely, therefore, that degeneration and secondary proliferation in the areas under consideration are induced by the primary response to cytost. The blood stasis induced by the latter interrupts the normal metabolism of the kidney cells and permits an accumulation of toxic metabolites, which in turn results in further tissue destruction and cellular proliferation. If the latter becomes excessive, then the damage is irreparable.

It is interesting to compare these results with the changes induced in the stomach by the application to the mucosa of emulsions of mustard. In the latter

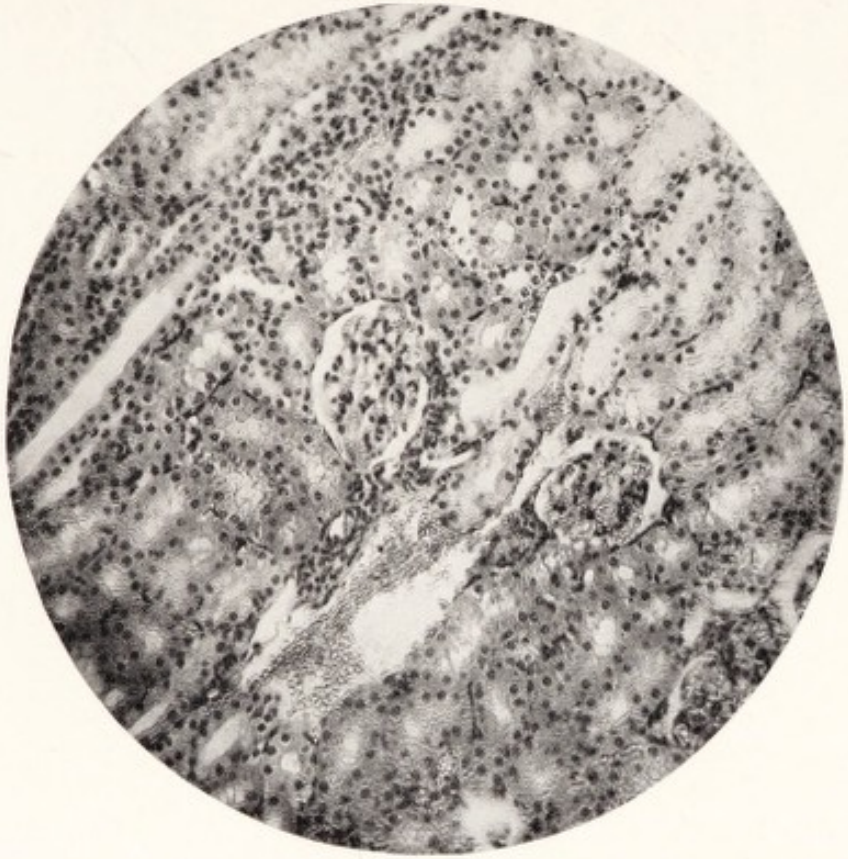

Figure 8.

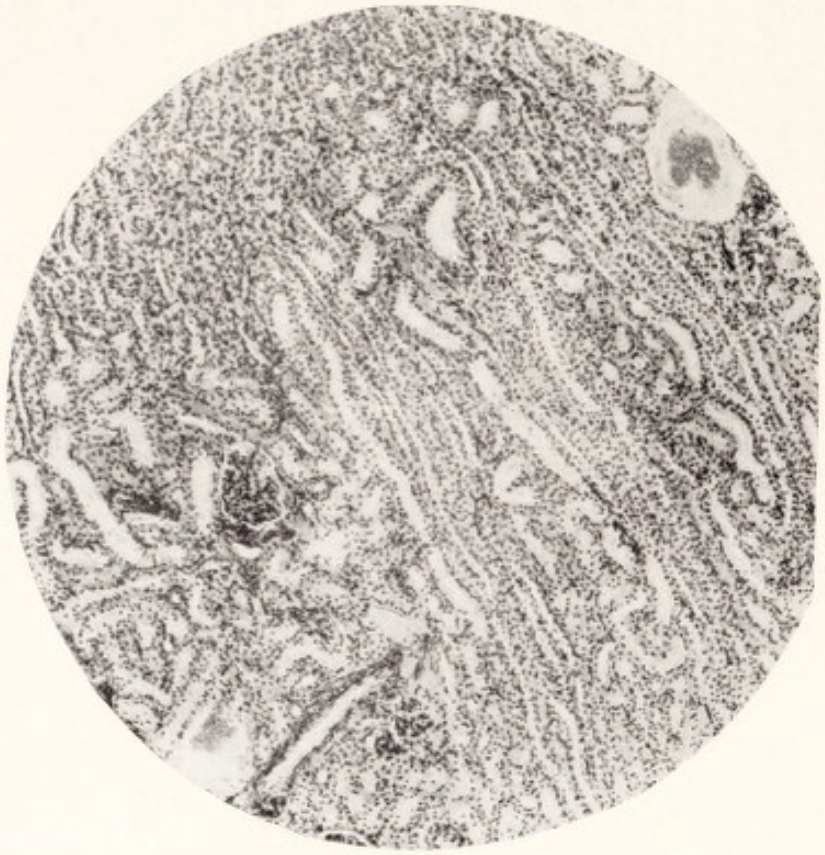

Figure 9.

Figure 8. Section of kidney of cat in which shock had been caused by injection of homologous cytost. Note the hemorrhagic areas, stagnation, and agglutination of blood corpuscles.

Figure 9. Section of kidney from cat in which nephritis had been induced by frequent injections of cytost for a period of two weeks. Shows retraction and destruction of the glomeruli.

experiments, it will be recalled, the mustard first induced merely a hyperemia. This was followed later by degenerative changes and subsequent active proliferation of the cells, as evidenced by the occurrence in sections of many karyokinetic figures. Further, these changes continued to take place even after removal of the mustard, the latter merely acting as a trigger to release the cytost from the mucosal cells in the same fashion that trauma or burning releases it from epithelial and muscle cells.

While burns and other injuries always produce acute lesions of the lungs and liver, these, if not extensive, may clear up rapidly, leaving little or no evidence of any tissue damage. This difference between the other organs and the kidney, wherein, as stated above, cytost may produce a chronic injury, may in part be attributed to the single functional blood supply of the latter, which depends almost entirely on the renal vessels and a few auxiliary anastomoses. During blood stasis, therefore, the kidney is more severely taxed than are the other viscera, which have a double vascular blood supply. Again, the kidney cells are known to be very sensitive to toxic substances, such as the salts of mercury and uranium and various organic compounds. In this respect they resemble the endodermal cells of the liver, lung, stomach, and intestines. Hence it seems probable that the kidney cells may be more easily injured by cytost than are other tissues. Photomicrographs showing congestion of the glomeruli, venous stasis, and clumping of the corpuscles in the region of the straight tubules are shown in the accompanying illustrations.

CHAPTER V

FURTHER EXPERIMENTS WITH CYTOST

THE investigations presented in the preceding chapters have shown that cytost, a product of tissue autolysis, when introduced into the circulation promptly induces a splanchnic stasis which is followed by shock and death. Now such effects are induced only by relatively large quantities of cytost. This is evident from the fact that shock does not follow minor trauma and burns, although from the preceding discussion it should be apparent that such injuries must provoke the liberation of cytost in precisely the same manner as does more extensive tissue damage. Such being the case, it is of interest to follow the reaction of animals to injections of sublethal quantities of cytost prepared by the *in vitro* autolysis of homologous tissue.

The cytost extracts used in the following experiments were prepared from cat's muscle, which was allowed to autolyze in chloroform vapor for 24 hours. The autolyzed tissue was triturated with an equal weight of sterile water, heated to boiling, and centrifuged to remove the insoluble matter. The supernatant fluid was then sterilized by passing it through a Berkefeld filter and used directly for injection.

The toxicity of such preparations was ascertained by injection into the femoral vein of anesthetized cats. When administered in this fashion, 1.5 cc. of such a preparation caused death of the animal within 1 or 2 minutes. As in the other acute experiments which we have considered, immediate autopsy always disclosed marked congestion of the lungs and abdominal viscera. When injected intraperitoneally, intramuscularly, or subcutaneously, such cytost extracts do not cause immediate congestion and death, but, depending upon the dosage, bring about different pathological conditions, as shown in the following experiments selected from many recorded in the author's laboratory notebooks.

No. 73

Male cat, weight 343 gms.

March 29, 1920. Temperature 101.4°. 1 cc. injected intraperitoneally and 1 cc. subcutaneously.
March 31. Similar injections. Temperature 102°.
April 4. Similar injections. Temperature 102.2°.
April 6. Animal died.

Autopsy: stomach congested, dilated; duodenum, congested; intestines hemorrhagic; spleen enlarged; kidneys congested.

No. 80

Castrated male cat. Weight 2980 gms.

March 31, 1920. 1 cc. injected intraperitoneally and 1 cc. subcutaneously. Temperature 101°.
April 4. Similar injections. Temperature 101.6°.
April 8. Sick, temperature 103.8°. Weight 2210 gms.
April 10. Weak, does not eat, temperature 102.5°. Weight 2000 gms.
April 11. Temperature 99°; exit.

Autopsy: stomach, few hemorrhagic spots. Duodenum, congested and atrophied; spleen, enlarged, infracts; liver, very hemorrhagic; lungs, characteristic lesions; kidneys, congested.

No. 86

Female cat. Weight 2900 gms.

April 8, 1920. 1 cc. injected intraperitoneally and 1 cc. subcutaneously. Temperature 101°.

April 13. Similar injections. Temperature 101°. Animal appears to be weak.

April 18. Similar injections. Temperature 100°.

April 19. Died.

Autopsy: Stomach and duodenum show erosions and what appear to be breaking down ulcers; liver, very hemorrhagic; spleen, enlarged; kidneys, congested; lungs, characteristic appearance.

Results similar to the above have been obtained a great many times. Occasionally, however, a cat was encountered which was apparently capable of tolerating such injections of cytost for a much longer period of time. Such animals however eventually succumb to the long-continued action of cytost, and when autopsied are found to exhibit degenerative tissue changes similar to those recorded in the preceding protocols. For example, consider cat No. 89.

No. 89

Castrated male cat. Weight 3250 grams.

April 26, 1920. Injected 1 cc. intraperitoneally and 1 cc. subcutaneously. This was repeated on each of the following dates unless otherwise noted.

May 2, 1920. Weight 3240.
9, Weight 3190.
18, Weight 3300.

May	25,	Weight 3300.	
June	17,	Weight 3490.	
	30,	Weight 2950.	No injections. Cat appears dull.
July	13,	Weight 3070.	½ cc. used for each injection.
	27,	Weight 3130.	½ cc. used for each injection.
Aug.	11,	Weight 3060.	No injections.
	25,	Weight 3500.	½ cc. used for each injection.
Sept.	14,	Weight 3500.	
	29,	Weight 3570.	
Oct.	18,	Weight 3690.	
Nov.	9,	Weight 3910.	
Dec.	7,	Weight 3940.	
Jan.	7, 1921.	Weight 3960.	
	23,	Weight 4010.	
Feb.	6,	Weight 3890.	
	15,	Weight 3890.	
Mar.	5,	Weight 3770.	
April	9,	Weight 3920.	
Aug.	6,	Weight 3240.	
Nov.	19,	Weight 3630.	
May	7, 1922.	Weight 3510.	
	13,	Weight 3640.	
	27,	Weight 3650.	No injections.
Aug.	22,	Weight 3480.	No injections.
Sept.	7,	Weight 3350.	No injections.
	22,	Weight 3480.	
Oct.	1,	Weight 3450.	
	10,	Weight 3490.	
Nov.	9,	Weight 3000.	
	16,	Weight 2990.	
Dec.	11,	Animal died.	

Autopsy: Lungs, chronic lobular pneumonia; heart, pericarditis and dilatation hepatitis; stomach, gastrointestinal catarrh, ulcer all over and in pyloric region; kidneys, chronic glomerulitis.

It will be noted that this animal was markedly resistant to the action of cytost.

As shown in the protocol, this animal (cat No. 89) withstood periodic injections of cytost for a period of more than two years. During this time several different cytost preparations were used, each of which was definitely toxic when injected into the femoral vein of other cats. It follows, therefore, that this animal possessed to a marked degree a natural immunity to the action of cytost. As this was also encountered in several other animals the author was led to attempt the production of an artificial immunity to cytost. This interesting aspect of the problem will be discussed later.

It has been shown previously (Chap. III) that cytost may be liberated *in vivo* by agents which bring about cellular destruction and autolysis. If such processes are induced continuously for a protracted period of time, we should expect to obtain results similar to those following the injection of tissue autolysates. Now *in vivo* autolysis may be accomplished by a variety of means, such as trauma, burns, and the application of caustic chemicals such as mustard oil, ether, chloroform, and alcohol. Of these the last three are most adaptable for prolonged experiments, since by regulating the amount injected one may to some degree control the extent of autolysis. Animals which died as a result of such treatment, or were killed after a number of such injections, quite uniformly were found to have suffered the same organic lesions as those injected with extracts of autolyzed tissues. Such observations confirm the hypothesis that the tissue damage caused by these reagents is responsible for the observed pathology.

The protocols following are representative of many of the author's observations.

Cat No. XII. Male.

March 1, 1919. 2 P.M. Temperature 40°. 5 cc. of ether injected subcutaneously on right side. No immediate effects although animal appears stupid.
3 P.M. Temperature 37°. Lying down, moaning. Remained this way for ¾ hour, then recovered.
March 4. 5 cc. ether injected on left side. In 10 minutes commenced to stagger.
March 8. Thin and weak. 5 cc. ether injected on right side.
March 13. 5 cc. ether injected on left side. Stupor, then coma.
March 14. Found dead.

Autopsy: Liver and spleen, enlarged and congested; omentum, sclerosed, hardened; stomach and duodenum, congested, stained with bile; lungs, red, solid, pneumonic; brain, normal.

Cat No. XVII. Male

Feb. 28, 1919. 2:50 P.M. Temperature 40°. Injected 10 cc. alcohol in each side.
3:00 P.M. Temperature 38°.
March 4. Injected 10 cc. of alcohol on each side.
April 17. Injected 10 cc. of alcohol on each side. Staggered about for ten minutes, then became comatose, remaining so for 8 hours.
April 18. Found dead.

Autopsy: Pneumonic spots, marked congestion; stomach and duodenum, congested, hemorrhagic; liver, very congested, omentum hardened; spleen enlarged, infarct; kidneys, marked congestion; brain, normal.

In such experiments, objection may be raised against the relatively large quantities of the reagents injected into the animals. In order to obviate this, the following procedure was devised. The animal's leg

was ligated and a *small* quantity of the chemical to be studied was injected below the site of the ligature. By such means, free diffusion of the reagent was prevented and the tissues adjacent to the site of the injection received the brunt of the action. By this means, a greater tissue damage and consequent increase in the amount of cytost liberated was achieved, for the oxidation or elimination of the injected substance was prevented. Upon removal of the ligature, the accumulated cytost was released into the circulation and carried to the viscera, which reacted in the manner previously described.

Cat No. XIX.

March 1, 1919. Leg tied off. 3 cc. alcohol injected below ligature. Two hours later ligature released. Leg swollen.
March 7. Swelling of leg has disappeared, animal becoming thin.
March 10. Animal failing rapidly. Skin excoriating over site of injection.
March 11. Found dead.

Autopsy: Lungs, partially consolidated; stomach, duodenum, and liver, markedly congested; brain, meninges congested; heart, ventricles normal.

Cat No. XX

March 1, 1919. Leg tied and 4 cc. ether injected below ligature. Latter removed after 2 hours.
March 4. Leg swollen, animal quiet and stupid.
March 10. Does not eat, thin, weak.
March 13. Found dead, body still warm.

Autopsy: very thin, fat and muscles gone. Lungs, congested, hemorrhagic, red, solid; stomach and duodenum, congested; liver, congested, fatty degeneration; kidneys, congested, fatty; spleen enlarged; brain, normal.

Cat No. LII

June 4, 1919. Leg tied off. Fifteen minutes later injected 1 cc. chloroform. After 1 hour ligature removed.

June 5, 9:15. Respiration rapid and weak. Râles in lungs. Mucous membranes pale. Taken from cage and laid on desk, is relaxed, does not move. Raises head for an instant but cannot hold it up.

10:15. Gasps for breath. Temperature 38.5°. Respiration and heart stopped.

10:20. Autopsy: Lungs, lower lobes engorged, hemorrhagic; liver, stomach and upper intestines congested.

When fluid was withdrawn from the site of the injection and injected into other animals, the latter showed a typical response, as demonstrated in the following protocol.

Cat No. XXII

March 1, 1919. 2:00 P.M. Temperature 40°. Four cc. of autolytic products, resulting from the injection of ether into the leg of another cat, were injected subcutaneously.

2:30. Temperature 38°.

March 2. Eats. Temperature normal.

April 14. Animal very thin.

April 20. Thin, coughing.

April 26. Extremely thin. Constantly coughing and sneezing.

May 6. Does not eat. Staggers.

May 11. Found dead.

Autopsy: Lungs, pneumonic spots, and spots resembling tubercules; liver, congested; stomach and intestines, congested, hemorrhagic areas and apparently ulcers in intestines; kidneys, congested.

Such experiments as these show definitely that cytost liberated *in vivo* by the action of chloroform, ether, and alcohol leads to the same pathological end

as does cytost from autolyzed tissues. In both instances the primary congestion results in damage of the tissues of endodermal origin.

When somewhat smaller quantities of cytost are injected or liberated *in vivo* much the same results may be obtained, but a much longer period of time is necessary before the pathological changes become apparent. In this connection the reader may recall the production of kidney lesions by superficial burns and by the repeated injection of small quantities of chloroform and ether (page 92). Similarly, repeated injections of small quantities of tissue autolysates result in the production of chronic lesions. The results of some experiments of this nature are summarized in Table IX. The cytost preparations were prepared as stated in the preceding protocols.

Aside from the resulting pathology, the marked loss in weight of the experimental animals is interesting. This may be taken as indicative of a lowered metabolism. On the basis of these experiments alone one cannot decide whether this is due primarily to the action of the cytost on the body cells, or to impaired circulation resulting from blood stasis induced by the cytost.

In the writer's experience, gastrointestinal ulcers are rare in cats; hence he is forced to conclude that those found in the animals injected with cytost were due to the prolonged action of the latter on the gastrointestinal mucosa, which, like the other tissues sensitive to cytost, arises from the endoderm of the early embryo.

As is now well known, in the adult animal ingested foods are for the most part utilized as a source of

energy for the organism, a relatively small fraction being used for the repair or replacement of tissues. While for the latter purpose the nature of the ingested fats and carbohydrates appears to be immaterial as

TABLE IX (TURCK, 1921)

Amount Injected	Mode of Injection	Interval	Period	Original Weight	Weight at Death	Loss	Pathology
1 or 2 cc.	Subcut.	2–5 days	6 months	2970	1420	1550	arterial sclerosis
1 cc.	Subcut.	daily	2 months	2335	1180	1155	arterial sclerosis
2 cc.	Intra-peri-toneal.	2–3 wks.	1 year	3960	2550	1410	arterial sclerosis
2 cc.	Subcut.	2 days.	2 months	3980	3090	890	chronic lobar pneu-monia, ulcers, nephtitis.
1 or 2 cc.	Subcut.	4 days.	1 month	3130	1950	1180	pneu-monitis, hepatitis, nephritis, dudodenal ulcer.

well as interchangeable, there is a definite protein requirement. This is necessary, as shown in the researches of Osburn and Mendel (see Mendel, 1924), to supply certain essential amino acids which the organism is incapable of synthesizing from other components of its diet.

During complete starvation, such essential amino acids are not available; hence the animal is forced to draw upon the proteins of some of its body cells in order to satisfy the nitrogen requirements of other

cells. That the body proteins are actually utilized during starvation is shown by the fact that a more or less constant excretion of nitrogen takes place during the period of starvation. Such being the case, it seems likely that during inanition the essential amino acids are liberated by autolysis of the tissue cells. This hypothesis is substantiated by the observations of Casa-Bianchi (1912), who found the histological changes incident to starvation to be similar to those observed during the autolysis of tissues. As we have seen, tissue autolysis liberates the toxic substance, cytost; hence we should expect to find some evidences of the latter's action in starving animals. In agreement with this conclusion, Asada (1919) found the capillaries of the lungs and other organs of fasting rabbits to be intensely congested—a typical "cytost reaction."

When animals are caused to suffer partial inanition (malnutrition), the changes have frequently been found to be more extensive than during total starvation. The reason for this is not clear, although autolysis made necessary by the deficient diet is seemingly an important factor. Some years ago the writer (1907) observed degenerative changes in all the organs of rats which had been kept for a month upon a diet of meat extractives, although control animals fed meat or extract-free meat for the same period showed no harmful effects. When dogs were fed in similar fashion with beef extract containing *B. coli* for from four to six months, the animals died from perforated ulcers of the stomach, or hemorrhage. At the time these experiments were performed the author's interest was directed towards other ends than those now under discussion; hence cultures of the

colon bacillus were added to the diet. However, since this organism is normally found in the alimentary tract and the heart's blood of the experimental animals was found to be sterile, we may for the moment attribute little significance to the presence of the bacilli in the food. As a result of such feeding the dogs were found at autopsy to have suffered multiple peptic and duodenal ulcers, and venous congestion of the liver and kidneys.

Upon histological examination, the liver cells, particularly in the central portions of the lobules, were found to have undergone degenerative changes, while in the peripheral portions numerous small vacuoles were observed in them. Similarly the stomach and intestines showed glandular and vascular changes, appearing edematous, with the blood vessels more or less engorged. The kidneys were also involved; the glomeruli were separated from the capsule, the capillaries were engorged, and the peripheral cells lining the tubules were swollen; in some instances, their outlines had disappeared. In some cases in which the period of feeding lasted for several months, arteriosclerotic changes were found. Judging by the failure of the nuclei to stain properly, all the organs showed evidence of autolysis. In none of the experimental animals could any evidence be found for the existence of a bacteriemia or inflammatory reaction. In consequence it appears that the colon bacillus played a minor rôle in these experiments and that the observed pathology was due to the products of tissue autolysis resulting from partial inanition. If the reader will compare the tissue changes induced by such treatment with those produced by cytost, either injected as such

or produced *in vivo* by the other means previously discussed, he will see that the effects are the same. These experiments therefore support the contention that cytost may be liberated *in vivo* by starvation.

Much the same end may be achieved by feeding animals a complete although unsatisfactory diet. For example, six monkeys were fed small squares of bread fried in cottonseed oil in addition to the usual daily vegetable rations. (Turck, 1917.) All six of the animals kept on such a diet died within twenty-eight to sixty-one days, although other monkeys kept in the laboratory and fed vegetables alone remained in a healthy condition. At autopsy all six of the fat-fed animals presented much the same picture. The splanchnic vessels were markedly congested, and the tissues, especially the liver, were found to have suffered fatty infiltration. Peptic ulcers were found in the pyloric region of the stomach.

Although these changes were induced by a high fat diet, it does not seem likely that the fats *per se* were responsible for the observed pathology. The latter suggest the action of cytost, whose liberation may be accounted for in the following way.

The normal diet of the monkey consists largely of carbohydrate. Consequently when forced to ingest relatively large amounts of fats, these animals, one may well imagine, are subjected to a severe acidosis and, as we have seen, an acidosis in the tissues leads to rapid autolysis and the subsequent liberation of cytost. Further, in these experiments, it seems likely that the fats may have interfered with the digestion and absorption of food; hence the animals may have suffered partial inanition which, as pointed out above,

leads to autolysis. Whichever explanation is correct, the fact remains that the histological examination of the body tissues disclosed marked autolysis of the tissue cells.

This fact is but the expression of a general rule that nutritive disturbances which hinder the normal course of metabolism always result in the degeneration of an animal's tissues. This is seen in the various forms of illness, which are of common occurrence in captive wild animals, and will be discussed more fully in Chapter IX.

The reader is probably familiar with the well known fact that disuse of a tissue, such as a muscle, leads to atrophy and cellular degeneration. With this fact in mind, the writer conducted the following experiments. (Turck, 1906.) The experimental animals were placed in small sterilized cages which prevented them from moving about. Ninety-six guinea pigs and thirty-six rabbits were caged in this fashion and given a normal diet. During the first few months the animals gained in weight, due to deficient exercise. Later, although supplied with sufficient food, they became emaciated. At autopsy, general tissue degeneration and autolysis were found, as well as peptic ulcers in some cases. During the course of these experiments, routine blood examinations were made and those animals showing any evidence of infection were immediately discarded. At the expiration of nine months but six guinea pigs remained alive. At autopsy general tissue degeneration and autolysis were found in all cases, as well as several instances of peptic ulcers.

More than three hundred years ago Borelli demon-

strated that the movements of the body fluids in the capillaries is entirely dependent upon the contraction of the surrounding muscles, and today physiologists are generally agreed that the venous return to the heart is dependent upon this factor. Hence, during enforced quiescence there must be some degree of stagnation of blood in the finer vessels, which prevents both an adequate nutritive supply and the removal of toxic waste products. From the preceding discussion it should be obvious that the former will cause an increased liberation of cytost, while the impairment of circulation must necessarily bring about the exposure of the tissue cells to higher concentrations of the toxin than would otherwise be the case.

In consequence, it is not surprising that the tissue degenerations and pathological findings in animals whose quiescence has been enforced should closely parallel those induced by the frequent injection of sublethal quantities of cytost.

Of all the results obtained in the writer's experiments, the following are the most interesting (Turck, 1921b) : A pair of two months old kittens from the same litter were injected subcutaneously with like quantities of the same cytost extract. One, which we may call "A," was injected once a week; while the other, "B," received the injections daily or every other day. Much to our amazement, "A" gained in size and weight more rapidly than the other, untreated kittens of the same litter, whereas "B" ceased to grow, and suffered a progressive decline in weight. This was accompanied by senile changes, until, after two months of such treatment, the kitten (then four months old) appeared as a decrepit old animal.

Figure 10.

Kitten B, age 4 months. Premature senescence and apparent rachitis induced by cytost injections as described in the text.

The kitten's coat remained undeveloped and it acquired a curiously dejected facial expression. The spine assumed an inward curvature and the bones of the paws did not develop properly. These malformations are clearly shown in the accompanying photographs, taken shortly before the animal's death, which occurred five months after the injections of cytost commenced.

At autopsy arteriosclerosis, general atrophy, arthritis and fibrosis of the kidney, liver, and lungs were apparent. Such tissue changes as these are commonly found in aged animals, and because the factor responsible for these changes is unknown, are usually classified as senile changes. (See Fox, 1923.)

Since in kitten "B" these pathological changes were induced by the injection of cytost, it seems possible that senescence in the metazoans may in part result from the accumulation of cytost. As stated previously, tissue culturists have found that cells appear to be immortal if provided with adequate nourishment and means for the removal of waste products. However, even though the former is provided, cells in culture rapidly degenerate and die if their metabolites are not removed. (See Lewis, 1924.)

While the writer has not encountered another case in which senile changes were induced as rapidly as in kitten "B," he has by repeated injections of cytost in many cats accelerated the onset of senescent changes, as shown by the high incidence of arteriosclerosis in animals so treated. (See Table IX.) In such cases, this results long before death in external changes such as we normally associate with old age. For example, cat 114 (see protocol below) showed graying of the

hair, sclerotic changes in the marginal vessels of the ears, and hemorrhagic zones at the junction of the gums and teeth.

Cat No. 114 (Abstract of Protocol)

Young female, white with black spots

Nov. 9, 1920. Weight, 1750 gms. Injected intraperitoneally 1 cc. of cytost extract prepared by boiling 10 gms. of autolyzed cat heart muscle with 50 cc. of water and evaporating to 25 cc.

Nov. 11, 12, 13. Similar injection. Sneezes, discharge from eyes. These symptoms subsided by the 20th.

Nov. 13–21. Similar daily injections.

Dec. 7. Weight 2015 gms., good appetite but has appearance and actions of animal about to break down. Hence allowed a month for recovery.

Dec. 13. Weight, 1835 gms.

Jan. 9, 1921. Weight, 2270 gms. Appeared normal hence injections commenced again. Used 1:25 extract of autolyzed cat muscle. Injected 1 cc. subcutaneously each day from Jan. 9, 1921, until Feb. 17. During this time the cat progressively gained in weight.

Feb. 17. Weight, 2640 gms.

Feb. 18. In heat, assaulted by tomcat.

Feb. 19–26. Daily subcutaneous injections of 1 cc.

Feb. 27. Weight, 2520 gms. Blood vessels around margin of ear appeared sclerotic. Hemorrhagic zone at margin of gums. Indicates vascular and nephritic changes. Daily injections of 1 cc. continued until

April 7. Aborted two kittens about 4 weeks gone. No injection.

April 8. Aborted another kitten. No injection.

April 10–May 26. Daily injections but no noteworthy changes. Weight, 2420 gms. Apparently the cytost extracts used were too weak. Hence changed to muscle extracts made by extracting 10 gm. portions of autolyzed muscle with 10 cc. of water. 1 cc. of the extract injected daily until

July 7. Unable to climb or jump, very sensitive, resents handling, poor appetite, receding gums and extensive dental caries. Daily injections continued till

Dec. 10. Gray hair becoming more and more pronounced in the patches of once dense black hair. Most marked in tail. Daily injections continued.

Feb. 4, 1922. Weight, 2270 gms. Black hair on head replaced by white hair. Injections continued.

Feb. 8. Weight, 2170 gms. Cat weak, anterior incoördination, eye discharge. Daily injections continued until March 15, 1923.

March 17, 1923. Cat died. Weight, 2050 gms. Autopsy performed five minutes after death.

Autopsy: Lungs, chronic fibrous pneumonia; heart, hypertrophied, dilated; liver, fatty degeneration; stomach, full of erosions, dilated, fibrosis; intestines, fibrosis, erosion, beginning ulcers; kidneys, chronic nephritis; adrenals, enlarged; bones, extremely brittle; brain and cord, congested; aorta and arteries, hard.

In no instance has the writer been able to elicit such degenerative tissue changes by the injection of similar quantities of heterologous cytost—that is, cytost prepared from the tissues of animals of another species. Even cytost prepared from a lion's muscle, when injected in similar fashion into cats, does not lead to any pronounced pathology such as induced by that prepared from cats' tissue.

In various experiments in which parallel groups of animals have been injected with homologous and heterologous cytost, only those treated with the former exhibit such tissue changes as have been described above.

It will be recalled that in the experiments with the kittens mentioned a few pages back, kitten "A," which received cytost injections spaced a week apart, gained in weight more rapidly than its litter mates. This suggests that, in small quantity, cytost may actually be capable of accelerating the metabolism of the tissue cells. In order to test this conclusion a series of mature

cats were periodically injected subcutaneously with a cytost extract prepared from autolyzed muscle as described on page 94. The injections differed from those used in previous experiments only in that the dosage was carefully regulated to 0.25 cc. per kilogram of body weight. The animals were injected for a period of from one to eight months. In each instance the cats gained in weight, while a series of control animals of approximately the same age and weights, kept under identical conditions, did not exhibit any marked increase in weight. Data concerning ten cats treated in this way are summarized in Table X. (Turck, 1921).

TABLE X

Number of Injections	Interval between Injections	Period in Months	Original Weight in Grams	Weight at End of Period	Net Gain in Weight
10	2 weeks	$2\frac{1}{2}$	4230	5355	1125
7	2 to 5 days	1	2880	3140	260
5	2 to 3 weeks	3	2950	3650	700
21	2 to 5 days	4	2180	3090	910
5	2 to 3 weeks	2	2840	3260	420
13	2 to 3 weeks	6	2830	3780	950
15	2 to 4 weeks	8	3000	4020	1020
12	2 to 4 weeks	6	3500	4520	1020
14	2 to 4 weeks	6	3020	4010	990
13	2 to 4 weeks	7	4250	5030	780

Aside from the increase in weight, these animals had a general appearance equalled by but few of the non-treated animals. Their coats were sleek and their muscles firm and free from fat. Likewise, they showed a playfulness and courage which are quite uncommon in old cats. When injured, their wounds healed rapidly without suppuration. Two years after these ex-

periments, seven of the ten cats which had been kept in the laboratory were still healthy and vigorous.

In order to follow more closely the rate of increase in weight of animals so treated a series of cats was injected every four days with 0.25 cc. of homologous cytost extract and the weights of the animals were determined at the time of each injection. In figure 11 data from three representative experiments have been plotted. In each instance it will be noted that the cytost injections led to a marked increase in weight up until the time of the tenth or twelfth injection, after which the animal's weight fell, only to rise again to a value which was maintained despite further injections of cytost.

In these experiments, the small quantity of cytost injected first causes an increase in weight, due presumably to increased metabolic activity; then a reaction sets in which causes a loss in weight although the original level is not reached. This is apparently due to tissue damage caused by an excess of cytost, for post-mortem examination of some of these animals disclosed evidences of chronic involvement of the lungs, and gastritis. However, such damage is slight and appears to be readily overcome by the animal, which then regains its lost weight. The final rise in weight may perhaps be attributed to the production of antibodies against cytost. It seems probable that it is the presence in these animals of the latter, which we may call anticytost, which prevents the development of pronounced pathological changes such as those found in other experiments previously cited. It is interesting to contrast this series of experiments with those summarized in Table IX on page 103. In the lat-

ter, larger quantities of cytost were injected and these led to a rapid decline in weight, and subsequent death.

During the course of the writer's experiments it was

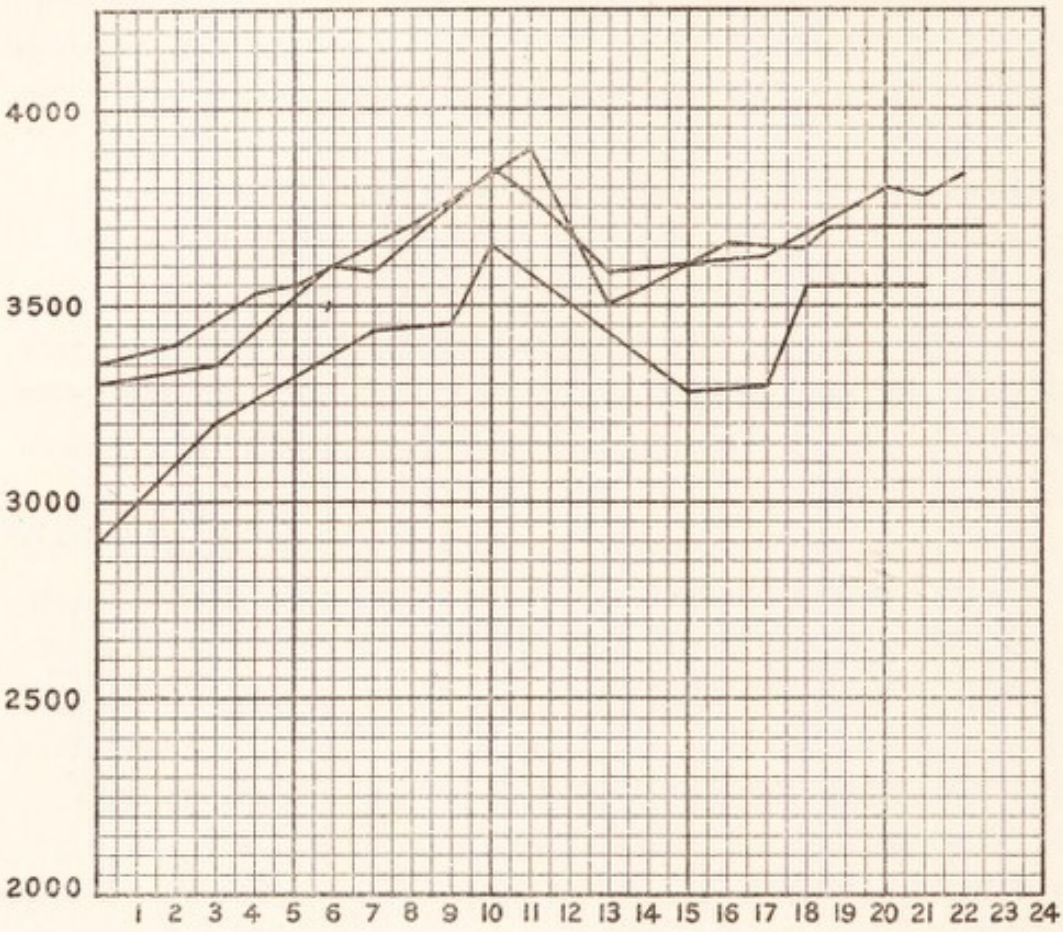

Figure 11. Graphs showing the effects of injections of homologous cytost upon the weights of three cats. The weights of the animals are shown as ordinates and the number of injections of 0.25 cc. of a 10% solution of homologous cytost as abscissae. These injections were spaced four days apart. (From a paper read by the author before Section D of the Brit. Assn. Adv. Sci., Sept. 9, 1930.)

noted that when pregnant cats happened to receive sublethal injections of cytost, they frequently aborted or gave birth to dead or weakly kittens. This led to a brief study of the effects of cytost upon pregnant cats and their offspring.

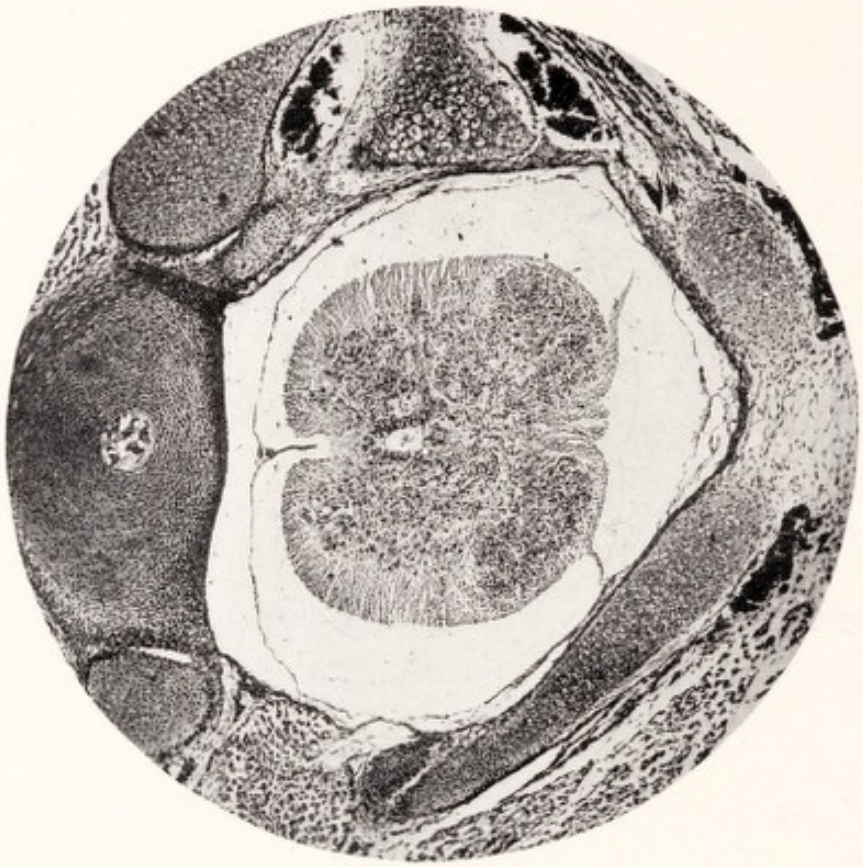

Figure 12.

Section through a pregnant guinea pig which had experienced severe shock. Clearly shows congestion and small hemorrhages in the entire visceral area excepting the brain and spinal cord.

Eight healthy pregnant cats were daily injected intraperitoneally with 1 cc. of cytost. As might be expected from what has been said above, the cats developed various pathological conditions of the viscera which caused the death of two of the mothers. Five of them gave birth to dead kittens, while all but one of the kittens born alive died shortly after birth. Data relative to these experiments is recorded in Table XI.

TABLE XI

EFFECTS OF LARGE DOSES OF CYTOST ON MOTHERS AND OFFSPRING

MOTHERS		KITTENS					
SURVIVED	DIED	DEAD IN UTERO	BORN		DIED LATER	SURVIVED	TOTAL
			Alive	Dead			
1	—	—	3	1	3	0	4
—	1	4	—	—	—	0	4
1	—	—	—	5	—	0	5
1	—	—	—	1	—	0	1
1	—	—	5	—	5	0	5
1	—	—	—	3	—	0	3
1	—	—	3	—	2	1	3
—	1	—	—	2	—	0	2
6	2	4	11	12	10	1	27

From these results it follows that the injected cytost affected the kittens as well as the mothers. That this is entirely possible is shown by the fact that when shock was induced in a pregnant animal, the embryo was found to suffer marked stasis of the blood in much the same manner as did the mother. In figure 12 is reproduced a photomicrograph of a guinea pig embryo *in utero* of a mother which had been subjected to fatal shock. Examination of this section discloses

marked congestion with small hemorrhages throughout the visceral area, although there is no evidence of congestion in the spinal cord. (Turck, 1918b.)

This result indicates that if for any reason a pregnant animal suffers an accumulation of cytost we may expect the fetus to be affected in some adverse way. Experiments which will be discussed in Chapter VIII have shown that such is the case.

Chapter VI

THE ACTION OF CYTOST ON SINGLE CELLS

Some years ago Leo Loeb (1897) observed that excised somatic cells and tissues could be grown in test tubes upon solid media such as blood agar, a method which presented a serious drawback in that the processes of growth and differentiation could not be continuously observed. This difficulty was surmounted by Harrison (1907), who found that isolated bits of nervous tissue taken from frog embryos could be kept alive for many days in a hanging drop of frog lymph. Such a technique permits the observation of the cells by means of a high power microscope, and present day methods of tissue culture are an elaboration of Harrison's method.

In general the technique is quite simple. Under aseptic conditions tissues are excised from an animal and immediately placed in a balanced salt medium, such as Ringer's or Locke's solution. While so immersed, the tissue is cut into pieces a millimeter square, or less. These are then transferred to a sterile cover slip, covered with a drop of suitable medium, and the cover slip is then inverted over a depression in a sterile slide and sealed in position.

After incubation, if the culture medium is suitable, the cells are found to migrate from the periphery of the tissue fragment or explant. This migration takes place only along solid supporting substances; hence blood plasma is usually added to the medium, since after coagulation of the drop, the fibrin threads form a suitable pathway for movement of the cells.

That the plasma *per se* is not essential to the cellular migration but simply offers a convenient mechanical support is shown by the fact that glass wool or spider's web immersed in a fluid medium offers a substitute satisfactory for this purpose. (Harrison, 1914.)

This migration of cells from tissue explants will take place in simple inorganic media such as Locke's solution,[1] although in such instances, because of the absence of suitable organic foodstuffs, the cells do not undergo appreciable proliferation and growth. Such experiments, however, demonstrate that the cells of recently killed animals are alive, and are capable of remaining alive in Locke's solution for some time. The duration of life under such conditions varies with the type of tissue and the temperature. For example, at 37° C. kidney epithelium and smooth muscle may live for ten days or longer, while nerve, and skeletal muscle may be kept alive but a few hours. (Lewis, W. H., and Lewis, M. R., 1924.)

Although migration of cells such as is referred to above increases the area of the explant, no growth as measured by an increased mass of living tissue takes place unless suitable foodstuffs are added to the saline medium. When, however, the composition of the cul-

[1] Locke's solution contains 0.9% sodium chloride, 0.025% calcium chloride, 0.42% potassium chloride, and 0.02% sodium bicarbonate.

ture medium is properly adjusted, a number of the cells which have migrated to the periphery of the explant soon show mitotic division.

As stated above, the presence of blood plasma appears to be of value principally because of its ability to form a solid matrix suitable for the migratory activity of the cells. So far as is known, it will not sustain unlimited growth, and if taken from an old animal may exhibit definitely toxic effects (Carrel and Ebeling, 1923), although when taken from young animals heterologous plasma appears to be almost as satisfactory as homologous plasma for migratory purposes (Burrows, 1910, 1911).

While a large number of substances have been examined with reference to their suitability as a medium for cell growth in tissue cultures, but one substance seems to be completely suitable for this purpose: namely, an aqueous extract of young embryos. (Ebeling, 1913; Carrel, 1913.) By the use of such an extract Ebeling (1922) has succeeded in keeping a culture of chicken fibroblasts more or less indefinitely—for fifteen years at the time these experiments were reported. In the course of this period the original chick embryo cells had passed through some two thousand generations, and no apparent diminution in growth rate was observed. The duration of this experiment was well beyond the average life span of the hen and in consequence offers excellent evidence of the potential immortality of somatic cells, to which we have referred previously.

Just how embryo juice keeps cells in culture alive is unknown. It is obvious that the growing cells demand a source of nitrogen for the development of

their unique proteins; yet when amino acids are added to non-dialyzable portions of embryonic extract, the mixture does not exhibit growth-stimulating properties analogous to that of the undialyzed extract (Baker and Carrel, 1926). On the other hand, Wright (1925) has obtained from extracts of chick embryos an active dialysate which markedly stimulates the growth of cells in cultures.

It follows, therefore, that the growth-promoting principle must be a substance of relatively low molecular weight; otherwise one should not expect it to pass through the collodion membranes used for dialysis. Such being the case, this substance cannot be a proteose, as suggested by Baker and Carrel (1928), who found that peptic digests of albumin and fibrin, in contradistinction to the pure proteins, augmented the growth of tissue cultures. Unlike the embryo extracts, such digests will not allow the tissues to live indefinitely; hence their growth-stimulating properties are more apparent than real.

Presumably such preparations offer the tissues nitrogen in a readily assimilable form, but do not possess some as yet unknown factor which is essential to the continual growth of the tissue. This factor contained in Wright's dialysates may be a stimulant which permits the cells to utilize the foodstuffs available in the media. Carrel (1928), Fischer (1925), and others have observed that embryo extracts lose their growth-stimulating activity if heated above 56° C. and in consequence it has been suggested that the mysterious growth factor is of enzymatic nature, since, as is well known, many enzymes are rapidly inactivated at this temperature.

An alternative explanation may, however, be offered, for at this temperature some of the water-soluble proteins in the extract are coagulated, and it is conceivable that the active constituent of the embryo juice may be absorbed upon the coagulum and thus become unavailable to the growing tissue. This concept is in harmony with Carrel's (1913) observation that the growth-promoting stimulant is removed from the embryo extracts when the latter are passed through a Chamberland filter, which presumably absorbs the active substance.

The conclusion to be drawn from these investigations is simply that cells in tissue culture require, aside from adequate food, some substance which stimulates in some fashion their metabolism and growth. We shall now pass to some related experiments conducted by the author (Turck, 1921).

The reader may recall the author's experiments, in which mustard infusions were introduced into the stomachs of dogs, and that such treatment, if prolonged, results, as stated previously, in severe inflammation of the gastric mucosa and eventual destruction of the mucous membrane and gland cells, although the surface epithelium is not so seriously damaged as are the deeper tissues. This fact, it may be remembered, led to the conclusion that the latter were affected, not by the mustard, but by substances released from the surface epithelium under the destructive action of the mustard. Further histological examination disclosed distinct evidence of mitosis in the affected tissues; hence we are led to conclude that cytost, aside from its toxic action, may actually be a stimulant of cellular metabolism and growth.

Again, when cytost extracts such as those frequently referred to in our previous discussion are injected into animals, the cells at the site of the injection are to a certain extent stimulated to multiplication, as shown by the presence of mitotic figures. This was observed in the following way: A homologous cytost preparation was injected intramuscularly into one limb of a cat, and a like quantity of sterile water was injected similarly into the parallel leg. After the lapse of six hours the tissues surrounding the sites of the two injections were excised, fixed, and sectioned. Upon microscopic examination the tissues surrounding the cytost injections showed distinct evidence of mitosis, such as is seen in a healing wound, while the tissues about the water injection showed no such evidence of proliferation.

Similarly when the heterologous cytost prepared by a rat or rabbit was injected in this fashion, localized mitosis did not take place. Hence these simple experiments offer further evidence of the specificity of cytost. It is interesting to compare these results with the growth-stimulating factor present in embryo extracts where such marked specificity is not shown, for Fischer (1925) has found that duck fibroblasts grow well in chicken embryo extract, while Carrel and Ebeling (1922) report that the tissues of the rat may likewise be grown in the same medium.

In view of these results, it became of interest to examine the effects of cytost upon cells in tissue culture. Quite unexpectedly, cytost added to hanging-drop cultures of various tissues was found to exhibit two effects: in very low concentration it stimulates the cells to growth in a manner similar to that of chick

embryo extract; in higher concentration, it exerts a definitely toxic action, in some instances completely inhibiting growth. (Turck, 1921.)

For the purposes of these experiments, the tissues were first grown in the usual blood plasma chick embryo extracts. In this fashion 34 cultures of chick cells and 25 cultures from a human fetus (about 3 weeks old) were prepared. As needed, transplants were made from these stock cultures to hanging drops of the media under examination. The cytost extract used in the following experiments was prepared by autoclaving 10 grams of autolyzed tissue with 10 cubic centimeters of water.

Bits of tissue were cut from the outgrowth of the stock cultures and placed momentarily in Ringer's solution, which served to wash away the media, and then transferred to a drop of homologous plasma on a cover slip. In the first series of experiments a minute quantity of cytost was added to the medium. This was accomplished by touching the plasma drop with the tip of a platinum needle which had been immersed in the cytost extract. After the cover slips had been sealed onto hollow ground slides, the cultures were incubated at 37.5° and transplants to similar media were made every 48 hours. After several days the cultures made in pure plasma showed very little growth, while the same tissues in plasma plus homologous cytost grew very nicely.

This was not an isolated result, but was duplicated in 308 successive transplants of both human and chick tissues. Hence we may safely conclude that cytost is capable of stimulating cell growth *in vitro*. The tissue fragments which did not grow in pure plasma

were not dead, however, for when transplanted to fresh plasma containing a trace of cytost they commenced to show growth, and after a few transplants in such media they underwent active proliferation and apparently could be carried along in such a medium indefinitely.

In one instance after the tissues had been grown in plasma plus a trace of cytost for eighteen successive transplants, bits of the tissue were implanted in pure plasma and in the pure cytost extract. In this case the tissues in the latter medium died within two days, while those in the pure plasma remained alive and grew slightly although not so well as cultures made with plasma and a trace of cytost.

This experience led to an investigation of the effects of higher concentrations of cytost upon tissue cultures. To this end transplants were made from the stock cultures to a medium consisting of two drops of homologous plasma mixed with one drop of homologous cytost. After incubation for 48 hours these transplants were found to be dead. This result was obtained a number of times and definitely shows the distinct toxic action of cytost when present in sufficient quantity. The lethal effect cannot be attributed to simple dilution of the plasma, for Carrel and Burrows (1911) have shown that a threefold dilution of plasma actually accelerates the growth of tissue cultures.

Now the interesting question arises: Will heterologous cytost behave in the same manner as the homologous extract? To answer this, the above experiments were repeated, using chicken cytost with human tissues and human cytost with chick tissues. Transplants from the stock cultures, originally obtained from the

human fetus, were made to human plasma containing a trace of chicken cytost, and after 48 hours such preparations were compared with control cultures containing human cytost. In each instance growth was apparent, but the cultures made with chick cytost did not show so much growth as the controls, from which it may be concluded that heterologous cytost is not so effective a growth stimulant as a similar preparation from homologous tissues.

This conclusion was substantiated by experiments made with chick tissues in a medium of chicken plasma plus traces of human cytost. These differences between homologous and heterologous cytost are much more clearly shown by the fact that when chick tissues were transplanted to a medium consisting of two drops of chicken plasma and one drop of human cytost, the resulting cultures underwent growth, although the latter was slight compared to growth obtained in control cultures made simultaneously. This result is in marked contrast with that obtained with chicken cytost in equivalent concentration. In the latter case, as stated above, the cultures were killed.

Thus we arrive at the interesting conclusion that whereas heterologous cytost has not the growth-stimulating potentialities of homologous cytost, it also does not exert the toxic effects characteristic of the latter in high concentrations.

The stimulating effects of tissue extracts upon tissue cultures have been in part confirmed by Drew (1922, 1923), who found autolyzed tissues to possess a growth-stimulating principle analogous to that found in embryo extracts. It will be noted, however, that the growth-stimulating substances present in the

cytost preparation differ from those in the embryo extracts in that they are not thermolabile, for, as stated at the beginning of this section, the extracts used by the author were autoclaved, a procedure which inactivates embryo juice.

This difference was made more strikingly apparent in the following way. It will be recalled that the author was able to obtain the typical systemic effects of cytost when extracts of charred tissues were injected into cats. Such being the case, it became of interest to investigate the action of such extracts upon tissue cultures. To this end 10 gram portions of tissue were heated in a crucible at 300° C. until a black ash was obtained. This ash was extracted with 1 cc. of water and filtered to remove the insoluble material, the resulting solution being used as "ash cytost" in the experiments described below.

As in the previous experiments, transplants of chick tissues were made from the stock cultures to a drop of homologous plasma and a trace of the "ash cytost" was added on the tip of a platinum needle. So far as could be judged, the cultures thus prepared grew as well as control cultures made with plasma and unashed cytost. Again, when transplants were made in a mixture of two parts of plasma with one part of the "ash cytost," the cultures were found to be dead at the end of 48 hours. When similar experiments were conducted with chick tissues and ash cytost, prepared from human tissues, decidedly different results were obtained. The heterologous ash cytost did not appear to augment growth appreciably, but it did not kill the culture, as was the case with the extract of homologous ash.

These rather startling results tempt one to speculate as to the possible nature of the growth-stimulating agent present in the ashed tissues. At first one might be led to believe that simple inorganic salts were responsible for the observed effects. This seems somewhat attractive, in view of the experiments of Loeb and Blanchard or amoebocyte tissue. They found that various inorganic salts exerted a marked influence upon the migration of cells from experimental amoebocyte tissue. The concentrations used by these authors, however, was considerably greater than could possibly exist in the cytost-plasma media used in our experiments. Further, in the present state of our knowledge it is difficult to conceive of any mechanism whereby inorganic salts would exert such a specific action as that observed in the tissue culture experiments. One is therefore forced to an alternative hypothesis: that the ash cytost contains some markedly heat-resistant organic compound which is not completely destroyed during the ashing procedure.

Admittedly this concept seems a wild stretch of the imagination, but no other postulation seems to fit the empirical results of the experiments. As far as the writer is aware, no organic compounds of biological importance can withstand the temperature attained during ashing, although my friend Dr. K. C. Blanchard informs me that he has encountered some synthetic organic compounds such as tetra substituted ureas and aromatic silicon derivatives which do not suffer appreciable decomposition at such temperatures. Of course these substances are of no biochemical interest, and are simply referred to here to illustrate the possibility of the existence of organic compounds

which are markedly thermostable. Be that as it may, we are at present in complete ignorance of the nature of the growth-promoting substance in cytost, and ash cytost, and must therefore content ourselves with the results of the experiments.

As was stated towards the beginning of this chapter, various tissues will remain alive for some time but will not grow in Locke's solution. In consequence it became of interest to ascertain whether or not the addition of cytost to such a medium would permit growth to take place. To this end bits of muscle from the thigh and heart of a chick embryo were carefully washed in Ringer's solution and then implanted in a hanging drop of Locke's solution with the addition of a minute quantity of chicken cytost on the tip of a platinum needle.

After two days in the incubator these cultures showed very little growth. They were, however, transplanted again to a similar medium, and controls were made in Locke's solution without the addition of cytost. After 48 hours the latter transplants showed practically no growth, while the cultures made in the Locke-cytost medium exhibited a small but distinct growth.

These cultures were then divided in two, one set being carefully washed in Ringer's solution to remove cytost, and then implanted in Locke's solution; while the second half of the cultures was transferred to the Locke-cytost medium. After two days' incubation, the latter were found to be growing nicely, whereas the former, washed free of cytost, showed but a very meagre growth. Upon again transplanting these cultures to their respective media, the cultures made with-

out cytost showed no signs of growth, whereas those made with the addition of cytost showed a satisfactory growth, although, because of the lack of suitable supporting structure, it was not so good as in controls made with blood plasma.

These experiments make it clear that even in such an unfavorable environment as Locke's solution, cytost may exert its growth-accelerating properties. Similarly in such a medium cytost may, if its concentration be too high, exert a definite toxic action comparable to that found in the plasma cytost mixtures. This was demonstrated experimentally by implanting bits of actively growing cultures in a mixture of one drop of Locke's solution and one drop of chick cytost. Such cultures failed to grow, and unlike those made in Locke's solution alone were found to be dead after 48 hours' incubation.

The lethal action upon tissue cultures of cytost in high concentration is perhaps not of any especial significance other than that it shows that a product of cellular disintegration may be toxic to contiguous normal cells—a fact in harmony with the numerous experimental results quoted in preceding chapters, which have shown that cytost introduced into the intact animal or liberated *in vivo* by a localized injury results in damage of the body cells, particularly those of endodermal origin. On the other hand the stimulation of cell growth by cytost is of very considerable interest because a number of statements found in the literature confirm the idea that cells influence the growth of one another by the liberation of some soluble substance. Thus, although many workers have attempted to culture single cells *in vitro* they have

quite uniformly met with failure. Witness for example the remarks of Harrison (1928): "It is a very interesting and at present inexplicable fact that single somatic cells isolated in culture media do not proliferate. Experiments to this end in my own laboratory some years ago but not published did not succeed and other workers have reported similar experience. As Fischer put it,"A colony of fibroblasts cannot arise from a single cell even when the nutrient conditions are most favorable. Likewise small groups of cells if isolated do not undergo division and their growth remains at a standstill. On the other hand certain tumor cells (Rous chicken sarcoma) are capable of multiplying and producing colonies when isolated singly."

It seems, therefore, that in order that a somatic cell may undergo division it must obtain suitable stimulants from its neighbors. In this connection, the most careful observations seem to be those of Haberlandt (1919–1922), who, working with plant tissues, found a direct correlation between the size of the transplant or the number of cells within it, and the frequency of mitoses. In consequence this investigator concluded that some substance arising from the injured cells, which he termed "division hormones," incited the intact cells to division. This is in direct confirmation of the writer's hypothesis—Haberlandt's division hormones being synonymous with cytost.

Similarly, other names have been applied to the growth-stimulating substances present in tissue extracts. They have been termed "trephones" by Carrel (1924), "desmones" by Fischer (1925), and "archusia" by Burrows and Johnson (1925). Since, how-

ever, the exact nature of these substances remains unknown, these various names are of no descriptive value. Therefore the writer prefers to retain the name "cytost" simply because it was the first one coined to describe this mysterious substance.

In a large mass of cells such as the transplants used in tissue culture it is inevitable that some suffer injury from one cause or another. In consequence, by the mechanism previously discussed cytost will be liberated from such cells. It seems likely, therefore, that the growth observed in cultures made in pure Locke's solution may be due to the stimulating action of the cytost so derived.

In agreement with this concept is the fact previously mentioned on page 130, that transplants of single cells, even in apparently suitable media, do not undergo division and growth. Further, an enormous number of papers have been published which present evidence that the crowding of tissues in cultures exerts a decidedly beneficial effect upon their outgrowth. (See Fischer, 1923.) This again may be attributed to the action of cytost derived from some cells upon other members of the colony.

In view of the experimental results obtained with vertebrate tissues, it became of interest to investigate the effects of cytost upon unicellular organisms such as protozoans and bacteria. A survey of the literature concerning cultures of such organisms discloses a number of facts which may be interpreted in a manner similar to that used to explain the empirical observations on tissue cultures. Thus, Robertson (1921), working with *Enchelys farcimen,* found that the division rate of this organism in a hanging drop is sig-

nificantly increased when two animals are present in the same drop. Similarly, Calkins (1926), working with *Uroleptus,* found the division rate to be inversely proportional to the number of protozoans introduced into a given volume of culture medium.

From these experiments it seems likely that these microscopic animals release a substance which accelerates the growth of the same species. With *Paramecia,* variable results have been obtained by different workers (reviewed by Allee, 1931). In part, this seems to be due to differences in experimental technique, although the recent work of Petersen (1929) has thrown considerable light upon the factors which influence the growth of this ciliate.

By alterations in experimental procedure Petersen has been able at will to obtain results which confirm or negate Robertson's conclusions, for she has shown that the volume of the medium employed is a decisive factor in the final results obtained. For example, she found that when the volume of media was slightly less than 1 cc., the division rate of individual *Paramecia* was markedly accelerated by the presence of other animals of the same species, whereas in larger volumes of fluid, the organisms were without apparent influence upon one another. This fact, of course, may be due simply to a dilution effect, for if a given concentration of the growth stimulant derived from a few animals is necessary in order to accelerate the division rate of others present, it is evident that simple dilution of the pericellular fluid will lower this concentration below the necessary minimum.

It has long been recognized that in mass cultures of protozoans, some metabolites are excreted into the

medium, rendering the latter unfavorable for the continued growth of the organism. In the case of *Paramecium,* this has been carefully investigated by Woodruff (1911, 1914), who has also demonstrated (1913) that the toxins which are elaborated in mass cultures are more or less species specific, for he finds that media from such cultures, which is incapable of supporting further growth of *Paramecia,* is satisfactory for the cultivation of *Stylonychia.* Robertson (1924), in discussing his experiments with *Enchelys,* states that "the ultimate cessation of reproduction in old cultures is attributable to the accumulation of a product of growth, and possibly of the same product that was originally responsible for the acceleration." This conclusion of Robertson's is similar to that advanced by the writer (Turck, 1921), to explain the action of cytost on cells of all types.

In order to obtain cytost from *Paramecium,* the organisms were grown in large numbers in small dishes for 10 days. The growths were then centrifuged, washed, and dried on a steam bath. The resulting mass was autoclaved with 10 times its weight of water and filtered in precisely the same fashion as that utilized for the extraction of cytost from other tissues. In order to ascertain the effects of such cytost upon the growth of *Paramecia,* six or eight of the latter were transferred from the stock cultures to each of several small tubes containing 1 cc. of water. Some of these tubes were then inoculated with paremecium cytost by adding as much of the dried organisms as could be held on the tip of a platinum needle.

After twenty-four hours at room temperature, the animals in the tubes containing the cytost were found

to have undergone an increase in number greater than that found in the control tubes to which no cytost had been added.

When this result had been confirmed several times, the experiment was repeated but the ash obtained by ignition of the dried animals was substituted for the cytost. In water containing traces of such ash the *Paremecia* were found to multiply more rapidly than in the control tubes, but the increment in number of the control cultures was nowhere nearly so marked as in the previous experiments with the dried animals.

Since, as is well known, traces of various mineral salts may markedly affect the behavior of protozoan cultures, it is impossible to state definitely that the ash cytost possesses any specific ability to accelerate the division rate of such organisms. On the other hand, the unashed material, when present in traces, exerts a distinct stimulation of growth as measured by increased numbers of organisms. Further, this effect is more or less specific, for when cytost prepared from chicken, rat, or human tissue was substituted for the *Paramecium cytost,* no such increase in division rate was noted.

As was found to be the case with tissue cultures, the growth-stimulating principle in the dried ciliates is water-soluble. This was shown in a series of experiments in which the 10% extract referred to above was used. Four or five *Paramecia* were transferred to tubes containing 1 cc. of tap water. A loopful of the cytost solution was added to several of these tubes and the remainder were kept as controls.

After 48 hours at room temperature, the number of organisms in each of the tubes containing *Paramecium*

cytost was found to be significantly greater than in the controls. Incidental to these observations, it was noted that the protozoans in the water containing cytost were considerably more active than those in plain tap water.

In order to examine this further, hanging drops containing a single *Paramecium* were touched on the periphery with a platinum needle which had previously been immersed in cytost solution. Under such conditions the single animals became very active, darting back and forth across the field. After approximately ten minutes, the magnitude of these movements diminished, shortly assuming a circular path whose radius became progressively smaller until finally the animals simply rotated on their own axis. Controls in drops of tap water did not exhibit such behavior.

It follows, therefore, that some component of the cytost influences the movement as well as the reproduction of *Paramecium*.

When a loopful of the aqueous cytost extract was added to a few drops of water containing several *Paramecia,* a similar behavior was noted. Following the onset of the circular movements, the axial rotation ensued. This became progressively slower, and, after the lapse of an hour and a half, the organisms assumed a spherical form resembling a cyst. It seems likely that this behavior, perhaps the beginning of of encystment, represents a response to the unfavorable environment created by too high a concentration of cytost in the aqueous medium. Similar results were obtained when the ash of *Paramecia* was substituted for the cytost extract, but not when heterologous cytost was employed in similar concentration.

From these experiments, each of which was re-

peatedly confirmed, we may conclude that homologous cytost affects protozoans in a manner similar to that found in tissue cultures: i.e., in very low concentration, it appears to exert a definite stimulation of activity and growth, whereas in higher concentrations it becomes distinctly toxic. The latter conclusion is further substantiated by the observation that the addition of large quantities of homologous cytost to hay infusion (a loopful per cubic centimeter) renders the medium unsuitable for the cultivation of *Paramecium*.

When we turn to the growth of bacteria, we find that an enormous literature has accumulated concerning the requirements for and the nature of bacterial growth. Owing to the non-uniform nature of the media employed in experiments concerned with such studies, and to numerous conflicting statements as to the results obtained, it is virtually impossible to draw any definite conclusions concerning the general nature of bacterial growth.

Two undisputed facts, however, are worthy of comment in connection with our discussion. It is now quite generally accepted that, following the inoculation of a bacterial culture, a definite time interval (the lag phase) may elapse before active proliferation begins.

Various hypotheses have been advanced to explain this phenomenon: some believe that during the lag period some substance essential for growth is liberated into the medium from the cells of the inoculum; others have imagined that the lag phase represents a period during which the organisms recover from injury caused by the accumulation of toxic metabolites in the parent culture. Penfold (1914) has suggested that a time period is necessary for the synthesis of certain

substances which must attain a definite concentration in the cells before active growth ensues. These and other explanations have been reviewed by Buchanan and Fulmer (1928), and we shall not therefore elaborate upon these ideas.

If, as suggested above, some accelerating substance is necessary for the rapid growth of bacteria, they can, if supplied with adequate food and kept in an optimal physical environment, synthesize this substance for themselves. This becomes self-evident when one recalls that cultures of bacteria may be obtained from a single bacterium isolated by the micro-pipette method first introduced by Barber (1904). In this respect, then, the bacteria differ from the somatic tissues of higher animals, which, as found in tissue culture experiments, demand a source of preformed cytost, trephone, archusia, or whatever one prefers to call the growth-accelerating substance.

This is not surprising, considering the relatively undifferentiated nature of bacteria and their lowly position in the scale of life. Nevertheless, it is of interest to ascertain if cytost prepared from bacteria is capable of accelerating the growth of such organisms.

For this purpose the author selected *Bacillus coli communis,* since, in the course of another investigation in 1912, he had accumulated some 80 grams of the dried organisms, which had been grown in mass culture on solid synthetic media. As the colonies had been removed from the surface of the latter by scraping, the mass of organisms was undoubtedly contaminated with some of the media. This unfortunately could not be avoided with the facilities at the author's

disposal. In consequence, in the experiments to be cited presently, the possibility exists that the observed effects were in part due to an enrichment of the media by this means. This objection, however, seems of doubtful significance, in view of the fact that the amount of additional nutriment introduced into the medium along with the cytost must have been of a negligible order.

Cytost was prepared from the dried *B. coli* mentioned above by autoclaving 1 gram of the dried organisms with 10 cc. of water. Plates were poured with 5 cc. of nutrient agar to which had been added 0.1 cc. of the aqueous extract of dried *B. coli*. These plates and controls were then inoculated with a standard loopful of a 24 hour broth culture of *B. coli*. After 24 hours, the plates made with the bacterial cytost showed a much better growth than the controls. Similar results were obtained using 0.15 cc. of the *B. coli* cytost per 5 cc. of medium. It seems therefore that although cytost is not essential to the growth of these organisms, its presence in the medium accelerates the growth of the latter.

Similar concentrations of *B. coli* cytost added to cultures of staphylococcus and streptococcus in nutrient media failed to cause any observable increase in growth; hence the specificity of cytost is apparent in even such lowly forms as the bacteria.

Chapter VII

NATURAL RESISTANCE AND FATIGUE

All forms of life from the lowest bacteria to man are so constituted that they will live only within certain definite limits of environmental changes. While it is true in many instances that the interval between such limits may be rather wide, it is well known that organisms live normally and longest only under optimal environmental conditions. This is a necessary consequence of the operation of the mechanism of natural selection, which has permitted the survival of only those individuals which are more or less adapted to the particular environment wherein the development of a given species has taken place. This has been due to the fact that natural selection operates according to the laws of chance, rather than purposefully, to produce and maintain a race of organisms best fitted to survive and reproduce its kind.

The various races of animals and plants resulting from such a process are therefore not ideally adapted to their environment, but only better adapted to it than their ancestors. By a similar process the constituent body cells of the metazoans and higher plants have become adapted to a definite *internal environment* which is comparatively constant for a given

species when the external environment of the organism is not permitted to undergo marked changes. Conversely, external changes will result in internal variations which tend to compensate for the former and thus to a degree prevent serious impairment of the body cells. This type of compensation is well illustrated in the warm blooded animals, which have developed during the course of evolution a temperature-regulating mechanism which permits them to maintain a body temperature very nearly constant throughout a wide range of external temperature variations.

Similarly all living organisms have developed to a certain degree various mechanisms which enable them to suffer environmental changes which do not vary too far from the norm to which they are best adapted, although in some few instances this inherent adaptive ability is exceedingly elastic. In general, individual cells such as protozoans and the cells of higher animals in tissue culture demand for their normal activities a rigorously constant environment, while higher animals, due to the compensating and interrelated activities of their component tissue and organs, are much better able to withstand marked environmental changes such as those of temperature, radiation, and oxygen pressure.

In many instances such ability is sufficiently pronounced to permit an actual acclimatization to a sudden shift in environment. To this end, living beings are provided with certain defense mechanisms which as a rule do not become apparent until called upon to defend the organism against an environmental change or a sudden new environmental factor which

the individual has not been previously forced to contend with. As a rule such defense mechanisms do not appear instantly when needed, but are developed as necessary from some latent source when the organism is appropriately stimulated by an environmental factor. As a simple instance of this in man, let us briefly consider the phenomena incident to sunburn. We are all familiar with the fact that after a winter indoors the exposure of our skin to the bright summer sun for a sufficiently long period usually results in a painful sunburn. If, however, we submit to a series of such exposures, of lesser duration, the skin becomes "tanned," after which prolonged exposure to the sun does not result in a burn. This is due to the fact that while our skin contains more or less pigment which hinders the absorption of solar radiation, the concentration of this pigment ordinarily is not sufficiently great to prevent serious consequences from a single prolonged exposure. On the other hand, the series of short exposures necessary to elicit a good tan stimulates the formation of an increased amount of the protective pigment within the skin. In this way, then, the human body can protect itself against highly intense solar radiation. Due to the slight amount of pigment normally present within the skin, all humans possess to some degree a natural resistance to sunlight, but, as the phenomena of tanning shows, this natural resistance may be intensified by the suitable stimulation of pigment formation—i.e., of a latent natural resistance.

The development of callus of the skin as a protection against a mechanical injury may be analyzed in a similar fashion. Our skin affords us a certain

degree of protection from the elements. Although this is by no means a perfect protection, nature has endowed the dermal cells with the ability to fortify themselves, and consequently the more delicate underlying tissues, when properly stimulated. To this end continual exposure of the skin to mechanical friction results in the formation of callus. The reader will readily recall other obvious examples of such devices, by which an animal or plant is enabled to cope with limited variations in an environment to which it is not perfectly adapted.

It will be noted that whereas the mechanism of the increased development of resistance to sunburn is readily understood on the basis of the increased formation of pigment, no such simple explanation is forthcoming to account for the formation of callus. In the majority of cases, the mechanism underlying an observed increase in natural resistance is not in the least obvious. Similarly, while individual variations in natural resistance are apparent at all times, we have little exact knowledge concerning the underlying causes of such variation. To illustrate: When a series of animals receive identical wounds inflicted by the same means and in the same manner, the various individuals respond differently. In some, the wounds heal uneventfully; in others, they may heal slowly; and in some individuals shock and other untoward disturbances are experienced. To what may such differences in reaction be attributed? We may answer, to individual differences in natural resistance. Quite obviously, we cannot logically postulate a natural resistance to the particular type of wound inflicted, or the particular device employed to produce the injury.

In consequence, the observed variations in the response to the wounds must be due to variations in the natural resistance of the animals to wounds and the sequelæ thereof.

In this connection it is interesting to note that, as the author and others have observed, battered street cats are usually better subjects than pampered house cats for laboratory experiments which involve surgical procedures. May we not conclude from this simple observation that because of his fighting existence and frequent bloody encounters, the alley cat's natural resistance to traumatic injury has been significantly raised above that of his brother, the protected house cat?

As has been shown in the previous chapters, the author's experiments upon shock produced in various ways have all led to the conclusion that cytost, a product of cellular disintegration, is the causative factor primarily responsible for the train of physiological changes which follow severe injuries. It seems, therefore, that individual differences in susceptibility to shock—i.e., natural resistance to shock, must be inherently due to differences in susceptibility to cytost —a concept in harmony with our observations relative to the differences in response which normal animals show upon injection of extracts of autolyzed tissue.

As has been stated above, an animal's natural resistance to a given environmental factor may be enhanced by appropriate stimulation by that factor at an intensity lower than that required to inflict severe injury. In the case of sunlight, the development of colored pigments in the skin is a tangible explanation of the increased resistance of the animal. On the other

hand, if the pigments so developed were colorless, but absorbed light of the near ultra-violet portion of the spectrum, we might be equally well protected from the sun; yet in all probability we should be in a quandary as to the mechanism by which increased resistance to sunlight is accomplished. Similarly, one is mystified by the vast majority of changes to which many animals, and man, are resistant or may become acclimatized. The observed resistance or acclimatization is usually attributed to natural resistance or adaptation—admittedly and necessarily vague terms.

However, although we may not completely comprehend the underlying mechanism by which increased natural resistance takes place, it seems necessary to postulate that such a physiological mechanism is absolutely essential to life.

While the tissue culture experiments previously referred to have shown life to be potentially immortal if provided with an adequately controlled environment, the existence of animals and plants in their natural environment is sooner or later terminated by death, natural or otherwise. This is necessarily so, because an organism's ability to increase its natural resistance towards all sorts of changes is not unlimited—if it were, we might reasonably expect all animals to be immortal.

The study of disease has disclosed a number of important facts concerning natural resistance, or immunity, as this is termed by medical men.

Following Pasteur's classic experiments on immunization to the virus of rabies, and Behring's discovery of diphtheria antitoxin, it became apparent that some organisms, notably the bacteria, when introduced into

the circulation of higher animals, were capable of eliciting the production of specific antibodies. The latter by one means or another afford the host some degree of protection against the invading parasites. In general, the antibodies which are formed in response to an infection are specific towards the species of microörganism which caused their formation. Thus, it is common knowledge that an attack of measles during childhood usually renders an individual immune to further infection by the causative organism; yet no immunity towards other infectious diseases results. In this instance the antibodies formed appear to last throughout the remainder of the host's life. If one wishes, this may be regarded as an adaptation of the host to a new environment—i.e., to one wherein the chances of infection with measles is ever present.

In many instances, antibodies produced during the course of disease are either negligible in amount or rapidly disappear from the body of the patient following his recovery. Witness, for example, the all too frequent recurrence of colds, grippe, and so on, in some individuals. Despite the fact that in such infections specific antibodies are not demonstrable, we nevertheless find many individuals who seldom are so afflicted even although they have been exposed to the same predisposing causes as their less fortunate brothers. Classical immunology offers no explanation other than natural resistance or natural immunity for this commonly observed fact. While such terms may suffice to indicate the observed differences, they denote no definite causative factor.

In order to effect a satisfactory explanation of such

observed instances of natural resistance, we must seek further than the antibodies of classical immunology. It is hoped that the reader will not misunderstand the above statement. We do not wish to deride the results of the brilliant investigators who have contributed so much to the conquest of disease and the advancement of our knowledge concerning the manner in which higher animals protect themselves against their invasion by more lowly forms of life. It is our desire rather to stress the fact that while the formation of specific antibodies towards invading organisms affords an animal some measure of protection against infection, other physiological factors are perhaps of equal importance in this respect.

As is well known, many animals are resistant towards diseases which ordinarily affect man; and conversely, man appears to be immune to some diseases of animals, although careful study has failed to disclose the existence of specific antibodies in the immune individuals. In consequence, the conclusion is forced that factors other than specific antibodies must be invoked to explain such differences in susceptibility. Judging from the remarks of such prominent investigators as Manwaring (1929) and Zinsser (1928), the immunologists themselves have recently staged an aboutface in the interpretation of such confusing facts.

According to the tenets of classical immunology, only specific antibodies should be capable of combating the invasion of higher animals by bacteria or other toxins. In recent years, however, there has been accumulated a considerable volume of evidence which shows that some infections may be overcome, at least

in part, by the injection of non-specific (i.e., heterologous) proteins which, so far as is known, bear no relation whatsoever to the bacterial products (see review by Petersen, 1928).

For example, numerous clinical investigators have reported that they have obtained encouraging results in the treatment of arthritis by the intravenous injection of divers substances, such as dead typhoid bacilli, or sterile milk. Such substances obviously bear no relationship to the causative factor in arthritis; yet in some mysterious way they are able to stimulate the animal's resistance towards the primary factor responsible for the pathological condition. The very nature of the treatment indicates that this increase in resistance is brought about in a non-specific fashion: that is, in some manner other than by the elaboration of antibodies specific for the invading organism causative of arthritis.

With these facts before us, it is of interest to examine their possible relationship to some observations of the writer concerning the nature of natural resistance.

Many years ago, in connection with the experiments previously mentioned wherein emulsions of mustard were introduced into the stomachs of dogs, at frequent intervals for a period of some months, the animals gradually developed a tolerance for the markedly irritating substance. This was evidenced by the fact that after several weeks of such treatment the animals failed to expel the stomach contents following the introduction of the mustard. While this observed fact may appear quite remarkable, it is of interest to note that it is analogous to the distinct tolerance which certain Oriental and tropical peoples have developed to-

wards the highly spiced condiments which they consume daily.

The development of this tolerance towards the introduction of mustard into the stomach naturally raised the question as to whether the animal's increased resistance towards this substance was localized in the gastric mucosa or was a general resistance to be found in all the body tissues. This problem was solved in part by the simple expedient of applying ordinary mustard plasters to the skin of the animal. By this procedure it was observed that coincident with the tolerance to mustard developed by the stomach tissues, the external application of mustard in the form of a plaster did not result in the usual vesicant effect. From this we may conclude that the tolerance developed towards the introduction of mustard into the stomach is due to a generalized increase in resistance towards this substance (Turck, 1905). Although the exact nature of this resistance has not been elucidated, it seems clear that no specific antibodies were produced, since all the evidence accumulated by immunologists shows that only proteins are capable of acting as antigens for the production of specific antibodies (Wells, 1925); while the irritant principle present in mustard is known to be a volatile oil of non-protein nature. Such being the case, the observed results must be interpreted on other grounds.

In an earlier chapter, evidence has been presented which indicates that the general toxic effects produced by the introduction of mustard into the stomach may be interpreted as due to the liberation of cytost from the mucosa cells injured by direct contact with the irritant. The tolerance to mustard developed by the

stomach might be accounted for by postulating the development of an increased resistance of the mucosal cells to the action of mustard. While such a concept would suffice to explain the development of an increased tolerance towards the introduction of mustard into the stomach, it would in no manner account for the development of a generalized resistance towards mustard, as evidenced by the experiments with the plasters. Consequently, we must seek further for an adequate explanation of the facts.

If it be accepted, as appears reasonable, that the mustard liberates cytost from the cells of the gastric mucosa, we may develop an hypothesis along the following lines. Let us assume first that the vesicant action of mustard when applied locally to a given tissue takes place in the following manner. At the site of contact the mustard injures a number of tissue cells which in turn liberate cytost. The latter, upon diffusing to the underlying cells, subjects them to its action, and so on. Eventually the capillaries are reached, and, as has been shown previously, their permeability is increased by the action of cytost. Because of this increased permeability, there is an exosmosis of fluid into the surrounding tissues, and bleb formation takes place. Now it is to be noted that in this chain of events the factor leading to vesication is the peripheral liberation of cytost and its subsequent action upon the underlying tissues and capillaries.

Since divers chemical substances can behave as vesicants, it would appear that in general their actions are similar to that postulated above for mustard. Indeed, that vesication of the skin is due to a product liberated by the tissue cells themselves is shown by the beautiful

experiments of Sir Thomas Lewis (1924), who has demonstrated that in young subjects wheals may be formed on the skin by firmly stroking it with a blunt instrument. The wheal formation is preceded by a red *tache* which indicates a dilation of the capillaries of the skin. These observations of Lewis offer irrefutable evidence that some product liberated from the injured tissues is responsible for the observed increase in capillary permeability and the subsequent localized edema.

Lewis and Grant (1924) believe the substance which is liberated from the tissues to be of a histamine-like nature because they found that a similar wheal formation follows the introduction of histamine into the skin. It may be remembered that some investigators have reached the conclusion that traumatic shock is the result of the liberation of histamine by the injured tissues; hence, on this basis, there exists a distinct relationship between shock and vesication. In this respect, then, these results are in agreement with the author's cytost theory, the only essential difference being the names applied to the tissue breakdown product responsible for the observed physiological changes. For the reasons given previously, the writer prefers to adhere to the name cytost until the exact chemical nature of the substance is known.

To return to the problem of the immunity to mustard found in the animals studied by the writer, it seems obvious that the resistance developed is not really an increased tolerance for this substance, but is rather the expression of an increase in resistance of the body cells to the substances liberated from the tissues by the action of mustard. That is, during the

course of the experiments the animal's resistance to cytost must have been raised.

These experiments, made many years ago, demonstrate that it is possible to increase the resistance of an animal to the breakdown products of its own tissues and open the way to a fascinating field of investigation,—the production of such an increased resistance by various experimental methods. This problem is of importance because in the battle between animals and their environment, the former are subjected to many conditions which tend to cause tissue damage and the consequent liberation of cytost. If the animal is not more or less resistant to the action of the latter, he will be subjected in a greater or lesser degree to the various ailments which, as has been shown in previous chapters, may be induced by the parenteral introduction of cytost. If such be the case, the animal will be less suited than his more resistant brothers to cope with the environment which nature presents to him. It follows, therefore, that an animal's resistance to cytost is a factor of considerable importance in determining his so-called natural resistance. In consequence, if one is enabled to raise an animal's resistance to cytost, it follows logically that we shall in some degree be able to raise his natural resistance. In other words, we may be able to increase the adaptation of a given animal to his environment and thus in some measure cause his earthly struggle for existence to be easier and, perhaps, more pleasant.

Resistance to fatigue may be regarded as one important aspect of natural resistance, for obviously the more easily an animal is fatigued by his daily routine, the less is his ability to cope with his environment.

Similarly, when a given organ is fatigued, it is rendered incapable of performing its allotted physiological functions in a manner prerequisite for maximal efficiency. Many years ago the writer reached the conclusion that numerous gastric disturbances might be attributed to fatigue of the digestive organs. In particular, let us consider the digestive disturbances and accompanying pain due to failure of the stomach to undergo the usual cycle of contractions and relaxations which are necessary for the complete mixing of ingested foods with the digestive enzymes of the gastric juice secreted by this organ.

Protein foodstuffs are digested in the stomach by the enzyme pepsin, and such digestion is only possible when the enzyme is brought into intimate contact with its substrate. In the normal animal this is accomplished by the churning of the stomach contents, which is brought about by the more or less rhythmic movement of the stomach walls. The lack of such movement also fails to cause ready movement of the stomach contents through the pyloric valve into the small intestine. Such being the case, the afflicted individual suffers from both faulty nutrition and physical discomfort varying from an uncomfortable sense of fullness to distinct pain. If long continued, these distressing sensations prevent the individual from living a normal physical life, and conceivably the continued discomfort may lead to a mental state which prevents the individual from having normal relations with his neighbors—an important factor in one's environment.

Every clinician has encountered patients whose principal source of complaint may be traced to gastric disorders similar to that outlined above, a condition

not difficult of diagnosis, for the salient characteristic is the atonicity of the stomach. Believing such atonicity to be due to the fatigue of the gastric musculature, the author injected intravenously into dogs extracts of material taken from the stomachs of fasting patients suffering from atony of the stomach. The animals receiving such injections exhibited a slight distress, and the results suggested the presence of a fatigue toxin in such stomach contents, although it must be admitted experiments of this type are not very convincing. Because of this, the stomach musculature of normal dogs was fatigued by means of a rapidly revolving cable introduced into the stomach by means of the gyromele, or by recurrent tension brought about by the alternate inflation and exhaustion of a rubber bag inserted in the stomach (Turck, 1903). Both these methods result in a stretching and relaxation of the muscles, treatment which von Uexküll found to be the most effective method of stimulating muscle by mechanical means. In the intact animal such treatment does not result in a rapid fatigue of the muscle, because the firm external abdominal muscles must be fatigued before the gastric muscles can be stretched sufficiently to cause a distinct state of fatigue. If, however, as in the experiments cited below, the animal's abdomen is opened, the continued intermittent tension which is caused by either of the two methods mentioned above causes the gastric muscles to show signs of fatigue, such as marked dilation, and failure to respond to stimuli which ordinarily cause rhythmical contractions of the organ.

The muscles of such stomachs were removed by dissection, minced, and extracted with normal saline so-

lution. Upon injection of such an extract into the gastric musculature of animals whose stomachs had not been fatigued, they rapidly developed signs of fatigue dilations, beginning within about half an hour following the injection, and progressing until the stomach had undergone distention to a volume some three or four times its normal size. This was accompanied by a loss of tone, and complete loss of irritability. These effects were not due to the volume of saline injected but must have been caused by some product extracted from the fatigue muscle, because, upon injecting a like quantity of saline into the stomach musculature of other animals, similar results were not obtained.

Further evidence for the presence of a "fatigue toxin" in the tissue extracts was found by injecting such extracts into the peritoneal cavity of normal dogs. Animals so treated developed an intense thirst some eighteen hours following the injection, but vomited water or milk as soon as taken into the stomach. Upon opening the abdomen two days after the injection of the toxin-containing extract, the stomach was found to be dilated and flabby. In another similar experiment, following the injection of the muscle extract the animal was fed bread, milk and bismuth, and examined by the X-ray, as described by Cannon (1902). In such experiments it was found that inhibition of gastric peristalsis began shortly after the injection; and three or four hours later it was found that the movements would cease entirely for a time, and then continue sluggishly. Such results were not obtained when an extract of unfatigued muscle was substituted for the extract of the fatigued muscle.

These experiments, the details of which were published almost thirty years ago (Turck, 1903), demonstrate that fatigue of the gastric muscles gives rise to the formation of a toxic entity capable of causing changes similar to those associated with fatigue in the muscles of other animals. Since such a fatigue syndrome may be produced by the parenteral introduction of the toxin into other animals, it follows that if this same toxic substance, or another substance of similar nature, is produced elsewhere in the body in sufficient quantity, then a similar reaction of the stomach is to be expected.

The reader will recall, perhaps, that in our discussion of shock produced by anesthesia it was shown that under prolonged anesthesia the stomach was found to become distended, flabby, and insensitive to stimuli. This was shown to be due to the action of cytost liberated by the action of the anesthetic upon various cells of the body. At the time the experiments upon the stomach, cited above, were made, the author's cytost theory had not been developed to the extent presented in our previous discussion. In view of the latter, however, it now appears that the "fatigue toxin" extracted from the stomach muscles is to be regarded as synonymous with the cytost liberated from tissues by the various means detailed in the preceding pages. If it be remembered that, as shown previously, the visceral organs of endodermal origin are peculiarly sensitive to the action of cytost, one can readily account for the behavior of the stomach under the conditions of these early experiments.

If the fatigue toxin formed in the musculature of the stomach by the experimental means cited above

is identical with cytost, we should expect to find histological changes similar to those observed in other tissues when induced to discharge cytost by any means. Such is actually the case, for microscopic examination of bits of muscle taken from the cardiac and pyloric ends of the fatigued stomachs showed some stasis, congestion, and distention of the capillaries. These changes appeared much more marked in the pyloric end of the stomach than in the cardiac end, a difference of some interest, inasmuch, as demonstrated by numerous observers, only the pyloric end of the stomach shows definite peristaltic movements.

Under higher magnification it was observed that the cytoplasm of the cells stained poorly and appeared to be less granular than that of normal muscle cells. Similarly, the nuclei did not stain well with methylene blue, as is the case in normal tissues. (Turck, 1903.) These observed histological changes are distinctly analogous to those observed in other instances of beginning cellular autolysis, which we have shown to be prerequisite for the liberation of cytost.

These observations are substantiated by the observations of Gilman (1903) on the nuclear changes incident to the fatigue of striated muscle.

From the above discussion it will be seen that muscular fatigue involves the production of cytost which in turn tends to inhibit the action of the muscle. This conclusion must not be interpreted as meaning that muscular fatigue is caused solely by the accumulation of cytost resulting from muscular activity. The recent remarkable advance in our knowledge of the chemistry of muscular contraction would nullify any such interpretation of the facts; for quite obviously

the exhaustion of available phosphagen, or any other substance essential to the activity of muscle, would likewise lead to fatigue. Similarly, the accumulation within the muscle of any toxic metabolite such as lactic or carbonic acids would likewise lead to fatigue. Fatigue due to the latter substances has long been recognized, but, as Bainbridge (1919, page 79) has recently pointed out, neither of these substances "ever attains during exercise such a concentration in the blood as to be directly toxic."

Vincent and Sheen (1903), and Bayliss (1918), have found that when injected into an animal a neutral extract of muscle may cause a loss of tone of the arterioles. This action, as has been shown previously, is a typical cytost reaction, and is of interest in connection with the earlier investigations of the author.

During violent muscular exertion such as rowing or running, the circulatory and respiratory increases necessitated by the increased muscular activity usually cease shortly after the muscular exertion has stopped, although other evidences of fatigue may persist for some time afterwards. This is perhaps due to the accumulation of cytost during the period of exercise. In this connection it is of interest to note that upon reviewing the available literature Bainbridge (1919, page 186) has been unable to find any evidence that metabolites formed as a result of muscular activity have any part in causing a fatigue of the central nervous system. This is of interest in the present discussion; for, as has been shown in earlier chapters, the parenteral introduction of even large quantities of cytost does not lead to any involvement of the central nervous system.

Nowadays it is universally recognized that regular exercise is necessary to insure a normal functioning of an animal's organs and their efficient coördination in bodily activities. Further, college and professional athletes after proper training engage almost daily in the most violent forms of exercise, and not only do not suffer any serious consequences, but seem to be actually benefited by their exhausting performances. Indeed, there is a prevalent impression that the mortality rate among athletes is lower than among non-athletic individuals. While a few statistical studies of college men have appeared to indicate the correctness of this opinion, we cannot seriously consider such data, because as a rule the college athlete is at the outset a selected individual—selected for his healthy and vigorous constitution. Admitting this source of error in such studies does not in any way invalidate the common knowledge that properly regulated exercise is distinctly beneficial to any animal.

At first glance this appears to be paradoxical in view of what has already been said concerning cytost and its possible relation to muscular fatigue. In the past much mystery has surrounded the mechanism by which exercise results in beneficial effects to the organism. While the increased blood flow through the various organs results in a more rapid removal of metabolites and a better nutritive supply, this cannot be the sole beneficial factor; otherwise violent anger with its concomitant increase in circulation should be as stimulating to the organism as is exercise.

During exercise an increased metabolism takes place, the energy liberated in excess of the body requirements being utilized to do the external work

demanded by the nature of the exercise, such as rowing a boat, or throwing a ball. Studies directed toward this end have demonstrated that with cessation of the physical activities, the excess metabolic activity is utilized in connection with the repair and synthesis of tissue; and the latter may and usually does take place to a greater extent than is warranted by the tissue substance loss during the period of exercise. This is attested to by the fact that an individual's muscular development is conditioned by the extent to which his muscles have been used in past performances.

Conversely, the disuse of muscles leads to atrophy.

The manner in which functional activity causes an increase in growth and incidentally in natural resistance has up to the present been shrouded in mystery, although the writer feels that at least a partial explanation of the observed facts may be effected as follows. Let us recall the facts demonstrated previously: that (a) cytost in low concentration stimulates the proliferation of cells in tissue culture; (b) cytost in too high a concentration brings about the death of cells exposed to its action ; (c) severe muscular fatigue is accompanied by an accumulation of cytost.

From the last it follows that cytost is liberated by the muscle cells as a consequence of their activities incident to contraction. If it be imagined that the amount of cytost liberated during muscular activity is determined by the extent of such activity, it becomes apparent that if formed in the proper amount the cytost so liberated should by analogy to the tissue culture experiments stimulate the growth of the cells of the muscle. In this manner we may account for

the growth stimulus induced by functional activity. Continuation of this line of thought seems to indicate logically that aside from mere muscular development, the general benefits of exercise upon the body as a whole may be traced to the action of cytost; for even though exercise brings about the liberation of cytost in the muscles alone, the substance may be carried to the other tissues of the body by means of the circulation; and if, as postulated above, the concentration of cytost in the body fluids is of such an order of magnitude as to cause an increase of metabolism of the cells bathed by these fluids, there will result a general increase of the animal's metabolism and growth, for each is an integration of the chemical activities of the constituent body cells.

If there is any truth in this concept, one would expect that the introduction of small quantities of cytost into the circulation of animals must result in an increased metabolic activity and general salutary effects akin to those resulting from regular exercise. Now this is precisely what has been observed in the author's laboratory when very low concentrations of cytost in the form of extracts of autolyzed tissue are injected into cats for a protracted period of time. If the reader will refer to the results tabulated on page 112 and summarized in the graph on page 114, he will note that as the result of such a series of injections the experimental animals underwent an increase in weight considerably greater than did the control animals of the same age group on a similar diet. When, as shown in the experiments just referred to, the cytost injections were continued for a much longer period of time, the animals suffered a decline in weight, and

eventually pathological changes more or less similar to those found in animals receiving large doses of cytost over a shorter period of time.

This result is of importance because it demonstrates that the tissue cells of the intact animal behave towards cytost as they do towards other alterations in their immediate environment. As is well known, tissues in general demand the presence of certain essential elements and compounds in their surrounding fluids. In general, the mere presence of these essential substances does not fulfil the needs of living cells. In order that the cells may live a normal life, the concentration of the essential elements and compounds in the surrounding fluids must be within certain optimal limits. When present in a concentration less than or greater than the optimal, such essential substances exert definitely toxic actions. This has been definitely shown by the exhaustive investigations of Jacques Loeb and others on the phenomena of salt antagonism.

Similarly the writer's investigations concerning the effects of cytost upon cells in tissue culture have shown that while the presence of this substance appears necessary for the growth of cells under such conditions, an excess of cytost rapidly inhibits growth and may actually lead to the death of the cultures.

From these results it may be reasoned that in the intact animal the normal functioning of the body cells is in part conditioned by the concentration of cytost. Such being the case, it follows that as a result of proper exercise the tissue growth which results from the liberation of cytost must be due to the fact either that cytost does not accumulate in the body fluids to such an extent as to manifest toxic actions;

or, if it does, its actions are counterbalanced by the development of factors antagonistic to cytost—i.e., by the development of an anticytost.

The name "anticytost" implies an antibody capable of overcoming the toxic actions of cytost. While it is true that we have been unable to demonstrate the presence of such an antibody by the usual methods of serology, a considerable mass of experimental evidence has been accumulated which substantiates such a concept. This evidence will be considered in detail in the succeeding chapter.

If for the moment the reader will accept the concept of anticytost, we may proceed a little farther with the discussion of some of the aspects of fatigue. If, as suggested at the beginning of this chapter, muscular fatigue is in part due to the accumulation of cytost in or about the muscles, it follows that, other things being equal, an animal whose body fluids contain an appreciable amount of anticytost will fatigue less easily than will an individual less well equipped to combat the toxic effects of cytost,—that is, having available less of the specific antibody necessary to counteract the toxic effects of cytost. Such being the case, it follows that since regular exercise leads to the development of a certain degree of resistance to fatigue, it must likewise lead to an increased production of anticytost; for only by this means could a resistance to the fatigue-causing cytost be developed.

As a corollary it follows that one reason a relatively inactive animal is readily fatigued by a moderate physical exertion is that his body cells have not developed a sufficiently marked resistance to cytost to enable them to withstand the sudden onslaught of this

substance from the active muscles. By proper training, however, such a resistance is developed; for the gradual liberation of small quantities of cytost by appropriate exercise may be considered to stimulate the formation of anticytost and thus endow the individual with an appropriate mechanism for combating fatigue.

On this basis one is enabled to account for the frequently remarked fact that oftentimes individuals of perfect muscular development are less able to withstand certain types of physical exertion than are those of distinctly less prowess. On the basis of what has been said above, distinct muscular development as seen in ex-athletes and captive wild animals depends upon past performances which have stimulated the development of the muscles, while resistance to fatigue depends upon the immediate presence of anticytost in sufficient quantity. Thus, an ex-athlete or captive animal may, due to forced inaction, fail to stimulate the formation of sufficient anticytost to overcome the fatiguing effects of what otherwise might be regarded as a relatively simple physical task. From these considerations it is seen that there is not necessarily a correlation between physical development and resistance to fatigue.

Before leaving this discussion, one more factor must be considered: the results attending complete physical inaction. Such results are well seen in individuals afflicted with some pathology which has caused a degeneration of some portion of the motor nerve paths—such, for example, as infantile paralysis, or, in experimental animals, section of the motor nerves passing to a limb. In consequence of such in-

volvement, nervous impulses are not carried to the affected limb and the muscles receive no stimuli from the central nervous system. Therefore, unless moved by external manipulation, the muscles of the injured member participate in little or no movement, and after a time they undergo degeneration or atrophy. Consequently the injured limb may dwindle to a size, measured in terms of tissue mass, considerably less than that of the opposite limb. The essential point to be observed here is that inaction leads to atrophy. No factor other than enforced inaction is necessary to bring about atrophy; it may be accomplished simply by splinting a limb in one fixed position for a few months.

Just why the degenerative changes described by the word atrophy should be caused by muscular inaction has never been entirely clear in the past, although it usually has been attributed to the impairment of circulation consequent to inaction. This is due to the fact, as first suggested by Borelli in the seventeenth century, that the venous return of blood to the heart is owing not to the heartbeat, but to the extrinsic circulatory mechanism of the veins; for their muscle contractions, by exerting a pressure upon the underlying veins and capillaries, force the blood by virtue of the unidirectional valves towards the abdomen and trunk. When the muscles are completely inactive this process cannot proceed at all; or when partially inactive, the magnitude of the venous return to the heart is seriously diminished. There follows then, as the case may be, either a complete or a partial stagnation of blood in the tissues of the immobilized part. Such being the case, the tissues suffer a lack of foodstuffs and

oxygen, and an accumulation of metabolites, including any cytost which may have been formed by their activities.

Since under the conditions specified above the activities of the tissues have been minimized as a result of inaction, the cytost arising from this source cannot in all likelihood attain a sufficient concentration in the body fluids to be of serious consequence. Nevertheless, the faulty nutrition of the tissues and their prolonged exposure to acidic metabolites must result in some degree of autolysis, as explained in an earlier chapter; and, as shown previously, the autolytic process results in the liberation of cytost in considerable quantities. In consequence, there will be an accumulation of this substance in and about the tissues of the inactive limb, and these tissues will therefore suffer. By this line of reasoning we reach the conclusion that the cause of atrophy in immobilized tissues is twofold, being due in part to impaired nutrition and in part to the accumulation of cytost.

If an animal is completely immobilized, then his entire system will be exposed to the toxic action of large quantities of cytost arising in the manner described above; for the general impairment of the circulation will also inhibit the excretion of any toxic products formed in the tissues. The writer has performed experiments in this connection by bandaging rabbits in such a fashion that all movements other than those incident to respiration were inhibited. In the writer's experience, all the animals so treated died within thirty-six hours following the application of the bandages. At autopsy the blood was found to have undergone coagulation and stasis in the viscera simi-

lar to that observed in other instances of acute death by cytost. These experiments represent an extreme case of cytost intoxication due solely to inaction.

Mention has previously been made of the experiments conducted by the writer in 1901, in which the less stringent method of confinement in close-fitting, sterile cages was used to cause inactivity. As previously recorded, it was found that under such conditions the vast majority of the animals so treated died within a few months, while the majority of those surviving this treatment were found at autopsy to have developed various lesions similar to those produced in later years by the repeated injection of homologous cytost.

The foregoing discussion offers a partial explanation for the numerous ill effects, such as malaise, ease of fatigue, and general lowering of the so-called natural resistance, which follow periods of enforced or willful inactivity in both man and animals.

Chapter VIII

INVESTIGATIONS CONCERNING NATURAL RESISTANCE

In the last chapter we were led to certain speculations regarding natural resistance and the rôle played by cytost in this important aspect of normal physiology. Let us now consider the results of various experiments which have been made by the writer in an attempt to test the validity of his conclusions concerning natural resistance. For convenience, such experiments may be divided into three groups: (a) those dealing with the susceptibility to cytost as evidenced by the ease of the induction of shock by the injection of that substance; (b) wound healing; and (c) experiments concerning the breeding of captive animals. In each of these groups we shall for the present limit our discussion to animals whose natural resistance has been raised by a preliminary series of cytost injections.

It will be remembered that when small graded doses of cytost are injected every few days for some weeks into laboratory animals, these animals undergo a progressive increase in weight and apparent well-being. If such injections are discontinued before the animals suffer a decline as shown in the graph on

page 114, we may suppose that their natural resistance to cytost has been increased beyond that of similar untreated animals which have been kept under otherwise identical conditions. As will be shown presently, this supposition is justified by the experimental results. Such being the case, we may reasonably speak of such animals as having been actively immunized to cytost by the treatment they have undergone. It must be stressed, however, that the term immunity is here used in a relative manner, for we cannot by any treatment render an animal absolutely immune to cytost. All we can do is to raise his degree of tolerance to cytost above that of his untreated mates.

If a series of cytost injections, which leads to increased weight and general health, really is capable of raising an animal's resistance to cytost, then animals so treated should be more resistant to shock than are animals whose resistance has not been so raised. This point is easily settled by experiment. A potent cytost preparation prepared as previously described from autolyzed cat's muscle was first assayed by injection into normal cats in order to find the dosage necessary to cause a severe shock. This dosage was then injected under parallel conditions into normal cats and into cats actively immunized to cytost, as above described. The untreated animals always passed into severe shock frequently resulting in death, whereas the actively immunized animals developed a shock of considerably lesser degree and but few fatalities resulted.

When the quantity of cytost injected was reduced to such an amount that it caused a severe but non-fatal shock in normal animals, the actively immunized animals were found upon injection to suffer shock in but

a slight degree, and to recover from it rapidly. In some instances, the immunized animals were able to withstand the intravenous injection of a quantity of cytost from 100 to 500 times as great as the amount found to cause the death of normal animals within three minutes. Furthermore, the immunized animals proved to be refractory to the usual procedures which are known to induce shock. (Turck, 1918, 1921.)

These striking results prove that the preliminary series of cytost injections cause the elaboration of some defense mechanism within the body of the treated animals. These facts, taken in conjunction with our previous observation that cytost is to a marked degree species-specific, suggest that the increased tolerance to cytost shown by the treated animals is due to the elaboration of a specific antibody or anticytost.

Two different sets of experiments tend to substantiate this concept. First, it was found that cats which had been subjected to a series of injections of heterologous cytost prepared from beef or rat tissues did not develop an increased resistance to cytost. This was demonstrated by the fact that animals so treated, when injected with homologous cytost passed into shock and died as rapidly as did untreated normal animals.

These results offer further evidence that the process of active immunization to cytost depends upon the elaboration of a specific anticytost analogous in some respects to the specific antibodies of classical immunology. By analogy, then, it appeared likely that such antibodies might be produced in the body of another animal, such as the horse or goat, and that possibly by injection of the serum of such an immunized

animal cats might be given a passive immunity to cytost. To this end horses and goats were given a series of injections of autolyzed cat tissue, and after some months blood was withdrawn and the serum separated as in the usual methods for the preparation of antisera, and preserved by the addition of tricresol (Turck, 1919).

The existence of anticytost in horse or goat serum prepared in this fashion was demonstrated as follows. A quantity of the antiserum was mixed with an amount of cat cytost known to be fatal when injected into cats. The mixture was then injected into normal cats. As a rule the animals receiving the cytost-anticytost mixture developed severe shock but did not die as did the animals receiving cytost alone. Alternatively, when injected first with the antiserum and then with a potent cytost preparation, it appeared that the antisera afforded the animals some measure of protection against cytost although the degree of immunity obtained in this manner was in no way as striking as that resulting from active immunization, as described above. While this result proved disappointing, it is in harmony with common experience with many bacterial endotoxins, where it has repeatedly been found impossible to convey a reliable passive immunity by the injection of antisera.

However, since some degree of success attended these experiments, the writer feels that the results mentioned above favor the concept of an anticytost as a defense mechanism of the organism. In this connection it is of interest to recall the investigations of Weichardt (1912), who claimed to have obtained an antitoxin against the toxin of muscular fatigue.

Since, as postulated in the previous chapter, it appears that cytost is concerned in muscular fatigue, Weichardt's fatigue antiserum may be closely related to or identical with anticytost. In view of our present knowledge, however, it appears more likely that more convincing experimental results can be obtained by active immunization with homologous cytost than by the transmission of antibodies elaborated in another animal. In consequence, in the majority of the experiments to be discussed subsequently, the various animals used were immunized actively rather than passively.

From the standpoint of general biology, the immunization to shock discussed above is of importance only in so far as it demonstrates our ability to raise an animal's resistance to the effects of high concentrations of cytost. From a general standpoint this is of interest because, as we have shown, various bodily activities as well as injuries lead to a liberation of this endogenous tissue product.

If, as postulated previously, an increased tolerance to cytost is a factor in an animal's natural resistance, then we should expect that actively immunized animals would recover from minor injuries more rapidly than non-immunized. In order to test this concept, the rate of healing of standardized wounds was compared in immunized and non-immunized cats. For this purpose an area back of the heads of the animals was shaved, and, under ether anesthesia, a triangular flap of skin was removed between the occipital tuberosity and withers. As far as possible, these injuries were made alike, the dimensions of the triangles being held to a standard size. The rate of wound healing

was then estimated by measuring the size of the wound and area of granulation from day to day. Without exception it was found that the wound healed much more rapidly in the immunized than in the non-immunized cats. These results are summarized in Table XII. (Turck, 1921.)

TABLE XII

RATE OF WOUND HEALING IN IMMUNIZED CATS (TURCK, 1921)

GROUP	FIRST WEEK	SECOND WEEK	THIRD WEEK	FOURTH WEEK	FIFTH WEEK	SIXTH WEEK
1	3×3×4	3×2.5×4	2.5×2×3	1.7×1.5×2	0.5×0.7×1	Healed
2	2.7×2.7×3.7	2×2.5×3	2×2×2.5	1×1×1.5	Healed	
3	3.5×3×4.5	2.7×2.5×3	2×2×2.5	1×1×1.5	Healed	

The figures given above represent the average measurements in centimeters of the unhealed portions of the standardized wounds (3 × 3 × 4 cm.) in three different groups of cats. The first group were untreated in any way. The members of the second group received injections of a few drops of cytost around the margin of the wounds, while all members of the third group had been actively immunized to cytost as described in the text.

These results indicate that the immunized animals can more readily cope with minor injuries than can the non-immunized. Since all animals during their existence are subjected to various minor injuries of one sort or another, it follows that an increased tolerance to cytost must be of considerable service to the animal in overcoming adverse disturbances in his environment.

It has long been recognized that, other things being equal, the rate of healing of a localized injury is in a large measure determined by systemic conditions of a so-called "constitutional" nature. While the term constitutional is admittedly vague, it nevertheless

conveys the idea with which we are all familiar,—that individual internal factors over which we have little control determine the physiological activity of an organism.

Thus, it is well known that captive wild animals do not breed well. Commonly this fact has been attributed to constitutional alterations arising from changed feeding habits and inaction enforced by caging which play an important rôle in their altered breeding activities and the viability of their offspring. We have seen previously that these same factors lead to a liberation of cytost and various pathological conditions which may be attributed to the action of this substance. Consequently, it became of interest to ascertain whether or not immunization to cytost would result in an increased fertility and possibly in a greater viability of the offspring. As captive wild animals were not available to the writer, he was forced to conduct his experiments upon domesticated and laboratory animals.

A series of pullets which had not begun to lay eggs were divided into three flocks. The first flock (A) of ten received a course of four immunizing injections of chicken cytost, spaced one week apart. The second flock (B) of twenty-five received similar injections throughout the period of observation, while the third flock (C) of ten were held as controls. The daily egg production of each flock was noted over a period of seven months, from January through July. During the first three months the number of eggs produced was about the same, although the members of flock A (the immunized birds) yielded a slightly greater number of eggs. At the end of the fourth

month, however, distinct differences in egg productivity became apparent in the various flocks. While the control birds reached and maintained an egg production of about 30%, the immunized birds showed a marked increase in the rate of egg production, attaining 60% at the end of the seventh month; whereas the members of flock B which had received the cytost injections every week throughout the period of observation, after the fourth month suffered a rapid decline in egg production, which fell almost to zero after the seventh month. (Turck, 1923.) The results of these experiments are represented graphically in figure 13.

These experiments with chickens recall those made with the cats, wherein it was found that although a few doses of cytost appeared to be distinctly beneficial to the animals, a long continued series of injections ultimately led to retrogression and pathological changes. It may reasonably be assumed that the diminished egg production observed in flock B is due to the production of circulatory disturbances and consequent tissue damage similar to that found in cats when treated in the same manner.

When attempts are made to breed cats under laboratory conditions it has been frequently observed that the kittens born are sickly and survive but a short time. Further, as we have shown previously (page 87), when apparently healthy cats are placed in cages contaminated with cytost, the animals rapidly develop respiratory disturbances, involvement of the lungs, and frequently pneumonia. Hence when one allows pregnant cats to give birth to kittens in cages contaminated with cytost, or places the newly born kittens in such cages, the young animals are subjected to

a combination of factors which early brings about their death. Obviously, then, such conditions may be utilized as a rather severe test of our ability to raise an animal's natural resistance by injections of cytost.

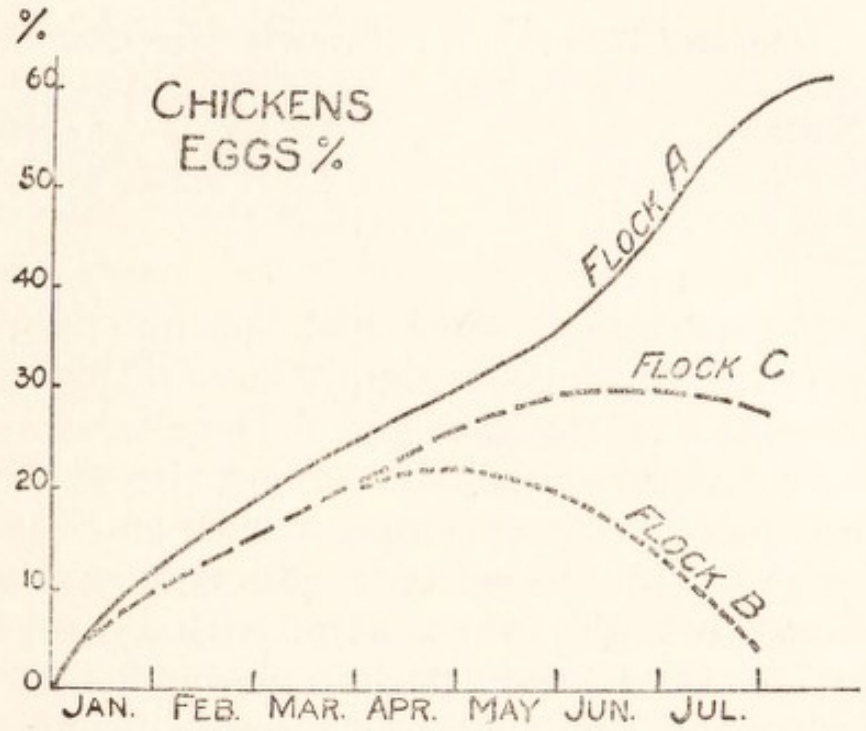

Figure 13. Graphs showing the effects of cytost injections upon the monthly egg production of hens. The ordinates represent the percentage of the hens which laid eggs each day and the abscissae represent the time after the beginning of the experiment. Curve A refers to the hens stimulated by cytost injections; curve B represents the hens in which a decline was caused by cytost injections; and curve C the control flock. (From Turck, 1923.)

Kittens born of normal mothers were divided into two groups. The members of one group were injected with a cat anticytost serum which had been prepared as above described, while no attempt was made to immunize the individuals of the other group. All the kittens were then placed in cages which had been sprayed with a potent cytost preparation. All

the kittens died within a month, although all the animals treated with the anticytost lived approximately twice as long as the untreated ones. (Turck, 1923.) The longevity of the members of both groups is summarized below:

Untreated kittens		*Passively immunized kittens*	
AGE	DEATH IN	AGE	DEATH IN
8 weeks	12 days	8 weeks	17 days
4 weeks	10 days	4 weeks	25 days
4 weeks	12 days	4 weeks	32 days
4 weeks	11 days		

These experiments show that specific anticytost sera afford the animals a slight degree of protection from the cytost infected cages. A large series of experiments has shown that the average life of healthy kittens exposed to cytost contact is from ten to twenty days, whereas if injected with anticytost serum before being brought into contact with cytost, they usually live for a longer period, although they ultimately succumb if not removed from such contact.

These experiments were continued in slightly different fashion, as follows. Female cats were actively immunized to cytost as above described, and allowed to give birth to their offspring in cages sprayed with cytost. In this case the kittens, although in contact with cytost, were found to live for a considerably greater period than did those born of non-immunized mothers. A number of the former received injections of anticytost as well, and the kittens so treated withstood the contact with cytost slightly better than did those born of actively immunized mothers but receiving no anticytost serum. Since, however, in these two groups of animals the length of life in the cages

did not differ very greatly, it seems likely that the tolerance to cytost exhibited by both groups of kittens is directly traceable to the fact that their mothers had been actively immunized. The length of life of the kittens born of immunized mothers is summarized below. (Turck, 1923.)

Untreated kittens born of actively immunized mothers		*Kittens from actively immunized mothers and injected with anticytost serum*	
	DEATH IN		DEATH IN
Born in cage	8 weeks	Born in cage	6 months
Born in cage	57 days	Born in cage	4 months, 11 days
Born in cage	18 days		
Born in cage	32 days	Born in cage	1 month
		Born in cage	11 weeks
		Born in cage	8 weeks

The fact that all the kittens in these two groups lived for a comparatively long period under conditions known to be rapidly fatal to kittens implies that the resistance of these animals was greater than is usually the case with young kittens, and from the very nature of the experiment can only be attributed to the fact that the mothers had been actively immunized to cytost. This fact suggests that the immunized mothers were able to transmit their acquired immunity to their offspring. This perhaps is simply another way of stating the well-known fact that vigorous healthy animals usually give birth to young which as a rule have a greater natural resistance than do the offspring of normally inactive animals.

Although the latter statement by itself conveys nothing new, it is of singular interest that immunization of the mothers to cytost enables us to insure the pro-

duction of offspring having a higher natural resistance than is ordinarily the case. This conclusion is not based upon the experiments just cited alone, for results of a similar and even more striking nature have been obtained with mice, rats, and guinea pigs as well. Before considering these in detail, it is worth while to outline the probable relation between the health of the mother and that of her offspring.

It is now generally accepted by biologists that acquired characteristics of the parents cannot be inherited by their offspring. This conclusion is based upon the results of thousands of experiments in this direction which have been made since the beginnings of scientific biology. While from time to time apparent evidence to the contrary has been recorded in the literature, subsequent investigation has in each case proved such evidence to be the result of either faulty experimentation or observation. Further, the modern theory of heredity, which has withstood innumerable onslaughts, furnishes an adequate basis for the non-inheritance of acquired characters. According to present day concepts, an animal's inheritance is uniquely determined by the genes passed on through the germ plasm from one generation to another. So far as is now known, an organism's inheritance can be modified only by factors capable of altering such genes. To date, the only known method of accomplishing this is by means of the X-ray which, as Muller (1925) has shown, may cause such alterations in the genes as to lead to the formation of mutants. Such being the case, it appears obvious that ordinary factors affecting the activities and health of the parents can in no wise influence the inheritance of the offspring.

At first glance these conclusions may appear to be at variance with the results of the author's experiments concerning the breeding of animals. Such, however, is not the case, as the following considerations will show. Supposing that, given an adult man who by right of inheritance is an excellent physical being capable of withstanding without difficulty the rigors of present day civilization, we suddenly transfer him to the Arctic regions and fail to equip him with proper clothing or housing to withstand the extreme cold. It is quite obvious that under such conditions the man would rapidly succumb to the intense cold, regardless of the genes he had inherited from his forbears. Again, if after a brief exposure to the intense cold and before death, we were to transfer him back to a more suitable environment, we know, from experience, that regardless of his genes, the individual would show the results of his unfortunate exposure, and would for a time be susceptible to various maladies, particularly respiratory infections. In other words, while the genes determine one's inheritance, environment determines largely the value of this inheritance to the individual concerned. Further, as shown in the simple example cited above, a relatively brief exposure to a distinctly unfavorable environment may so alter one's natural resistance that for a longer or shorter time he is unable to cope with simple variations in an environment to which he is normally relatively resistant.

There is no reason why these simple concepts should not be applied to the developing fetus *in utero*. In other words, regardless of the genes which have been passed on to the fetus by its parents, the immediate environment of the fetus must, to a great extent, de-

termine the resistance of the fetus both before and after birth. Similarly the development of the fetus in the body of the mother must alter her internal environment. The latter fact is easily made apparent in the various anatomical and physiological changes which accompany pregnancy, and by the vomiting and other evidences of toxemia frequently to be observed.

Such unpleasant effects, which are commonly observed in women and higher animals during pregnancy, may in part be ascribed to the action of cytost liberated from the uterine tissues as a result of the activities of the developing fetus. For the first week following insemination the developing egg is a free body, but later attaches to the uterine wall. In the case of the monkey, the changes incident to this attachment have recently been described by Hartman (1931). According to this author the embryo of the monkey begins to implant itself in the tissues of the mother about eight or nine days after fertilization, and eventually becomes half buried in the uterine wall. Hartman's experiments show that this implantation is accompanied by an erosive action which for a time causes the maternal tissues literally to "melt away" before the egg and thus affords a mechanism for the attachment of the egg.

The "melting away" of the mother's tissues, as Hartman terms the process, is undoubtedly autolysis—the usual procedure by which cells disintegrate. It seems, therefore, that some secretion of the egg must act as a trigger which initiates such autolysis and thus permits implantation to take place. Such an autolysis, in common with any other, must liberate cytost into the circulation of the mother and cause her to show the effects

of cytost intoxication—in this case the so-called toxemia of pregnancy. On a similar basis we may explain the occurrence of such toxemias in pregnant women; for in their case it has long been known that the human embryo becomes completely embedded in the uterine tissue, and this must take place by a mechanism similar to that in the monkey. For obvious reasons, embryologists have not been able to follow this process in the woman as Hartman has done in the monkey; but in this instance it does not appear fallacious to argue by analogy.

The concept of toxemia outlined above is easily subjected to experimental verification; for symptoms similar to those accompanying the toxemia of pregnancy should be elicited by the parenteral introduction of cytost into pregnant animals. This has been shown by the writer (1921, 1922) as follows: When homologous cytost preparations were injected intraperitoneally into healthy pregnant cats, the animals showed signs of acute nausea within from thirty seconds to two minutes after the injection, and violent retching continued for thirty to fifty minutes, frequently accompanied by symptoms of eclampsia. As has been stated previously, such injections, if continued, may induce abortion. On the other hand, injections of heterologous cytost into pregnant cats does not result in the onset of any of these difficulties. These results seem to prove unquestionably that the toxemia of pregnancy is actually due to the release of cytost brought about by the activities of the developing embryo *in utero*.

In a normal pregnancy the majority of the symptoms of toxemia disappear long before the end of

gestation. This fact signifies one of two things: either the mother develops an immunity towards cytost, or the production of cytost by the fetus ceases. In order to analyze these concepts, let us return to Hartman's observations on the monkey, where it was found that the egg digs into the maternal tissues until half buried. Since, as we have seen, this implantation is accompanied by autolysis, we may inquire why this autolysis does not continue until all the uterine tissues have disappeared. Two answers to this perplexing question suggest themselves. Either the stimulus of autolysis, the fetus, has undergone a change, or the mother's tissues have become resistant towards the invasive action of their offspring. Each of these answers contains an element of truth, as is evidenced by the following considerations.

In the previous discussion we have shown that the repeated injection of small quantities of cytost leads to the development of an active resistance towards this substance, presumably through the development of an anticytost. With this in mind we may postulate that because of the slow and continual absorption of cytost from the disintegrating uterine tissues, the mother's resistance to this substance is raised in a manner analogous to that resulting from the regular injection of small quantities of homologous cytost. That such a resistance is developed during pregnancy is shown by the following considerations.

Injection of cytost in suitable quantity into pregnant cats causes nausea and retching which usually pass off within an hour. When like quantities of cytost are injected into male cats kept under the same conditions in the laboratory, vomiting and retching

are likewise induced, but as a rule the onset of such unpleasant symptoms is found to be more rapid and their duration more prolonged than in the case of the pregnant females. (Turck, 1921b.) This observation appears to substantiate the hypothesis that the females, as a result of their pregnancy, develop a certain resistance towards cytost.

It may be remembered that in our experiments concerning the action of cytost on the abdominal viscera it was shown that once autolysis is started the process appears to pass from cell to cell, gradually involving greater areas of tissue. With this in mind, it seems evident that the autolysis of the uterine tissues due to the activities of the embryo would spread not only to other tissues but to the embryo itself, unless checked in some way. It follows from the foregoing discussion that such a check is afforded by the anticytost developed by the mother. In those all too numerous instances where toxic symptoms are maintained throughout pregnancy, especially in eclampsia, it appears that for some reason or other the mother is incapable of elaborating sufficient anticytost to combat the activities of cytost in her body. This unfortunate circumstance may lead to two difficulties, one affecting the mother and the other the offspring. The first leads to various pathologies such as kidney involvement, and the second to ill health and perhaps faulty development of the young. Indeed, with regard to the latter, Holland (1920) has concluded—"that the toxemia of pregnancy is the principal cause of antefetal death."

This conclusion, which is the result of empirical observation, cannot be questioned; hence it is of inter-

est to seek the cause underlying the observed facts. To this end let us attempt an analysis of the facts in the light of the foregoing discussion.

In an earlier chapter attention has been drawn to the fact that when shock is induced in a pregnant guinea pig the embryonic tissues suffer congestion in much the same manner as do the viscera of the mother (see Fig. 16). Since shock, as we have shown, is due to the action of cytost, such an observation may be taken as evidence that the presence of an excess of cytost in the circulation of the mother will be reflected in the developing fetus. This may well lead to faulty development of the latter, and such faulty development *in utero* may seriously affect the animal's antefetal life. Thus we are able to see a connection between the toxemia of pregnancy and the life of the young from a rational point of view.

It follows, therefore, that anything we can do to lessen the toxemia of pregnancy, that is, to combat the action of cytost in such instances, will enable us to offer a measure of protection to young animals. The importance of this possibility lies not solely in protecting the young against cytost during their comparatively brief period *in utero,* but in the fact that by so doing we may, barring accident, insure the development of normal young which will better be able to live in accordance with the heritage of their germ plasm. To accomplish such a result, we must take advantage of all available means to combat the action of cytost *in utero* before as well as after birth.

One means available toward this end is the immunization of mothers against cytost so far as is possible. This will have a dual effect, for not only will it

decrease the tendency towards toxemia in the mother, and hence the possibility of attendant damage to the developing fetus, but, as shown in the experiments with kittens in cytost-sprayed cages, the offspring will be relatively more resistant to cytost than are the young born of non-immunized mothers. That a course of immunizing injections actually does decrease the liability towards the toxemia of pregnancy follows from the fact that the nausea and retching which follow the intraperitoneal injection of small quantities of cytost is not observed when a similar injection of cytost is administered to pregnant female cats which have been actively immunized by the procedure previously described. (Turck, 1921, 1923.)

From a laboratory standpoint cats are not convenient animals for investigations concerning breeding activities, and it was exceptionally fortunate for the writer that through the kindness of the late Dr. Edwin J. Banzhof we were able to conduct such experiments on a large number of guinea pigs used for breeding purposes in the laboratories of the New York City Department of Health. For a period of years it had been observed by members of the Health Department Laboratories that a large proportion of the breeding stock died in pregnancy, and that the young, when born, were often feeble and did not survive for long. This situation occurred time and time again during the winter months, although the animals were kept in a warm, well-ventilated room. At first it was thought that the unusually high mortality was due to some infection, and attempts were made to combat the situation by sterilizing the cages and by transferring the animals to other rooms. Such means, however, proved

of no avail; hence the conclusion was drawn that the breeding stock through generations of domestication had become unusually susceptible to the action of cytost liberated during pregnancy. In order to test this concept, it was decided to attempt immunization of the guinea pigs to cytost.

To this end, a cytost solution was prepared by extracting autolyzed guinea pig muscle with ten times its weight of water, and after removal of the insoluble material by centrifugation, the extract was passed through a Berkefeld filter in order to insure sterility. This preparation was then used directly for the immunization experiments reported below.

In the first experiment twenty old females of the same age and weight were taken from the breeding stock which had been doing poorly. Breeding was started on December 2, 1923. The animals were divided into two groups of ten each. Beginning February 4, 1924, each of the members of one group received a series of seven subcutaneous injections of the cytost extract, spaced one week apart, while the members of the other group were held as controls. The amount of the cytost extract used for each injection was two or three minims. In this experiment it was desired to ascertain the effect of the injections on the mothers at the time of parturition and shortly thereafter. All the animals in both groups gave birth to young at various times between February 3 and March 10. By the end of April all the members of the injected series were still alive, whereas four of the members of the controlled group had died on the following dates: April 4, 12, 14, and 18. Subsequently all

these surviving animals, including the young, were sent to the writer's laboratory, where they were kept under continued observation. By December, 1924, all the mothers of the control series had died, while all the members of the immunized group were still alive.

This result leaves no doubt but that the attempted immunization was actually effective in protecting the mothers against cytost liberated during pregnancy. Further, this result is of interest in that it shows again that animals may actually be immunized to cytost by such a series of injections—perhaps because of the elaboration of a specific anticytost, as previously suggested.

It may be suggested that these rather striking results are due to some factor other than the cytost injections. Such a possibility would demand serious consideration were it not for the fact that essentially the same result was obtained time and time again on repetition of the experiment.

In these early experiments the cytost injections were begun too late during the course of the pregnancies to warrant any conclusions as to the effect upon the offspring; hence other experiments must be considered. Before passing to these, we may interpolate an experiment made under the writer's direction by Dr. Banzhof and his associate, Mr. A. M. Klein. A number of guinea pigs was given a series of three cytost injections spaced a week apart, and was then set aside in the animal house for a period of fifteen months. At the end of that time, they were large and healthy, while, according to Dr. Banzhof, animals of the same age and size usually show a mortality rate

of fifty per cent within a year when kept under the same conditions in his animal house. These results of Dr. Banzhof offer striking confirmation of the writer's results with cats (page 112), and again demonstrate that it is possible to raise the natural resistance of animals by a properly graded series of cytost injections. Unfortunately Dr. Banzhof did not record the gain in weight of his animals; therefore we cannot compare this aspect of their growth with that of controls, as was done in the writer's experiments with cats.

Old guinea pigs were found to be less resistant to injections of large quantities of cytost than younger animals. This fact may be taken as indicative of the fact that the older animals suffer from a greater accumulation of cytost than do the younger ones; consequently the former are more apt to die as a result of the toxemia of pregnancy than the latter, a conclusion in agreement with observations from experience. That older animals actually are more sensitive to cytost is evidenced in that they succumb more rapidly to injections of large quantities of cytost than do younger animals.

Turning now to the influence of the immunization of the mothers on the offspring, we have found the following: When female guinea pigs receive a series of immunizing cytost injections, as above described, but given prior to pregnancy, it is found that the offspring are more vigorous, have a healthier appetite, grow more rapidly, and present a healthier appearance than do the offspring of non-immunized animals. A series of young females was divided into two groups, one being immunized and the other held as control.

During the course of the experiment both groups of animals and their offspring were kept under identical conditions in the laboratory and were given the same rations.

Following the birth of the young, the following differences were noted: The litters of the immunized mothers were larger than those of the controls, and the young were more active and took to the breast more rapidly than did those of the non-immunized mothers. In a few instances, some of the latter died shortly after birth from what appeared to be acute inanition. This difference in appetite became much more marked a month after birth, when the animals born of immunized mothers showed the voracious appetite common to healthy young animals, while the members of the control group showed much less interest in food.

The difference in food intake was shown by the fact that one month after birth the average weight of the young of the immunized mothers was 60 grams greater than the average weight of the young born of the control group, whereas after the lapse of two months the difference in weight averaged 80 grams. Again, a distinct difference was noticeable in the appearance of the fur, that of the control group appearing rough, while that of the members of the other group was sleek and glossy, an indication of better health. During this period a distinct difference in natural resistance was evidenced by the fact that several of the young in the control group succumbed to respiratory disturbances which caused their death, whereas none of the animals born of the immunized mothers suffered from this cause or from any other. In other

words, the young born of immunized mothers appeared superior in every way to the controls.

These differences recorded above cannot be attributed to differences in the germ plasm, since all the animals used in these experiments were derived from the same breeding stock. Further, the observed differences between the offspring of non-immunized and of immunized mothers were sufficiently consistent to justify the conclusion that the immunization of the mother to cytost is decidedly beneficial to her offspring.

When we attempt to interpret this result, we are confronted with two possibilities, for as a result of the immunization we may consider that the observed results evidenced by increased resistance of the young are due solely to the lack of toxic effects upon the embryo during intrauterine life, thus permitting a normal development; or on the other hand, it may be supposed that the increased resistance developed by the mother is passed on to her offspring. From an experimental standpoint the latter conclusion appears the more likely for several reasons.

In the first place, it has been pointed out above that by immunization cats may be rendered less susceptible to the respiratory disturbances which are induced by keeping the animals in cytost-sprayed cages. Further, it was found that in such cages the longevity of kittens born of immunized mothers was greater than that of those whose mothers had not been immunized. This result can only be explained on the assumption that the kittens during their intrauterine existence had acquired, in a degree, the immunity induced in their mothers.

Bearing this in mind, it seems likely that the increased natural resistance of the young guinea pigs discussed above must likewise be due to the passage of the mothers' increased resistance to the offspring. This conclusion does not controvert the generally accepted doctrine that acquired characters are non-inheritable, for it may be effectively explained on the basis of the concept of anticytost. It will be recalled that some evidence has been offered that the immunization procedure accomplishes its desired end by causing the elaboration of a specific anticytost. If the placenta is permeable to anticytost, than the fetus may acquire this substance by diffusion from the blood of the mother. Consequently at birth the young animals are endowed with a certain supply of anticytost which offers them protection until such time as they become able to develop anticytost of their own.

Such a concept is in complete accord with the results of the experiments discussed above, and is in agreement with the findings of Ehrlich (1891, 1892), who found that when female mice were immunized to the toxins ricin and abrin, their progeny possessed a resistance to these substances which was greater than that normally found in the offspring of non-immunized mothers.

Ehrlich further observed that the increased resistance to ricin and abrin was transmitted to the offspring when only the mother was immunized to the toxin, whereas immunization of the father alone did not result in an increased resistance of the young to these toxins. A similar finding is recorded by Behring (1889) in the case of immunization to diphtheria toxin.

These results argue in favor of the placental transmission of antibodies, and it seems logical to conclude that when a pregnant animal is immunized to cytost, the anticytost formed passes in similar fashion to the fetus.

Chapter IX

THE BREEDING AND MANAGEMENT OF ANIMALS

In the foregoing chapters we have advanced a number of concepts and experiments relative to the action of cytost in the animal body. It has been shown that, depending upon its concentration, this substance may exert both stimulating and toxic effects, so far as an animal's health and well-being are concerned. Let us now consider the application of the information so far deduced to the problem of animal breeding.

The object of the latter, whether for laboratory or farm purposes, is to produce under more or less fixed conditions uniformly healthy and fertile animals which are as resistant as may be expected to unavoidable alterations in their environment. It is obvious that in order to achieve this end as economically as possible the breeding stock must likewise be kept in a healthy resistant state. If the surroundings natural to a given species cannot be completely duplicated the breeder must devise various ways of compensating for this enforced change in the animal's mode of living.

To this end intelligent breeders have always tried, in so far as possible, to duplicate climatic conditions, provide exercise, and give adequate attention to

dietary considerations. Nevertheless such attempts have frequently met with lack of success, even in the hands of experienced husbandrymen. It is of interest, therefore, to inquire into the relationship between such factors as those mentioned above and the various aspects of the cytost problem that have been considered in the preceding pages.

First consider the question of diet. As is well known, a given diet may be entirely adequate for an animal when regarded solely from the standpoint of energetics, and yet be inadequate in so far as it is lacking in essential components which the animal is incapable of synthesizing from other components of its food. It is evident, then, that before attempting to breed a given species on a large scale one should carefully study the dietary requirements of the animals at hand. Information as to the types and quantities of food demanded by the ordinary domesticated animals is usually obtainable, although it must be admitted that in most instances such needs have been determined as a result of empirical experience rather than by carefully planned investigations such as those Osborne and Mendel have made upon the rat.

The accumulated experience of years has taught husbandrymen that aside from an adequate diet, captive wild animals and domestic animals must be provided with adequate facilities for exercise, and kept under conditions which preclude crowding. The necessity of considering such factors in the care of animals is easily understood in the light of the various experiments conducted with cytost, and we may profitably consider them from the standpoint of the cytost theory.

As regards the question of diet, the reader should recall the experiments on partial inanition which have been discussed in Chapter V, where it was shown that as a result of malnutrition an animal is forced to call upon some of its body cells for essential foodstuffs omitted from its diet. In consequence, along with the nitrogenous products liberated as a result of cellular autolysis, it may be expected that an excessive amount of cytost may find its way into the circulation, and, as has been shown previously, this may result in various pathologies.

Besides containing an adequate quantity of essential foodstuffs, an animal's diet must be so adjusted that it cannot interfere in any way with the normal processes of digestion and absorption, and thereby prevent the proper utilization of the various dietary components. Such a necessity is attested to by the results of the writer's experiments with monkeys, which were fed bread fried in oil, together with the other foods commonly given to such animals. As stated on page 106, the monkeys on the high fat diet died within a few months, although other monkeys kept under similar conditions but not fed any of the bread fried in oil continued to live an apparently normal existence. In other words, although the diet was adequate from a nutritional standpoint, the addition of the heated oil brought about an end result similar to that induced by partial inanition. Indeed, we may reasonably take the view that the oil so inhibited the normal processes of digestion and absorption that the experimental animals were in the predicament of "starving to death at a banquet," and hence suffered an accumulation of cytost in much the same manner as occurs on an incom-

plete diet. Similarly any other means which promotes faulty absorption in one way or another leads to the same ends.

Some years ago it occurred to the writer that such faulty absorption of essential foods might be brought about by the admixture of various substances with an otherwise satisfactory diet. Various irritants such as mustard and silver nitrate were tried without any especially gratifying results. Shortly thereafter, it was found that the desired result could be achieved by adding large quantities of beef extractives and bouillon cultures of *B. coli* to an otherwise satisfactory diet. Rats kept on such a diet died within a month. As the blood was found to be sterile, death was not due to a bacteriemia. In agreement with this, it was found that the same end resulted when the bacteria were omitted but the feeding of large quantities of beef extract was continued. (Turck, 1906, 1908.)

Dogs treated similarly usually died in about three months. At autopsy the blood was found sterile, but marked evidences of cytost action were apparent. The viscera were found to be markedly congested, and in many instances multiple peptic ulcers were found. These latter were indicative of the fact that autolysis had taken place. At the time it was noted that "there was no bacteriemia, no inflammatory reaction in the form of round cell infiltration such as one would expect in a reactionary inflammation induced by pyogenic microörganisms or toxins. It was not the picture of reaction to an infection, not the picture of a local acting agent, but rather of a systematic condition, and of an induced cellular change." (Turck, 1906.)

The question of exercise has been fully discussed

in Chapter IV; therefore it will not be necessary to consider this factor again.

Turning now to the question of crowding, we find that as a result of experience it has long been known that such a condition acts adversely on caged animals, although in the case of higher animals little actual experimentation has been directed towards a study of this factor. What literature exists on this topic has recently been reviewed by Allee (1931), and the reader is referred to his book and the excellent monograph on the rat by Greenman and Duhring (1923), for the details of experiments made by others.

At first it is rather difficult to understand why the overcrowding of cages should lead to the ill health of animals except in so far as it limits their freedom of motion, thus preventing a normal amount of exercise. Undoubtedly this is an important factor in the effects of overcrowding, but, as will be shown below, it is not the only one.

The reader may recall that it has been shown that the toxic effects of cytost and the respiratory involvement attendant thereto may be induced in cats simply by spraying their cages with an extract of homologous cytost, or by the application of a paste of autolyzed tissue to the paws of the animals. This finding demonstrates among other things that when present in the air surrounding the animals cytost may enter the respiratory tract and there set up a congestion.

Now other things being equal, the extent and severity of such a congestion will be dependent upon the amount of cytost inspired and this in turn must bear some relation to the amount present in the air. On this basis, it follows that as a result of inhaling air con-

taining cytost an animal may suffer an involvement of the lungs varying in severity from nothing to a true pneumonia. This is especially interesting because respiratory involvements of one sort or another are frequently responsible for a high mortality amongst small mammals, particularly rabbits and guinea pigs. Such troubles are usually attributed to bacterial infections, although as the writer has pointed out elsewhere (Turck, 1919), it appears that the bacterial invasion usually follows the congestion of the lungs.

The fact that animals may suffer from the toxic effects of homologous cytost in the surrounding air suggests that this is an important factor in producing the effects of overcrowding. Various experiments which we shall now consider substantiate this conclusion.

When the dust which accumulates about animal pens or cages was collected, converted into a sterile extract, and injected into homologous animals, it was found that the animals died rapidly, in some instances almost immediately, the time of death depending upon the quantity of the extract injected. A similar finding was obtained when concentrated extracts of the hair and epithelial tissues were substituted for the dust extract. Since all of the substances used as a source of cytost in these experiments will commonly occur in animal cages, pens, and runs, it is obvious that such places constitute a potential source of trouble unless frequently cleaned. Obviously in crowded cages this potential source of cytost is increased, due to the greater number of animals per unit area. In consequence we may conclude that the ill effects attendant upon overcrowding are in part due to the greater chances of exposure to air containing a relatively

higher concentration of cytost than would be the case if fewer animals were confined within the same area. Further, it is possible that cytost finds its way into the air of the cage by way of the effluvia of the breath.

Further evidence that in crowded cages cytost may be present in sufficient quantity to elicit various respiratory disturbances was obtained as follows: Pregnant animals were allowed to give birth to their young in cages which had previously been inhabited by a number of adult animals. Under such conditions it was found that the newly born offspring usually died within two weeks, from respiratory disturbances.

The results of experiments of this type confirm the hypothesis that the ill effects of overcrowding are due in part to the resultant relatively high concentration of cytost which accumulates in the cages or pens.

In raising small animals in the laboratory, it almost inevitably results that a certain degree of crowding is necessitated by the requirements of space. This condition should not present any serious difficulties if the cages are cleaned thoroughly at regular intervals, preferably daily. Such a cleaning should involve not only the removal of excreta and the like, but the complete removal of hair and dust. As a further means of minimizing the dangers of contact with cytost, the rooms in which the animals are kept in their cages should be well ventilated, as this will prevent the accumulation of cytost-laden air.

This fact has been appreciated by the laboratory workers engaged in researches on the white rat, and a visit to laboratories where this animal is raised in large numbers will show that the conditions stated above are scrupulously observed. For some curious

reason breeders of other species of animals have not seen fit to employ the same care in the treatment of their charges. Indeed, such breeders are inclined to scoff at the pampering which the white rat receives in the larger laboratories. They argue that the rat is a hardy animal naturally acclimated to life under conditions usually regarded as unhygienic for other species. While this is true, the scoffers forget that the rat breeders would not indulge in the care and attendant effort which they expend on their animals unless it had been found to pay, not only financially, but also in insuring the production of a constant supply of hardy animals suitable for purposes of experimentation.

It may not be amiss to suggest that the ill health all too common amongst humans in congested districts may in part be due to the same cause as that found in the case of caged animals. Everyone has heard the virtues of "country air" extolled, and I believe that most of us are ready to admit that there is something invigorating about country air that is lacking in the city. By analogy with the animal experiments discussed above, it seems probable that in densely populated areas the atmosphere must contain a certain amount of human cytost which is lacking in the country air. On this assumption the invigorating nature of extra-urban atmosphere lies in its comparative freedom from cytost. This inference, of course, is only speculation which cannot be proved by direct experiment, but several facts of human experience appear to testify to its correctness. Thus, it has long been recognized that patients suffering from respiratory infections recover more rapidly outside the larger

cities. Again, most individuals have at one time or another experienced a feeling of discomfort when together with a number of people in a poorly ventilated room or hall. This discomfort may in part be due to the accumulation of small quantities of cytost in the air of the room; and if this be true, it is obvious that the greater the number of people in the room the more rapidly will such an accumulation take place.

We have no direct evidence that humans actually discharge cytost into the air, but that they must give off something which is characteristically human is evidenced by the fact that bloodhounds can trail a man; and that many other animals, such as the deer, can scent a human at some distance, providing the wind is in their direction. Indeed, many animals exhibit a remarkable sense of smell, which enables them to seek their own kind. Since, as has been shown previously, cytost is distinctly species specific in its action, it is conceivable that such species specific scent as is shown by the dog is in part due to a special sensitivity to cytost rather than to a sense of smell as we ordinarily understand it.

The foregoing discussion, although admittedly somewhat speculative, suggests that the animal breeder should utilize every means at his disposal to combat the action of cytost upon his animals. Aside from the selection of an adequate diet, the prevention of overcrowding, the preservation of cleanliness, and the provision for adequate exercise, it is conceivable that the professional breeder might profitably consider the possibility of immunizing his animals to homologous cytost. By so doing he might be able to lower the mortality rate of his breeding stock and of

their offspring. This would seem particularly important when the animals are of necessity kept in highly artificial surroundings, such as is usually the case with laboratory animals.

In the preceding chapter, we have presented experimental evidence that immunization to cytost is of considerable value in the maintenance of a guinea pig colony, since it appears to insure the birth of young animals having a higher natural resistance than is normally the case under similar conditions of breeding.

Similar experiments have been conducted with white mice. These experiments are especially instructive, since the mouse is an animal with a span of life shorter than that of most other laboratory animals; and since they reach maturity more rapidly than do other species, it is comparatively easy to follow through several generations under fairly constant environmental conditions. Further, it is well known that white mice show a very high mortality even under apparently ideal conditions of temperature, feeding, etc. This fact, which frequently proves troublesome to the breeder, is ideal for our purposes, since a comparison of the mortality rates of mice immunized to cytost and those of non-immune animals offers a ready means of testing the validity of our conclusions.

In the experiments to be discussed presently, the mice were kept in clean, spacious cages in a well ventilated, sunny room, and all of them were given the same food. They were in charge of an experienced breeder of mice who for a period of seven years had kept careful records of the food requirements, morbidity, and mortality. At times the latter had reached

the discouraging proportion of 50%. During the period post mortems, bacterial cultures, and similar data had yielded no information concerning the high death rate, and the conclusion was drawn that death was due to "natural causes" incident to breeding under domesticated conditions.

As ordinarily used, the term "death from natural causes" signifies little short of a confession of ignorance. As it seemed reasonable to suppose that the high death rate was due to cytost intoxication, experiments were begun with this in view.

For this purpose mouse cytost was prepared from autolyzed mouse muscle in a manner identical with that used for the preparation of guinea pig cytost, as described in the preceding chapter. Initially the extract was prepared by the extraction of the autolyzed muscle with an equal weight of water. Such preparations proved too toxic for the experiments, for upon the injection of 0.5 cc. of such an extract subcutaneously or intraperitoneally, the mice always died within 12 to 48 hours. When the dosage was halved (0.25 cc.), the mice were found to withstand a series of five or six such injections spaced a week or ten days apart. In this instance, the usual birth rate prevailed, but 95% of the young died within two months after birth.

After considerable experimentation of this sort it was finally discovered that it was necessary to employ a considerably more dilute tissue extract in order to achieve any distinctly beneficial effects upon the breeding of such animals. In our final series of experiments, which we wish to discuss here, it was found that a cytost preparation obtained by extracting the

autolyzed mouse tissue with forty times its weight of water proved the most serviceable. Mice were able to withstand a series of several injections of 0.25 cc. of such an extract without any apparent harm.

A series of mice were immunized by means of six injections of 0.25 cc. of such a cytost preparation, spaced 10 days apart. The second series received eight injections of 0.06 cc. of the cytost preparations, spaced one week apart. These two groups of mice, together with untreated controls, were kept in spacious cages under identical conditions, and allowed to breed. The efficacy of the immunizing treatment was judged by the mortality rate of the offspring of the three groups. During the period of observation, which extended over several months, 30% of the young born of non-immunized mothers died; whereas in the two immunized groups the mortality rate of the young was 5% and 0 respectively.

The lower mortality rate found in the cytost-injected animals indicates that the immunization procedure adopted was actually effective. As it had been observed that during the winter months many mice died of pneumonia when their cages were placed on the floor of the breeding room so as to be exposed to draughts of cold air, cages containing immunized and non-immunized mice were kept on the floor throughout the winter and spring. Under these conditions many of the non-immunized animals died, although all the immunized lived through this period. This difference was especially noticeable in the case of the newly born, for the mortality of the offspring of the non-immunized mothers amounted to 50%.

On comparing the young born of the two groups

of mice, distinct differences were noticed. Those born of immunized mothers exhibited a thick, sleek fur, in marked contrast with the rougher coats of the young members of the non-immunized colony. Similarly the former surpassed the latter in activity and virility. Indeed, in these respects the young born of the immunized mothers approached the behavior of young wild mice. This is especially interesting, since Donaldson has pointed out that in the case of the albino rat, the young behave similarly to wild rats if they are born of hardy stock under ideal conditions. The young born of the immunized mothers also gained in weight more rapidly than did those of the other group. This is in accord with the findings in the experiments with guinea pigs referred to previously.

Greenman and Duhring (1932) in their discussion of the breeding of albino rats state that in addition to the observance of the various environmental factors noted in the preceding discussion, one should "select animals for breeding which shall have been reared for one or more generations in an exercising cage during the period of rapid growth from 25 to 130 days of age." This advice is based upon the empirical observation that by so doing one obtains a breeding stock having a higher natural resistance than is otherwise the case. In view of the discussion in Chapter VII, and the experiments with guinea pigs and mice, it should be clear that the utility of exercise in improving a breeding stock must depend upon the release of cytost by this means. This, as we have seen, results in the production of anticytost, and a consequent increase in natural resistance.

Greenman and Duhring, as well as King (1919),

have pointed out that the popular conception that inbreeding results in the degeneration of a stock is erroneous. The latter author calls attention to the fact that the dire effects usually associated with inbreeding are due to environmental rather than genetic factors. In other words, when animals are inbred in breeding establishments, sufficient attention is not given to the prerequisites for successful breeding, which have been discussed in the beginning of this chapter. Indeed, provided sufficient attention is paid to such factors as diet, cleanliness and provision for exercise, it is entirely possible to breed from poor stock a strain showing a high natural resistance. In this connection Greenman and Duhring remark, "If for any special reason it is necessary to breed from a poor stock, it is by no means a hopeless task to rebuild lost vigor." And again, "Brothers and sisters may be mated with no unfavorable results when breeders are carefully selected." This means simply that provided the brothers and sisters are in a high state of natural resistance we may expect their offspring to exhibit a similar natural resistance.

The conclusions of the investigators just cited are in harmony with the experimental results of the author. In the case of the latter, however, the increased resistance of the breeding stock was achieved by means of cytost injections rather than by the more natural methods employed by Greenman and Duhring. The fact that both methods of stock improvement lead to the same end argues well for the validity of the author's hypothesis that so-called natural resistance, however developed, is due to an increased tolerance towards cytost.

In the case of the colonies of mice investigated by the author, no special facilities for exercise were provided. Injections of cytost, however, proved an effective substitute, since our colonies were carried on for a period of three years without the introduction of any new stock. Prior to the application of cytost to these colonies it had been found necessary to clear out all the stock, old and young, every year or two and begin again with fresh breeding stock in order to insure the production of healthy, vigorous young.

It has been stated previously that experiments indicated that in both cats and guinea pigs the immunity to cytost developed in the mothers was transmitted to the offspring. This was also observed in the case of mice, as noted above. With the latter animals we have been able to follow the effects of cytost immunization through several generations. This was made possible by the fact that young mice mature rapidly. Thus by observing the mortality, appearance, activity, and fecundity of succeeding generations of mice, it was found that the beneficial effects of cytost immunization appear to be transmitted through at least three generations.

Perhaps it is erroneous to speak of the transmission of cytost immunity, for this seems to imply the alteration of a genetic factor. What we mean to convey is simply the experimental fact that succeeding generations of animals having an increased natural resistance result from the immunization of mothers. This may be due to the fact that, as stated above, the first generation born of immunized mothers shows a greater activity than do the offspring of those not immunized. Such an increased activity must of neces-

sity result in effects akin to those resulting from any other form of exercise. In consequence we may assume that the increased activity of such young animals suffices to stimulate anticytost production to a degree comparable to that which results from active immunization.

Some exceedingly interesting results have been obtained by the immunization of old guinea pigs. As is well known, with the onset of age females tend to become non-fertile; or, if fertile, they frequently abort, or give birth to young which die shortly after birth. When such animals, which are normally discarded by breeders, were subjected to a series of cytost injections identical with those used to immunize younger animals against the toxemia of pregnancy, it was found that they became fertile and proved useful as breeding stock for another year. This apparent rejuvenation suggests that their lack of fertility prior to immunization was due to the toxic effects of cytost which had accumulated in their bodies during the course of their lives. On the other hand, if such an assumption is true, it is difficult to see why the additional cytost administered in the immunizing procedure should not result in a distinct toxicity.

This difficulty may be circumvented by assuming that the apparent poor fecundity of such animals is due not to a persistent accumulation of cytost, but rather to an anticytost deficiency. Indeed, this is made manifest by the extreme susceptibility of such animals to the toxemia of pregnancy and abortion. In other words, prior to insemination they do not suffer from an accumulation of cytost, but when the embryo begins to attack the uterine tissues, the resulting libera-

tion of cytost proves too much for their powers of resistance. On this basis it is readily seen that when such animals become pregnant their abilities to produce normal, healthy young must be seriously impaired.

In captivity the guinea pig at best is not a very active animal; hence it is easy to imagine that, due to its limited muscular activity, it fails to stimulate anticytost formation in the natural manner we have postulated. From this brief discussion it should be clear that the cytost injections employed in immunizing such aged animals of poor fecundity must function solely by stimulating the formation of anticytost in quantity sufficient to overcome the effects of any cytost liberated during the course of a pregnancy. In consequence such animals are permitted to carry the developing fetus to term in a normal fashion.

From the preceding discussion it appears likely that much the same results could be achieved by subjecting old, apparently sterile females to a course of exercises of gradually increasing severity. So far as the writer is aware this type of experiment has not been investigated, but the results should prove of interest.

The conclusions reached above may be summarized as follows: In order that domesticated and captive wild animals may live a normal, healthy existence in accordance with their birthright, every precaution should be taken to inhibit the formation of cytost or contact with cytost in *excessive* quantities. Opportunity must be offered to develop anticytost in a normal fashion, since this will protect the animals against cytost intoxication. These ends may be achieved by

supplying the animal with an adequate quantity of its natural foods, with proper housing conditions, and with facilities for exercise. If for any reason such conditions cannot be adequately fulfilled, then this lack may in part be remedied by active immunization with homologous cytost.

The writer makes no claim that such immunization offers a panacea for all animal ills, but suggests, rather, that the results of his experiments indicate that the stimulation of anticytost formation offers a powerful weapon for the prevention of such ills. Of course, like all preventive measures, it is not infallible; but the experimental results enumerated in this chapter suggest that it is worthy of a trial in otherwise obscure cases, and in connection with the breeding of animals under the conditions enforced by captivity.

It would be exceedingly interesting to ascertain whether or not immunization to homologous cytost would stimulate breeding in those wild animals which are known to breed poorly or not at all when held in captivity. In this connection it is opportune to note the methods being used in rearing the baby gorilla at the New York Zoölogical Park. According to current press accounts (March, 1932), those in charge of this young animal have departed from the time-honored methods of raising gorillas in captivity and have permitted their charge to romp in the snow during the winter time. According to some, this should prove suicidal, since the gorilla is naturally acclimated to a warm environment. With the animal in question, however, now five years old, this procedure has proved actually beneficial, and he seems to be withstanding the rigors of New York weather

better than any of his predecessors at the Zoo. In common parlance we should state that this young gorilla has become acclimatized. This is undoubtedly true—by his continual exercise his natural resistance has been raised to such a level that he can withstand our climate without succumbing to the ills which affect gorillas in captivity.

Such animals frequently contract pneumonia and die. As we have shown elsewhere, a low resistance to cytost is a predisposing cause of respiratory infections. It will be interesting to watch the New York gorilla during the next five or ten years. If during this period he escapes the various respiratory infections, may we not attribute it in part to an increased resistance to cytost developed as a result of his unorthodox unbringing at the New York Zoo?

Chapter X

THE RELATION OF CYTOST TO BACTERIAL INFECTION

THE relation between bacterial invasion and cytost may be viewed from three different aspects: the release of cytost as a result of injury caused by bacterial infection; the cumulative effects of bacteria and cytost acting together; and the relationship between cytost immunity and bacterial immunity. At the outset we should expect that any microörganism capable of effecting the destruction of the tissues of a higher animal would bring about the production of cytost in much the same fashion as would any other means of injury. In consequence, the infected animal should exhibit symptoms of cytost intoxication as well as those due to the bacterial toxins *per se*.

When subtlethal quantities of exocellular bacterial toxins, such as diphtheria toxin, are injected into the skin of an animal, a local reaction sets in after the lapse of several hours. The reaction usually commences as a slight reddening at the site of injection, indicating a localized capillary dilatation. This is followed by a localized edema, which is due to an increased permeability of the capillaries. Since, as we have seen, both these effects are caused by cytost, and

since they appear only some hours after the injection of the toxin, it seems likely that they are not caused directly by the toxin, but by the action of the toxin on the surrounding tissues, in the same manner as was found to be the case when irritating chemicals, such as chloroform, were injected. Such reactions are by no means limited to the bacterial exotoxins, but follow the intradermal injection of living bacteria, dead bacteria, and bacterial extracts. (Simmonds, 1928.)

In agreement with this concept is the fact that the proteolytic organisms produce such localized reactions much more rapidly than do the non-proteolytic. This is especially interesting because, as has been shown earlier, the autolytic process which leads to the liberation of cytost is essentially proteolytic in nature. In agreement with this is the well known fact that infection with highly proteolytic organisms such as the Welch bacillus frequently leads to shock, apparently identical with that which results from the liberation of cytost by any other means.

The shock which sometimes follows such infection is really due to cytost liberated as a result of the activities of the microörganisms upon the tissues of the host; for if the infected tissue is dissected out, extracted with water, sterilized, filtered, and injected into another animal, shock frequently ensues. This result must be due to cytost present in the extract, and not to the bacteria or their exotoxins, since the latter are respectively removed and inactivated by the processes of filtration and the temperature incident to sterilization. When a similar experiment is conducted with tissues infected with weakly proteolytic or non-

proteolytic organisms, shock does not usually follow the injection of the tissue extract.

Such experiments show conclusively that bacterial infection may lead to cytost intoxication, provided the infecting organism is capable of causing the liberation of cytost from the infected tissue of the host. In such a case, then, the host may suffer from the summation of two harmful factors: the cytost liberated by the activities of the organism on the tissues, and the specific toxins elaborated by the bacteria themselves. On this basis it should be clear that if prior to infection the animal has a high degree of resistance towards cytost, it may suffer less from the action of the infecting organism.

In connection with some experiments upon the diffusion of bacteria through the intestinal wall, the writer (Turck, 1914) has made some interesting observations which indicate that the joint action of bacteria and cytost leads to a distinct potentiation of the latter. Before discussing the reason for this, let us consider the experimental findings.

Dogs were opened under anesthesia, and a culture was taken from the lumen of the intestines. The contents (secretions) were removed from the duodenum and jejunum, diluted with four volumes of normal saline solution, and placed on ice until needed for subsequent stages of the experiment. This extract will be referred to below as S. The intestines were then removed, and divided into two sections, the first comprising the duodenum and upper jejunum and the second the lower jejunum and ileum. The mucous membranes were removed from these by scraping and then ground with four volumes of saline. These ex-

tracts will be referred to below as M_1 and M_2 respectively. Lastly, the muscular coat of the intestines was ground in a meat chopper and extracted with four volumes of saline for a few hours and then centrifuged to remove insoluble material. This extract of the muscle coat will be designated as C.

The *B. coli* obtained from the intestinal lumen was grown in broth culture for 24 hours; 10 cc. of the culture was mixed with 0.2 cc. of extract S and incubated for 30 minutes; then 0.1 cc. of M_1 was added, and incubation continued for another 20 minutes.

One tenth of a cubic centimeter of this mixture was injected into the marginal vein of a rabbit's ear, whereupon the animal developed symptoms identical with those of anaphylactic shock and died in three minutes. Injection of a similar preparation containing M_2 in place of M_1 led to the same end, although the onset of the anaphylactic reaction was considerably delayed. Similar injection of an equivalent quantity of the coli culture without the addition of the intestinal extracts did not cause the appearance of shock or death. Injection of a mixture of S and M_1 or M_2 in quantity equivalent to that employed in the first experiment led to a mild shock but death did not follow. Injection of a like quantity of a mixture of 10 cc. of the extract of the muscular coat (C) and 0.2 cc. of S caused anaphylaxis and death, although the onset of symptoms was not so rapid as in the experiments with the mixtures containing *B. coli*.

Essentially the same findings were obtained in the case of dogs, provided the amount of the injected material was increased proportionately to the difference in weight of these animals.

In another series of experiments the technique was modified to permit of a quantitative evaluation of some of these findings. Cultures of *B. coli* were obtained from the feces of the dogs used in this experiment. Samples of blood were taken from the femoral vein and from the branches of the mesenteric vein near the junction of the jejunum and duodenum. The jejunum was ligated at the duodenal junction and at a point 10 cm. below this. Sterile broth was placed in the jejunal loop so obtained and allowed to remain sufficiently long to obtain a rich culture of the microorganisms present on the intestinal wall. This broth culture was then removed by aspiration and replaced by a 48-hour broth culture of the *B. coli* previously obtained from the animals' feces and the abdomen was closed. After ten hours the abdomen was reopened and portions of tissue were removed from the duodenum near the pyloric junction and from portions above and below the ligatures, for histological examination. After washing, the mucosa of the duodenum, jejunum, and the upper part of the ileum were scraped from the muscle walls.

The blood samples, the mucosa and submucosa were frozen in carbon dioxide to prevent decomposition, and then dehydrated *in vacuo* over sulphuric acid. The dry residues so obtained were weighed and then brought into 2% suspension in 8% saline. These stock suspensions were then diluted from twenty to two hundred fold for subsequent experiments. One-cc. portions of these various dilutions were mixed with 0.5 cc. of a forty-eight hour broth culture of *B. coli* and incubated for 30 minutes. One-tenth cc. of these mixtures was then injected into the marginal ear vein

of young rabbits. When the anaphylaxis-like symptoms occurred within three minutes, shortly followed by death, the results were considered positive, whereas very slight convulsions, followed by recovery, or death after 24 hours or a week, were regarded as negative.

This decision appears to be legitimate, since very slight shock followed by rapid recovery frequently follows the injection of many substances; and the delayed death was usually found to be due to a generalized infection which obviously could not take place within the brief time necessary for attainment of the positive results following the injections.

The results obtained in this experiment are summarized in Table XIII below. The column headed Dilution refers to the dilution of the stock suspensions of the various extracts which were mixed with the *B. coli* culture as described above.

TABLE XIII

Dilution	1:20	1:50	1:100	1:200
Mucosa and submucosa	+	+	+	+
Submucosa	+	+	+	+
Serum from mesenteric vein	+	+	−	−
Serum from femoral vein	−	−	−	−

The plus sign denotes the appearance of distinct anaphylactic like symptoms within 3 minutes followed by death while the minus sign denotes that the animals showed but slight convulsions and subsequent recovery.

As a control upon these experiments it was found that double the amount of *B. coli* culture or of the various extracts employed could be injected singly without the onset of any untoward symptoms. Since,

however, *B. coli* and the mucosal extract together form a highly toxic combination, we must admit that a definite potentiation of some sort is brought about by the mere mixing together of these components. Apparently the presence of the microörganisms increases markedly the toxicity of the cytost initially derived from the mucosa.

That this is true is indicated by the fact that in the more concentrated dilutions of the extract of the serum taken from the mesenteric vein a toxic action was observed when it was used in conjunction with *B. coli,* whereas the serum from the femoral vein exhibited no such toxicity. This result indicates that the blood leaving the intestine contains a toxic moiety, presumably cytost, which is either excreted or altered in some fashion before it reaches the general circulation.

That the actual toxic agent in these experiments is cytost is evidenced from the fact that immediate autopsy of the rabbits which died a few minutes following the injection of the various toxic mixtures disclosed an acute venous dilation of the splanchnic vessels. Presumably, as in other cases of shock, this was the cause of the sudden collapse and death.

Just why the presence of the colon organism should so markedly intensify the action of the cytost is not entirely clear, although it seems highly probable that such intensification is due to a surface adsorption of the cytost by the microörganisms. As is well known, colloid particles are in general stabilized by an adsorbed ionic layer and the mutual precipitation of oppositely charged colloids involves the interaction of such surface layers. Since, as pointed out elsewhere

in the text, the splanchnic stasis which is so characteristic of cytost action appears to involve the agglutination of blood cells, it may be that the agglutinating effect of minute quantities of cytost is greatly magnified by the adsorption of the latter on various blood colloids or upon bacteria, which in many respects behave as colloids.

In agreement with this concept, it was found that a similar potentiation of tissue extracts containing cytost could be achieved by admixture with a dilute agar-agar suspension. That is, if, by preliminary assay one ascertains the maximal quantity of a potent cytost preparation which may be injected into an animal without the production of any noticeable evidence of toxicity, and then injects this quantity of cytost in admixture with a small quantity of a dilute solution of agar-agar, the symptoms of shock are found to follow immediately.

This finding strengthens our contention that the rôle of the colon organism in the experiments cited above is essentially that of a colloid which, by adsorption of the cytost, markedly potentiates the latter. This is of considerable interest, since it opens the way to a possible explanation of the interesting mysteries of the well known phenomena of anaphylaxis and anaphylactic shock. As is well established, these promptly follow the injection of a foreign protein into the circulation of any animal which, some weeks previously, has received a sensitizing dose of the same protein. Various ingenious hypotheses have been advanced to explain this puzzling phenomenon, but as yet no single theory has been found capable of accounting for all the known facts of anaphylaxis.

Whereas we cannot offer a comprehensive theory of anaphylactic reactions, the experiments cited above indicate that such a theory should include a recognition of the possible rôle of cytost in such reactions. In this connection it may be imagined that the initial or sensitizing dose of protein, bacterial or otherwise, brings about in some fashion the liberation of cytost in an amount sufficient to cause shock when adsorbed upon an appropriate colloid. Then the parenteral introduction of the second or intoxicating dose of the protein may furnish the necessary surface for the adsorption of and consequent potentiation of the cytost. This hypothesis does not account for the well known specificity of anaphylactic reactions, is necessarily vague, and is offered merely as a suggestion for the consideration of those interested in this field of investigation.

The essential point is that regardless of the mechanism of anaphylactic shock, it seems to be ordinary shock, such as we have discussed at some length, which is brought about in an indirect fashion involving the foreign protein introduced into the circulation of an animal. This fact has been recognized by Zinsser (1931) in his recent discussion of anaphylaxis. He calls attention to the fact that the typical symptoms of anaphylactic shock may be elicited by a variety of injuries which can have nothing in common with a true anaphylactic reaction. Zinsser suggests that the reason for this may be that the union of the antigen and its specific antibody on the surface of the body cells causes a surface injury similar to that brought about by trauma or other means.

From the above discussion it should be clear that

when any highly invasive microörganism gains access to the body we may expect an analogous behavior —that is, a cytost effect in some respects comparable to that obtained in the experiments cited. Obviously the severity of such an effect will depend upon both the cytost available for adsorption upon the bacteria and the invasiveness of the infecting organism. Further, if, as we have shown, the latter is capable of causing the disintegration of tissue and thence liberating cytost, the host is forced to cope with a threefold difficulty.

In previous chapters it has been shown that various chronic disturbances, especially of the organs of endodermal origin, may be brought about by the continued injection of sublethal quantities of homologous cytost. From this, it may be deduced that, by the combined action of bacteria and small quantities of homologous cytost for a protracted period of time, similar chronic conditions may be induced. This is a concept rather difficult to subject to experimental test, since animals vary so in the rapidity of their response to sublethal quantities of cytost. Nevertheless the conclusion seems logical.

It is relatively easy to understand the importance of all these considerations in the case of animals actually suffering from an acute infection, although at first sight they do not appear to be of special importance in the life of an animal not suffering from infectious disease. There are, however, a number of diseases known to science which have all the earmarks of being infectious, but for which up to the present time no specific causative factor has been found. In some instances such ailments may be traced to a non-

specific microörganism, while in others no microorganism appears to be concerned. In the first instance, it is known that a number of unrelated microörganisms may give rise to the same type of pathology. This of course indicates that the particular pathologies observed in such instances are due, not to the specific activities of the microörganisms concerned, but to a common factor resident within the host.

Such considerations suggested to the writer that cytost might be this common factor. With this in mind, experiments have been conducted towards this end, and it has been found that several so-called non-specific diseases may be simulated in animals by the injection of cytost. These experiments, which have been discussed elsewhere (see Chapter XIII), will not be treated of here, since they are not of general interest. In this connection, however, it should be noted that the rôle played by the non-specific organisms frequently associated with such diseases is probably akin to that of *B. coli* in the experiments described earlier in the chapter: that of an adsorbant which intensifies the action of cytost.

So far we have considered the relation of bacterial infection to cytost only from the standpoint of cytost formation and intoxication. Passing now to a consideration of the possible relationship between immunity to cytost and to disease, it is at once apparent that in the case of infection by proteolytic microorganisms which can liberate cytost, the degree of an animal's immunity to cytost will be a determining factor of some importance in his offensive against the invading bacteria. If, then, we consider all members of a given population to have equal chances of infec-

tion, it is obvious that those individuals having the greatest resistance towards cytost will be the best equipped to offset the ravages of the microörganisms. Consequently, the employment of any of the methods discussed in the chapter on natural resistance, which may raise an individual's resistance to cytost, may be expected to result in an increased resistance towards bacterial invasion.

It must be noted that such an increased resistance is non-specific, and would not offer an immunity towards specific toxins, such as diphtheria toxin. Nevertheless, that cytost may play a rôle in the development of specific immunity is indicated by some recent experiments of Bresredka (1931), who found that the application of friction to the shaved skin of a rabbit was sufficient to endow the animal with a local non-specific immunity towards bacterial toxins, which lasted for from twelve to fourteen hours. If during this period an ointment containing diphtheria toxin is applied to the skin, the animals are found to develop a specific immunity to the toxin. This is at first purely local, and later becomes general.

In a previous chapter we have called attention to the experiments of Lewis and his associates, which demonstrated that prolonged gentle friction on the unbroken skin leads to a localized reaction identical with that which follows the intradermal injection of tissue extracts. As previously stated, this indicates that such gentle friction is sufficient to cause the liberation of cytost.

Such being the case, it seems clear that the immune reactions observed by Bresredka were due to the release of cytost, which was brought about by the rub-

bing of the animal's skin. This seems all the more probable since he records that the immunity developed in this way is non-specific. Perhaps the specific immunity which follows the inunction of the diphtheria toxin is due to the combination of the toxin with the non-specific anticytost or cytost whose formation is brought about by the mild injury attendant upon the friction. Be this as it may, the experiments of Bresredka make it appear highly probable that a close relationship exists between cytost immunity and bacterial immunity.

These considerations clearly justify the statements of Zinsser (1931) that "Antibody production is after all only the expression of underlying cellular activities," and, "The forces of natural resistance are normal reactions." The writer feels that his investigations on cytost and anticytost reactions lend considerable weight to such views.

In a paper read before the Pan-American Congress in 1901 and published in 1904, the writer directed attention to this fact. To quote: "In another series of experiments, I found that when an animal is stimulated by heat applied within the splanchnic area by methods previously described, immunity or resistance against infection was thus produced. When the serum of such an animal was injected into another animal there was obtained an increased resistance or partial immunity to infection. Animals so protected could not always be saved but death from infection was retarded, sometimes for one or two weeks." And again: "These facts establish a most important point in the pathology of shock, namely the alteration of the tissue cells and blood in shock."

As shown early in the text, excessive heat applied to the splanchnic area results in varying degrees of shock which, as we have seen, is due to the liberation of cytost from the tissues. The increased resistance to infection which was observed in the animals so treated must have been due to the cytost released into the circulation by the thermal injury of the splanchnic tissues. In this respect then, these early experiments of the writer are in harmony with the more recent findings of Bresredka cited above. Indeed, as one examines the recent writings of prominent immunologists, one becomes impressed by the fact that the experts in this field are beginning to realize that cellular products liberated as a result of tissue damage are of paramount importance in the development of immunity, both specific and non-specific. In this connection, the reader will find much of interest in the reviews of Manwaring (1930), Zinsser (1931), and Irons (1931).

The localized formation of cytost due to the action of bacteria in the tissues of a higher animal may lead to the operation of other defense mechanisms. As is well known, when a local infection resulting in abscess formation takes place, a migration of phagocytes to the site of infection occurs, and the body attempts to protect itself against a generalized infection by walling off the infected tissues by the development of a barrier of connective tissue.

The importance of both these types of defense mechanism has been recognized for a long time, but the manner in which they are brought into play is obscure. The migration of leucocytes to a site of infection is usually classed as a chemotactic response,

although in general little definite commitment has been made as to the exact nature of this response. Such a migration of leucocytes is found to take place following any kind of tissue injury, such as mechanical mutilation or the application of irritating chemicals such as chloroform or croton oil, as well as after bacterial infection. It would seem, therefore, that the specific nature of the cause of injury is of no importance in determining the migration of the white cells, but that an endocellular substance liberated from the tissues is responsible for the chemotaxis.

It is possible that cytost may be such a substance, for we have frequently observed an increased leucocyte count in animals at short intervals following the injection of extracts of autolyzed tissue. This observation suggests that the migration of leucocytes to the site of infection is brought about by the cytost liberated from the tissues of the host by the activities of the invading organisms.

We have shown elsewhere in the text that when present in appropriate concentration cytost stimulates the rate of proliferation of cells. With this in mind, it seems likely that the connective tissue barrier which is thrown about a localized infection may result from the stimulus afforded by the cytost liberated by bacterial action.

From the above it should be clear that as a result of bacterial infection cytost may be liberated, and in consequence the animal may suffer from a cytost intoxication as well as from the specific disease engendered by the bacteria. The liberation of cytost in this manner may cause serious consequences, or it may stimulate the defense mechanisms of the host in such

a fashion that an effective barrier to generalized infection results.

This conclusion may appear paradoxical, but it is no more so than the well established relationship between toxins and the formation of their specific antitoxins.

The colon of higher animals always contains large numbers of various bacteria; indeed, it has been estimated that from 5 to 20 per cent of the dry weight of the fecal mass consists of bacteria. Under normal conditions the bacteria present in the colon do not appear to cause the host difficulties of any sort. Occasionally, however, these organisms do gain access to the body and infection results. Many years ago the writer had occasion to investigate this matter. It was found, by the microscopic examination of appropriately stained sections of the intestinal tract of animals which had been fed cultures of *B. coli,* that the bacteria may diffuse into the intestinal wall, passing between the epithelial cells and between the muscle cells of the muscular mucosa into the areola tissue. (Turck, 1909, 1910.) Later it was found that this process could be followed very easily in fetal animals after injection of bacterial cultures into the intestine. (Turck, 1908, 1914.)

In all instances it was observed that as the diffusing bacteria entered the submucous zone rapid bacteriolysis took place. In consequence, this zone of bacterial destruction was named the "Zona Transformans." Presumably it is the zone which normally protects an animal from infection by the organisms present in the intestinal tract. If for any reason the Zona Transformans ceases to function in its normal capacity, then

it ceases to exercise its protective function and the varied intestinal flora may gain access to the body.

When shock is induced in animals it is found that the nature of the intestinal wall is so altered that it becomes readily pervious to bacteria. Thus the abdomen of animals under ether anesthesia was exposed to an air blast until marked venous stasis and shock were produced. After recovery from the latter, some of the animals were fed cultures of *B. coli*. A day later sections were obtained from the intestine. Both in animals that had been fed the cultures of bacteria and those which had not, it was found that bacterial invasion had taken place.

A similar result was obtained with animals which had been brought into shock by prolonged anesthesia. (Turck, 1914, 1917.) Curiously enough, the bacterial invasion was not accompanied by leucocytic infiltration or any other evidences of an inflammatory process.

The essential point to be noted here is that interference with the splanchnic circulation which always occurs in shock lessens the ability of the Zona Transformans to cause the bacteriolysis of the invading organisms. It follows from this, and the fact that cytost intoxication always leads to a greater or lesser degree of splanchnic stasis, that such intoxication must be regarded as a predisposing cause of infection by way of the intestinal route.

The bacteriolysis which we have found to take place in the Zona Transformans opens the way to an understanding of the diffusion or non-diffusion of other substances through the walls of the intestine. As is well known, protein molecules do not pass through

the intestines in most individuals. Since, however, the size of the protein molecule or micelle is very considerably less than that of the bacteria used in the author's experiments, one might expect them to diffuse even more readily. When it is remembered that the process of bacteriolysis may involve the action of proteolytic enzymes, it seems likely that the non-absorption of unhydrolyzed protein is possibly due to the proteolytic activity of the Zona Transformans.

Some individuals are sensitive to certain foodstuffs which upon ingestion give rise to anaphylactic reactions of one sort or another. It would seem that this effect must be due to the absorption of specific unhydrolyzed protein which for some reason or other succeeds in passing the Zona Transformans in the same fashion in which we have found bacterial invasion to take place in animals wherein the shock was produced.

At this time we do not wish to stress the medical aspects of these concepts, but it may not be amiss to point out that the treatment of infectious diseases might be more intelligently prosecuted if the ideas developed in this chapter were kept in mind by the practitioner. By this we do not wish to imply that the use of specific antisera and medication should be superseded, but rather that such methods of proven value might reasonably be supplemented by such methods as lead to a lessened susceptibility to cytost. Those interested in the writer's conclusions in this regard should consult Chapter XIII, wherein this topic is more fully discussed.

Chapter XI

INVESTIGATIONS CONCERNING THE NATURE OF CYTOST

It has been pointed out in previous discussion that extracts of autolyzed tissue, if boiled for some time, maintain unimpaired their toxicity for animals. This indicates that the substance in such extracts which is responsible for the various effects described in preceding chapters is quite stable. Furthermore, if solutions of cytost are made either alkaline or acid and then boiled, the toxic substance is unaffected. Consequently its molecule cannot be readily hydrolyzable, as is that of a simple peptide.

Sterile solutions of cytost have been kept in sealed ampules for years without any apparent loss in activity, and dry samples of tissues and tissue extracts likewise maintain their toxic nature.

Recently confirmatory evidence of the extraordinary stability of cytost has come from a quite unexpected source. The noted archeologist, Flinders Petrie (1932), in his book *Seventy Years in Archeology,* records that contact with the mummy dust caused the onset of various respiratory disturbances, headaches, and colds. Two statements of Petrie are illuminating (page 32): "All work in the pyramid

was unwholesome, owing to the dust raised which always produced feverish headaches after a few hours of it." And (page 94) "The effect of being surrounded with so much organic dust from the mummies was to give me a bad infection of the breathing passages."

Now it may be recalled that we have found that the endodermal cells of the lung are peculiarly sensitive to cytost and that various respiratory disturbances varying from sneezing to a true pneumonia could be induced by the introduction of minute quantities of cytost into the respiratory tract of animals. This result was accomplished by the simple expedient of spraying the cages of animals with a potent extract of autolyzed tissue as well as by coating the paws of cats with a paste containing cytost.

It would seem that the discomfort experienced by Petrie and his associates when in contact with the mummy dust was essentially the same as that experienced by the cats whose cages had been sprayed with cytost. One may raise the argument that exposure to any atmosphere containing finely particulate matter will cause some respiratory distress. This of course is true, but in general unless the dust contains some toxic substance its effects are purely mechanical and pass away very shortly after the exposure.

Such, however, is not the case with cytost, for, as our experiments have shown, cytost-laden air induces pathological conditions which last for some time. In this connection, it is of interest to note that Petrie complains that the mummy dust caused headaches and "colds" which prevented further work for a few days, and this is what we should expect from exposure to cytost, but not from exposure to a dry, non-toxic dust.

There have been a number of deaths among those engaged in the excavation of ancient tombs, and it is said that the Arabs consider this as the vengeance of the dead upon the excavators. Be this as it may, the observations just mentioned indicate that the dead may retain their cytost intact for centuries, and one might reasonably imagine that some of the deaths among the archeological explorers have been due to overexposure to the cytost-laden dust of which Petrie speaks.

To return to the experimental aspect of the problem, we have noted in our discussion of the induction of shock by severe burns that extracts of the charred tissue, when injected into other animals, caused shock. This observation suggested that the tissue might be charred *in vitro* with the same results, and this was subsequently confirmed by experiment. Naturally this finding evoked an interest in the determination of the degree to which charring could be carried without loss of cytost activity. To our amazement, it was found that excised tissue could be burnt to a gray ash without greatly diminishing its potency.

Such an ash when dissolved in water to form a 10% solution and injected intravenously into animals caused shock and death, provided a sufficient quantity was injected. Thus, 1 cc. of a 10% solution of the ash obtained from cats' muscles, when injected in this manner into cats weighing approximately 2500 grams, will always cause the immediate onset of shock and usually produce death within a very brief time after the injection.

In order to obtain tissue ash of such toxicity, it is necessary that the tissue be fired at relatively low tem-

peratures—in the neighborhood of 300° C., for at higher temperatures the ash loses its toxicity. It becomes practically inactive if fired at 700° C. in an electric furnace. In the experiments discussed below, the various tissues were always ashed at a temperature approximating 300° C.

In order that we might compare the toxicity of various cytost preparations, we have adopted as a convenient reference standard a solution containing 0.112 gm. of ash in 1 cc. This was prepared by extracting a weighed quantity of a given ash with sufficient water to yield a solution of this concentration, boiling for a few minutes, and then removing the slight quantity of insoluble material by filtration.

Various quantities of a solution of the ash of guinea pig muscle prepared in this way were injected subcutaneously into a large number of guinea pigs. As much as 1.5 cc. of the solution per 100 grams was injected in this manner without the occurrence of a single death.

Animals which received such large injections were ill for a day or two following the injection, but made uneventful recoveries within a few days, although the skin sloughed off at the site of injection.

Much more striking results were obtained by injecting the solution of the ash intraperitoneally. When injected in this manner, $\frac{3}{16}$ cc. of the solution proved lethal to all of 10 guinea pigs which received this quantity. A few minutes after the injection the coats of these animals appeared ruffled, and their respiration rate increased. Many of them vomited, and all died within from 15 minutes to 2 hours following the injection of the cytost. Slightly larger doses—i.e.,

¼ cc.—always brought about death within fifteen minutes, and in many instances within several seconds following the injection. At autopsy, immediately following death, all the animals killed in this way exhibited the typical pathology of "death by shock"; the lungs and abdominal viscera were always found to be markedly congested.

This extreme rapidity of the onset of symptoms, and death, shows that the ash itself must be toxic, and that the typical cytost effect obtained is due to the ash and not to the liberation of cytost from the animal's own tissues by the action of the ash thereon. Further evidence for this argument is found in the marked difference in the response of the animals to the ash solution when injected subcutaneously. In the latter case the sloughing of the skin at the site of injection shows that the ash may act locally, causing the death of tissues. The rate of absorption, however, is considerably less than in the case of the intraperitoneal injections; hence the viscera are not suddenly exposed to a sufficiently great concentration of cytost to cause congestion, stagnation, and death.

Similar experiments were carried out upon rats and cats with the ash of homologous tissues, and qualitatively the experimental findings were essentially the same.

We have stated earlier that saline extracts of autolyzed tissue appear to have a certain species specificity when the power to elicit shock is determined. Naturally, this raised the question as to whether or not the tissue ash would show a similar specificity in this regard. To this end experiments, of which the following are representative, were conducted:

A solution of the ash obtained from cats' muscles was prepared, containing 0.112 gm. of the ash per 1 cc. of solution. This was injected intraperitoneally into guinea pigs in order to determine the minimal lethal dose of the solution. Proceeding in this fashion, it was found that the minimal lethal dose of the solution when injected intraperitoneally was from 3/8 to 1/2 cc. per 100 gms. The lesser quantity caused the death of 50% of the injected guinea pigs within 48 hours, and 75% within 72 hours, whereas the higher quantity always caused death within 15 minutes. In consequence, it must be concluded that qualitatively the guinea pig and cat tissue ash both contain something toxic to guinea pigs. When, however, one compares the minimal lethal doses of the two solutions, it is noticed that on a weight for weight basis the solution of guinea pig tissue ash is more toxic for guinea pigs than is the solution of cat tissue ash.

This is made evident by comparing the quantities of the two solutions which are necessary to cause the death of guinea pigs within fifteen minutes. In this respect, 1/2 cc. of the cat tissue ash solution containing 0.056 gm. of ash is equivalent to 1/4 cc. of the solution of guinea pig ash containing 0.028 gm. of ash. Considering the weights of the ash involved, it is apparent that the homologous ash is twice as potent as is the heterologous.

The same result was obtained when the ash of beef, dog, and rat tissue was substituted for the cat tissue ash used in the above experiments. In each instance it was found that solutions of those ashes were all capable of causing the death of guinea pigs when injected intraperitoneally, but that the amounts necessary to

cause death within fifteen to twenty minutes was always greater than in the case of guinea pig tissue ash.

Such differences in the toxicity of the tissue ash of various species was also observed when they were injected intravenously, the route which causes the most rapid onset of shock. For comparative purposes 10% solutions, in normal saline, of the ashes of various animal tissues were employed and were injected into the jugular veins of the experimental animals.

When injected in this fashion into rats weighing 150–200 gms., a 10% solution of the ash of rat tissues was found to cause the onset of shock and death in from 5 seconds to 2 minutes. When rats of the same size received ¼ cc. of a 10% solution of cat tissue ash, they developed a slight shock, but usually recovered in about 10 minutes. This result shows definitely the existence of a species specificity in the ash.

A large number of similar experiments were carried out with cats, using 10% solutions of the ash obtained from rat, horse, beef, and lion tissues. When 1 cc. of such a solution of homologous tissue ash was injected into cats weighing from 2500 to 3000 gms., it was found that the animals always passed into severe shock and frequently died immediately. When such was not the case, the cats always died within two or three days after the intravenous injection of this quantity of cat tissue ash. When like quantities of the tissue ash of horse, dog, beef, or lion muscle were substituted for the cat ash solution, the injected cats of similar weight often showed no ill effects whatsoever. At other times they developed a mild shock from which they made uneventful recovery within less than an hour.

For some curious reason the ash obtained from rat

tissues was remarkably toxic to cats. Three cats weighing respectively 2790, 2115, and 2470 grams died within less than a minute after the intravenous injection of 1 cc. of a 10% solution of rat muscle ash, while a fourth cat, weighing 2850 gms., developed a mild shock from which it recovered in 10 minutes, and remained in good health for two weeks, when it was used in another type of experiment.

The species specificity which is evidenced in these experiments is also brought out by the reaction of cats to intraspinal injections of solutions of homologous and heterologous tissue ash. Ten per cent solutions of the various ashes were injected intraspinally into cats. The solutions of homologous tissue ash when administered in this fashion caused shock, paralysis of the hind limbs, and in some instances death, whereas equal quantities of the ash of heterologous tissues caused only slight transitory symptoms from which the animals promptly recovered. The results of representative experiments of this type are recorded in Table XIV. (Turck, 1921.)

These experiments again show the same type of species specificity observed in the other experiments. While 0.25 cc. of the solution of cat ash equivalent to 0.025 gm. of ash evokes distinct reaction, four times this amount of the ash from human, horse, and lion tissues causes the recipients but little inconvenience. It is especially interesting to note that of the three heterologous ashes employed in equal quantities, the ash from lion tissue caused the most distinct reaction, although this was far less severe than that caused by an equivalent quantity of cat ash. Phylogenetically the lion is closely related to the cat; hence this find-

TABLE XIV

EFFECTS OF INTRASPINAL INJECTIONS OF ASH SOLUTIONS

CAT NO.	ASH	AMOUNT INJECTED	RESULT
149	Cat	1.0 cc.	Deep shock for 30 minutes. Complete paralysis of hindquarters. Died in 8 days.
155	Cat	1.0 cc.	Deep shock, prolonged coma. Died in 12 hours.
158	Cat	0.25 cc.	Slight shock, paralysis of hindquarters. Died in 7 days.
192	Cat	0.25 cc.	Deep shock, delirium, marked incoördination. Recovered.
150	Human	1.0 cc.	Slight delirium only. Recovered in 30 minutes.
157	Horse	1.0 cc.	No evidence of any reaction.
161	Lion	1.0 cc.	Very slight shock. Recovered completely in 1 hour.
180	Lion	0.25 cc.	Marked delirium. Recovered in 30 minutes.

All the injections were made in the last lumbar space.

ing suggests the existence of a possible similarity between the composition of the ash of closely related species. (See Figure 15, facing page 262.)

One must not, however, confound the apparent species specificity of the various ashes with the remarkable protein specificity of different species which has been so carefully investigated by Nuttal (1904) in his studies on the correspondence between immunological relationships and accepted zoölogical classifications. Thus we have shown that the ash obtained from rat tissues, when injected intravenously, is about as effective as cat tissue ash in causing shock and death. Further, in the experiments on guinea pigs it was shown that following intraperitoneal injection all

the heterologous ashes could cause death, provided the dosage was sufficiently high. The relative species specificity became strikingly apparent only when we compared the minimal lethal doses of the heterologous ashes with that of the homologous ash.

It follows from this that the apparent species specificity which we have observed in our experiments is due not to a particular component of the ash unique to a given species, but rather to the quantitative distribution of a component common to the ash of several species. Thus if it be assumed that a certain concentration of this component per unit weight of ash is necessary to elicit in the cat the various symptoms which follow injection, it may be imagined that the concentration of this component in the ash of rat tissues is comparable to that which exists in the ash of cat tissue, in consequence of which solutions of the same strength of these two ashes will be about equally toxic when administered to cats by any of the routes we have mentioned above. Conversely, we may assume the ash of other heterologous tissues which we have used in our experiments to contain less of the active component than cat or rat tissues; hence when injected into cats a larger dosage of such ashes will be necessary to evoke reactions comparable to those obtained with cat and rat tissues.

This concept is purely speculative, but is suggestive of further experiments. Thus, if it is true, one should be able to ascertain a quantitative relationship between the toxicity of a series of heterologous ashes for animals of different species.

As yet we have no inkling as to the nature of the toxic component present in tissue ash. One is strongly

tempted to postulate that it is inorganic in nature, since it resists the temperatures incident to ashing. Our experiments have shown this to be unlikely, however, for, as mentioned earlier in this chapter, the ash may be inactivated if heated to too high a temperature during the ashing procedure. While very high temperatures might lead to the slow volatilization of some inorganic compounds, it is highly improbable that this would be great enough to cause the complete loss of an inorganic compound which determined the toxicity of the ash. Furthermore, injection of various salts of the metallic elements commonly found in tissue ash does not elicit shock in animals; hence it seems that such substances cannot be responsible for the effects observed in the experiments we have discussed above.

Since the ordinary methods of chemical analysis might fail to detect the presence of minute quantities of various elements present in the ash, we have subjected various samples of the active ash to spectrographic examination. This was carried out with a Hilger quartz spectrograph and by the arc method. For the most part high purity copper was used as electrodes, although in some instances aluminum and carbon were substituted. Typical spectrograms of the ash obtained in this manner are shown in the accompanying spectrographs, wherein the lines corresponding to particular elements have been marked with their respective symbols.

Ash obtained from the tissues of the cow, cat, hen, fox, guinea pig, horse, lion, rabbit, rat, dog, and human were examined. Examination of the spectrograms discloses the presence of sodium, potassium, calcium,

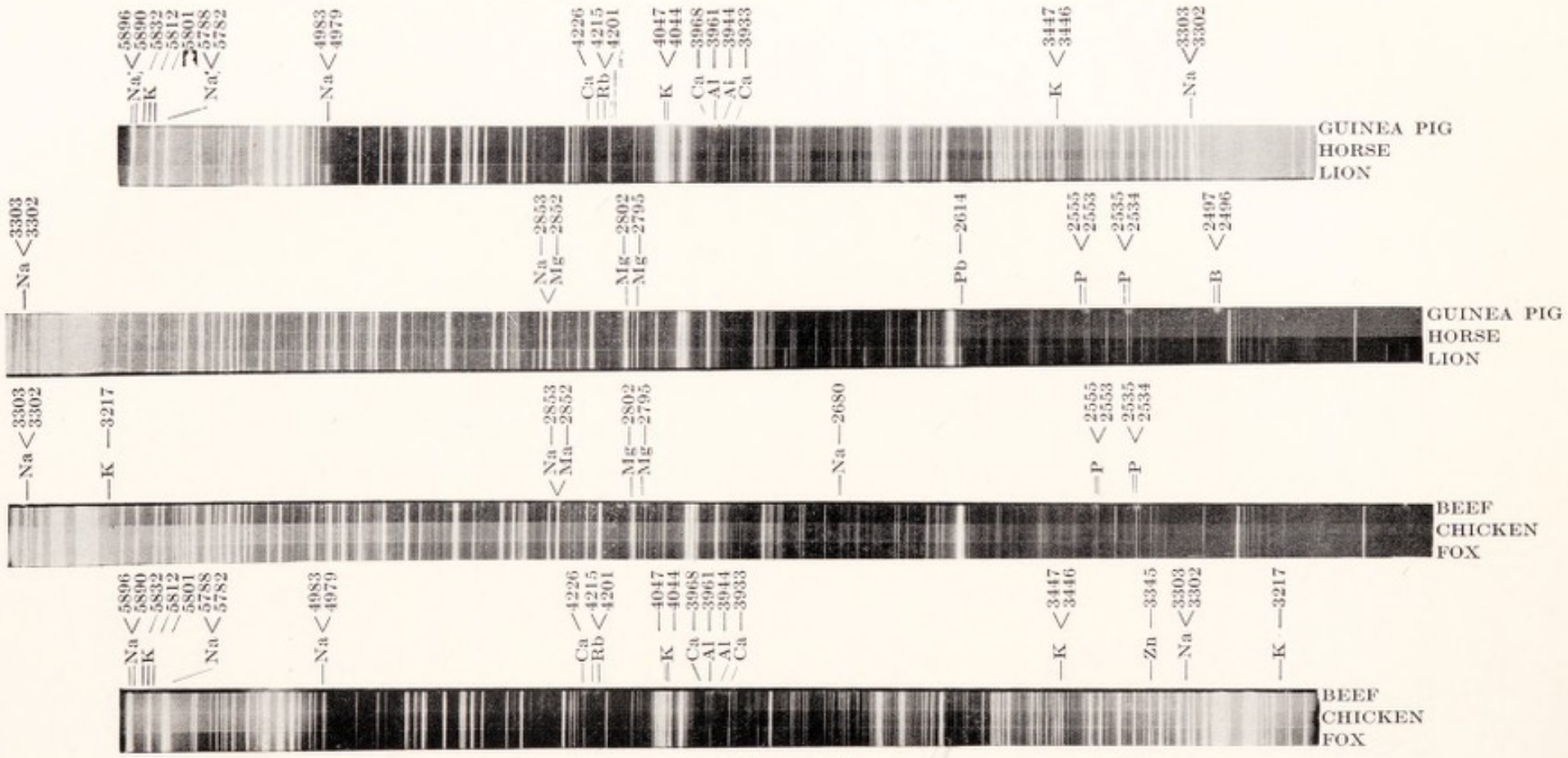

Figure 14.

Spectrograms of the ash of guinea pig, horse, lion, beef, chicken and fox muscle. These spectrograms were taken with a Hilger E1 quartz spectrograph with an aluminum arc. The principal lines corresponding to particular elements and marked with the symbols of these elements.

magnesium, and phosphorus in all samples examined. Rubidium was present in all the tissues except that of the dog. Traces of boron were found present in the ash of rabbit, rat, dog, and human tissues, traces of lead in the lion tissue, and traces of zinc in the fox tissue, and of aluminum in the cow and horse tissues.

While these findings were of no especial interest in connection with the cytost problem, the presence of rubidium in all the tissues examined excepting those of the dog is interesting. So far as the writer is aware, no biochemical significance has been attached to this element, although various investigators have from time to time noted that rubidium salts may be substituted for those of potassium in so-called physiological balanced salt solutions. The traces of heavy metals found in some of the tissues examined are probably attributable to the retention of metals derived from the utensils used in feeding and watering the animals.

For the present we must conclude that when tissues are ashed at low temperatures in the neighborhood of 300° C., they retain some markedly thermostable organic constituent which is responsible for the physiological reactions which follow the injection of solutions of the tissue ash into animals. As we have mentioned before, heating of the tissue ash of all species to 700° in an electric furnace causes the ash to lose its toxic properties. This is exactly what one would expect if the toxic moiety present in the ash were really an organic compound.

While, as was pointed out at the beginning of the text, we are in complete ignorance as to the exact chemical nature of cytost, the facts stated above testify

to its unique nature and will perhaps serve as an orientation for any chemically minded investigator who wishes to extend our experiments. At any rate, the study of the effects of tissue ash upon the activities of cells and tissues appears to offer an interesting field of experimentation.

In this connection it is of interest to note the recent investigations of Alexander, Weaver, and McConnell (1930), who found that the size of wheals caused by the subcutaneous injection of histamine was considerably enlarged if the latter were mixed with an aqueous extract of skin prior to the injection. In a later paper the same authors report that the substance in the skin which augments histamine wheals resists ashing temperatures, and on the basis of their experiments they suggest that it is calcium sulphate. (Weaver, McConnell, and Alexander, 1931.) Further, they found that the wheal forming powers of atropine and codeine are likewise augmented by skin extracts, and they state that it is likely that constituents of the ash other than calcium are capable of augmenting the localized action of histamine.

At first glance these conclusions may not appear relevant to our investigations. But if the reader will recall that it has been pointed out previously in connection with our discussion of shock that extracts of autolyzed tissue possess a histamine-like action, this discussion may strike a responsive chord. Various considerations discussed in earlier chapters have led to the conclusion that cytost is always present to a greater or lesser extent in the normal animal. Such being the case, it might be imagined that the introduction of tissue ash into the circulation of an animal

brings about an intensification of the action of cytost already present in much the same fashion as that in which Weaver and his associates have found the ash to intensify the localized action of histamine.

While this is an hypothesis which merits serious consideration, the author feels that it is incorrect. This objection is based upon the observation previously mentioned that ignition of a tissue at 700° C. leads to the formation of a virtually non-toxic ash. Such an ash must contain all the inorganic substances which were present in the original tissue. Consequently the toxicity of the ash obtained at lower temperatures cannot be due solely to the augmenting effects of such inorganic substances upon the cytost already present in the tissues of the experimental animal. It is obvious that further experimentation will be necessary to settle this point definitely.

Chapter XII

CYTOST IN THE PLANT WORLD

In the preface the writer has drawn attention to his earliest experiments with plants, conducted over forty years ago. At that time nothing was known of the rôle of cytost in animal physiology. In view of the experiments with animals it is interesting to recall these early experiments, for, curiously enough, they are capable of interpretation from the standpoint of the cytost hypothesis.

For many years it has been generally recognized that plants cannot be grown in the same soil for an indefinite succession of years, a fact commonly expressed in the agriculturists' "law of the minimum," which states that as soon as the concentration of certain elements in the soil has reached a minimum level, plants are unable to withdraw those elements in a quantity sufficient to permit growth. If, however, such elements are added to the soil, normal growth of the plants ensues. This is the essence of the well-known effect of commercial fertilizers on plant growth.

In the author's experiments referred to above it was observed that upon the addition of rich virgin soil to exhausted earth, healthy growth of transplanted

fronds did not ensue until the ratio of virgin soil to exhausted soil exceeded one half or two thirds. Of itself this observation is of no moment; but incident to the comparison of growth in various mixtures of exhausted and virgin soil it was observed that transplanted fronds grew more rapidly in a mixture of virgin soil with 5 to 10 per cent of the exhausted soil than in the virgin soil alone. This curious observation, which went unexplained for some years, may be readily understood if viewed in the light of our experiments with animal cells.

It will be recalled that in the latter case it was found that small quantities of homologous cytost appear to stimulate the anabolic metabolism and growth of cells, whereas higher concentrations of the same substance exert a distinctly toxic action. Transferring this concept to the plant world, we may reasonably assume that the accelerated growth of plants in the soil mixtures containing small quantities of the exhausted soil is due to the fact that the latter contains homologous cytost, which stimulates the growth of the plant cells.

Again, as noted above, considerable virgin soil must be added to exhausted soil in order to render it capable of supporting a rich growth. Much smaller quantities of the virgin soil should suffice to replenish the exhausted essential elements; hence we must conclude that the high ratios of virgin soil found necessary to stimulate plant growth play a dual rôle. Aside from supplying essential elements, it must have acted as a diluent for toxic products present in the exhausted earth. That is, by the addition of the virgin soil, the concentration of cytost present in the exhausted soil

must have been lowered to a level compatible with the growth of the plant.

Recently H. E. Dolk and K. V. Thimann (1932) have been able to isolate a substance capable of accelerating plant growth by culturing *Rhizopus suinus* in such manner that a constant stream of culture fluid passes through the vessels in which growth takes place. The continual passage of the culture fluid through the apparatus washes away the "growth hormone," as it is formed. From their experiments the growth-accelerating substance which they obtained appears to be an organic acid easily susceptible to oxidation, whereby it loses its growth-accelerating activity. This easy destructibility by oxidation indicates that this substance differs markedly from the active principle present in the various cytost preparations used in the author's experiments. But, as has been pointed out previously, in the case of animal cytost a marked species specificity appears to exist. Fundamentally such species specificity must depend upon differences in the chemical constitution of the various cytosts. In consequence, there is no reason why the chemical constitution of plant cytost or "growth hormones" should not vary markedly from that of the corresponding animal compounds.

Unfortunately, since the development of the cytost theory the author has not had available the facilities of a greenhouse wherein further experiments with plant cytost could be made. Nevertheless a considerable literature has accumulated in recent years which indicates that endocellular products such as cytost are of importance in plant physiology.

In the first chapter attention was drawn to the fact

that phototropic, stereotropic and chemotropic responses in plants depended upon the release of some endocellular agent by light, contact, pressure, or cauterization. This view was championed by the late Jacques Loeb and the earlier literature on this subject is reviewed in his book *Forced Movements, Tropisms and Animal Conduct.* (1918.) More recently, Cholodny (1927) has reviewed this problem, with particular reference to plants. He points out that all the various tropistic responses of plants, which are essentially growth changes, can best be explained upon the assumption that, due to pressure, injury, or light, the plant cells give rise to a "Wachshormone" which, by diffusion to various parts of the plant, brings about alterations in growth, the asymmetrical growth characteristic of tropic curvatures being due to an uneven distribution of the growth-promoting substance.

A few years ago Paal (1918) presented evidence for the presence of such a substance in the tips of coleoptiles of grasses. Later Went (1928a) succeeded in extracting such substances from the coleoptile tips and devised a quantitative method of estimating the activity of such extracts in accelerating the growth of plants. The latter author (Went, 1928b) has also demonstrated that the quantitative differences in growth on the two sides of a seedling which follow unilateral illumination must be due to changes in the concentration of the growth-promoting substance on the illuminated side.

When plants are subjected to continual friction, fungus infection, or chemical irritants, they frequently give rise to pathological outgrowths or intumescences.

Wallace has recently made a careful histological examination of such outgrowths, following the action of ethylene on the buds and stems of the apple, *Pyrus Malus,* Var. Transparent. Under the action of 1% ethylene in air, it was observed that the walls of the cells were corroded away by a sort of solution process (autolysis) induced by the ethylene. Individual cells and groups of cells then separated from the tissues. This process involves all the living elements between the cambium and phellogen, and the cells may be stimulated to division or become hypertrophied. In some instances cell counts in the intumescences produced at the ends of cutting showed a thirty-three per cent increment in numbers.

It is interesting to compare these findings of Wallace with the writer's observations on the prolonged irritation of the gastric mucosa by mustard. In the latter case, it may be remembered that a somewhat analogous situation was found, the mustard oils first causing a degeneration and loosening of the mucosal cells, whereas later even though the mustard was removed, the underlying tissues were found to show evidences of active proliferation. It was deduced (page 33) from the experimental findings that the proliferation and other changes were secondary changes which arose not from the direct action of the mustard but because of the presence of cytost liberated from the mucosal cells injured by the mustard.

If we transfer this concept to the problem of plant intumescences, we may imagine the phenomena observed by Wallace to take place as follows: The plant cells in contact with the ethylene suffer injury in much

the same fashion as the mucosal cells in contact with mustard. As in the latter instance, the injury leads to autolysis and the consequent liberation of cytost. This substance in turn attacks adjacent cells, thereby giving rise to the "solution effect" described by Wallace, and those cells not exposed to too high a concentration of the plant cytost are stimulated to division.

Conceivably any other injury capable of liberating cytost from the plant tissues should bring about a similar situation. This is testified to by the well known fact mentioned above, that the stimulus to intumescence formation may take the form of abrasion, fungus infection or chemical substances; although so far as the writer has been able to ascertain the effects of such stimuli have not been examined histologically in a manner such as Wallace employed in his study of the action of ethylene. This would appear to open an interesting field of study and it is hoped that such investigations will be carried out.

The localized stimulation of growth which takes place in the formation of intumescences is presumably identical in nature with the various growth processes which accompany the tropistic bendings of plants under various physical influences, and it may be worth while to consider a few of these. The coiling of the tendrils of phanerogams, the most sensitive of all plant structures, has long been of interest to botanists. The investigations of this curious phenomenon by Darwin (1882) and Pfeffer (1885) have thrown considerable light upon the conditions prerequisite for such coiling. At the outset it was determined that tendrils are stimulated to depart from a linear path of growth by contact with a solid body

such as a stick or plant stock. This of course suggested frictional irritation as the factor responsible for the onset of a more or less circuitous growth path. Experiments, notably those of Pfeffer, disclosed that contact with any rigid body suffices to induce tendril-coiling, but that contact with moist gelatin, water, oil, or mercury did not lead to a similar thigmotropic response. Further experiments of Peirce (1894) showed that, while contact with glass rods led to thigmotropy, such was not the case if the rods were covered with a layer of moist gelatin. On the other hand if sand is incorporated into the gelatin, then a normal coiling response ensues. Peirce found further that dry gelatin stimulated the coiling of tendrils in much the same fashion as did any other solid body; therefore the failure of the gelatin coated rods to excite such coiling cannot be attributed to any specific chemical character of the gelatin.

In consequence it must be concluded that the surface of the gel covered rods was not possessed of sufficient rigidity to permit the development of the degree of friction necessary to excite the usual thigmotropic response.

It seems reasonable to assume that such frictional force must attain a certain minimum value in order that the surface cells may become sufficiently irritated to discharge their cytost. In this connection it is of interest to recall the experiments of Sir Thomas Lewis and his collaborators on the production of a skin tache and subsequent wheals by stroking the forearm with a blunt instrument. A considerable amount of such stroking is necessary in order to elicit capillary dilatation and subsequent wheal formation. Nor is this

surprising, since the human skin is constantly subjected to mild friction from clothing, etc. In other words, from Lewis's experiments it appears that the degree of friction is important in determining the extent of the cellular irritation which leads to the discharge of the endocellular substance responsible for wheal formation. There seems to be no valid reason why a similar state of affairs should not exist in the plant, although of course the cells of the tendrils must be considerably more sensitive to friction than are those of the human skin. Indeed, it is recorded by MacDougal (1896) that the tendrils of *Echinocystis* will respond to the frictional forces incident to contact with the delicate thread of a spider's web.

Similar stereotropic responses in the roots of seedlings have been observed by Sachs (1873) and many later investigators. This author found that when a pin was placed in contact with the rapidly growing roots of various seedlings the growing region became concave within about ten hours. More recent investigations have disclosed that in such stereotropic responses the actual growth processes leading to the tropic curvature takes place, not at the site of contact between the stimulus and the growing root tip, but at a short distance from it. In this respect the behavior is comparable to that which obtains in phototropic bending.

Curiously enough, however, it is only the delicate growing root tip which is able upon contact to give rise to the growth-promoting substance—a phenomenon comparable to the observation that decapitated coleoptile tips do not respond to light. In this connection the experiments of Cholodny (1926) are il-

luminating. This author found that when the root tips of *Zea* were decapitated stereotropic responses could not be elicited. When, however, coleoptile tips from the same plant were transplanted to the decapitated root tips, restoration of stereotropism followed. Conversely, when the root tips were placed upon the decapitated coloeptiles, it was found that restoration of growth ensued.

These ingenious experiments show conclusively that the sensitive tissues of both coleoptile and root tips are capable of elaborating a substance which controls the growth of plant tissues, and the inference follows that in both instances the growth-stimulating substance is the same. If this be accepted, then it is clear that both phototropic and stereotropic behavior depend upon a common factor, a substance liberated from the sensitive tissues by the external physical stimulus.

Traumotropism, or responses to wounding, is closely akin to thigmatropism, and this we should expect, on the basis of the cytost hypothesis; for, as has been shown, in the case of animals the same cell substance appears to be liberated regardless of the agency employed to bring about the injury of the cell. Long ago Darwin (1881) injured bean radicles by the localized application of silver nitrate. Within a day the radicles exhibited a marked curvature away from the wounded side. A variety of other caustics, such as copper sulphate and potassium hydroxide, have been found to lead to the same type of response; and Spaulding (1894) achieved the same end by the cutting of a thin slice from the tip of the radicle, and by allowing steam to strike on one side only.

As a result of these experiments Spaulding concluded that traumotropism follows the application of any agent which irritates strongly without killing the plant. This conclusion coincides nicely with the deductions made by the author as a result of his experiments with animals.

Numerous investigations by various botanists have shown conclusively that the actual growth process which accompanies the phototropic, thigmotropic, and traumotropic responses of plants take place, not at the site of injury, but at some distance from it, due presumably to the diffusion of cytost through the plant. In this respect, then, we find a rather crude analogy to the behavior of cytost in the animal body, where, it will be remembered, the distinctive action of cytost becomes manifest in the splanchnic area at a considerable distance from the original site of injury.

Loeb (1924) in his exhaustive experiments on regeneration in *Bryophyllum calycinum* has shown that a quantitative relationship exists between the amount of regenerated shoots and roots and the mass of leaves and stems exposed to illumination. This was to be expected, for the mass of material employed in the regenerative process must arise from the photosynthetic activities of the leaves. Loeb accounted for the fact that mutilation leads to growth in portions of the organism where no growth would have occurred otherwise by assuming that, following mutilation, an increased concentration of sap took place at the site of the injury. These conclusions of Loeb's are satisfactory in so far as they account for the accretion of the material necessary for the new growth, but they

do not explain why the new growth follows. In other words, given an adequate supply of the basic substances necessary for the formation of new plant tissue, one must also account for their utilization in the formation of new cells—that is, for the excitant that leads to cellular multiplication at the site of injury.

In our discussion of the tropistic responses of plants we have seen that injury apparently leads to the liberation of a growth-promoting substance, presumably derived from the injured cells. Hence one is tempted to postulate that it is such a substance which is liberated from mutilated tissue that leads to regeneration at the site of injury. The accretion of sap at this point would tend to hinder the diffusion of the growth excitant from the site of injury, and thus permit the neighboring cells to receive the full brunt of its action. This is, of course, only speculation; but it should be capable of experimental test in some fashion.

In view of these conclusions it is of interest to recall the early observations of Jost (1893), who found that if potatoes were injured in close proximity to the eyes, germination was accelerated. A similar end was achieved by Schneider (1925) by the simple expedient of inserting near the eyes sticks coated with various chemicals. Schlumberger (1926), in a reinvestigation of this curious phenomenon, came to the conclusion that the chemicals employed by Schneider were of little or no consequence, the accelerated germination being due to the trauma incident to the insertion of the sticks into the potatoes. In conformance with this conclusion, Schlumberger found that a simple mechanical jolting, or the application of pressure to seed

potatoes, leads to a noticeable increase in the rate of germination.

If it be conceded that such injuries lead to the liberation of cytost, we may draw a somewhat crude analogy between the acceleration of germination and the beneficial effects of homologous cytost upon the breeding of laboratory animals. It would be exceedingly interesting to study the effects of cytost extracts prepared from potatoes upon the growth of the latter. In this connection it may not be amiss to suggest that the current practice of plowing under those portions of plants which are of no economic importance may possess benefits aside from the return of nitrogen and mineral salts to the soil. Conceivably the autolysis of such plant material within the soil may lead to the accumulation of a certain amount of cytost which is actually beneficial to the growth of succeeding generations of plants.

Before leaving the topic of trauma in plants, it may be worth while to note that Buenning (1926), in an investigation of the effects of mechanical irritation on *Aelium ascolonicum,* has observed that such injury and excessive heat both lead to the liberation from the injured tissues of some substance which is capable of affecting neighboring cells, leading to coagulation and increased permeability. This is in harmony with the author's observation that similar effects follow the exposure of animal cells to the abnormal concentrations of homologous cytost.

As a result of his experiments, Buenning suggests that the substance responsible for the histological changes which he observed may be inorganic salts freshly liberated from the plant tissues as a result of

the injury. This may be true, but as yet the experimental data available are insufficient to warrant any definite statement on this point.

Taken together, the experimental facts enumerated above appear to substantiate the hypothesis that in the plant world some endocellular product plays a rôle similar to that of cytost in the animals.

Chapter XIII

CYTOST IN MEDICINE

One can easily imagine that the introduction of cytost into the human body would result in the onset of pathological changes similar to those induced in the lower animals which we have investigated in the laboratory. Direct experiments concerning this conclusion are impossible for obvious reasons. There are, however, a number of pathological conditions commonly found in man that closely parallel those produced in animals as a result of cytost intoxication, and one can hardly escape the conclusion that many of these are due to just this cause.

For instance, the shock which may be easily produced in animals by the methods we have described earlier is of common occurrence in men who have been subjected to trauma, particularly crushing injuries, severe burns, and unduly prolonged anesthesia. Such shock is unquestionably due to the cytost released from damaged tissue, as is the case in lower animals. Early in the text it has been pointed out that the excision of injured tissues, if performed before extensive autolysis has progressed, is a fairly effective prophylactic against the onset of traumatic shock. This fact is well recognized by surgeons, who fre-

quently employ débridement, cauterization, and electrocoagulation as practical means of preventing the onset of shock during the repair of traumatic injuries.

Our experiments have shown that the continued introduction of sub-shock producing quantities of cytost into animals may result in degenerative changes in the organs of entodermal origin. Clinical experience suggests that an exactly parallel situation may exist in man. For example, individuals who have suffered severe burns frequently develop a nephritis which very often proves fatal. It seems clear in such instances that the nephritis is caused solely by toxic substances released from the damaged tissues. While this has long been surmised by pathologists, we wish to make it clear that such kidney damage closely parallels that which may be induced in animals by a properly regulated series of cytost injections.

This is of interest because it indicates that a diseased condition of non-bacterial origin may be caused by an individual's own tissue products, or cytost. Since clinical experience with burns justifies this conclusion in the case of kidney degeneration, it seems entirely logical to extend this concept to other pathological conditions involving the organs of entodermal origin.

There are a number of diseases, sometimes classified as metabolic, whose obscure origin strongly suggests that cytost is their causative factor. Among these we may include such chronic ailments as arthritis deformans, neuritis, certain types of paralysis, arterial sclerosis, and nephritis. At the outset we wish to make it clear that such diseases may be caused by factors other than cytost, but that, as will be shown

presently, they may be caused by cytost also. For an example we may consider nephritis, which may be caused experimentally by various means. Various metallic poisons, such as mercury and uranium, may cause the onset of this condition. Likewise there is considerable evidence that kidney damage may result from infections of one sort or another. In the case of nephritis following burns, however, it is clear that such factors are not the causative agent, and one is forced to assign this rôle to cytost. One may reason similarly concerning the other pathological conditions enumerated above.

In the case of arthritis deformans, this has been recognized by Nathan (1917), for he states:

"The principal gross and microscopical findings in both acute and chronic joint disease, whether of known or unknown origin, are fundamentally *simply phenomena of inflammatory reaction.*

"They are all of osseous origin. There are certain peculiarities characteristic of so-called Arthritis Deformans which are apparently very difficult to bring into correlation with an infectious origin. Indeed the peculiar deformities of hands and fingers, the acroparesthesia and premonitory weakness and spasm, cannot be ascribed to an infectious focus within the joint at all. It may show involvement of cord and spinal roots, definite signs of peripheral nerve abnormalities,—at most Perineuritis, Ulnar neuritis, etc.

"It must be *assumed* that all polyarthritis (neuritis), is due to infections, or, that *all deleterious substances* cause fundamentally the same general changes in articular structures. It cannot be denied that there

is some foundation for the interpretation of these phenomena, for it is well known that traumatic conditions near, or in the epiphysis, induce the phenomena of inflammation, congestion, vascularization, bone decalcification, and absorption, and, at times, tissue proliferation, which may lead to fibrous or bony ankylosis."

Similarly Harding (1921) reports that in a study of 300 cases of arthritis he found blood cultures to be negative, and states that he is "not at all convinced that germs in tooth or tonsil are causative factors in joint conditions."

Failure to obtain positive blood cultures does not necessarily indicate that focal infections are of no importance in connection with arthritis. Indeed, various statistical studies have shown that there exists a close correlation between foci of infection and various pathological conditions. On the other hand competent men have frequently failed to find any evidence of focal infection in patients apparently suffering from the same maladies as those in whom foci are found. Further, it frequently happens that the removal of foci of infection does not result in alleviation of the patient's condition. Such findings as these are disconcerting. If, however, we assume that the cellular degeneration is caused by cytost these apparent discrepancies may be obviated. In our discussion of the relationship of bacterial infection to cytost, it was pointed out that proteolytic microörganisms may cause the liberation of the tissue toxin. Obviously then, a focus of infection may cause a more or less continual release of cytost into the circulation, and this may lead to the onset of various pathological con-

ditions. Now the same end should follow the release of cytost by any means whatsoever.

In consequence one may regard a focal infection simply as one means of causing the accumulation of excessive quantities of cytost in the individual. If this view be accepted, we may readily account for the absence of a bacteriemia in individuals supposedly suffering from the effects of foci of infection. Further, this concept enables one to understand the fact that the removal of such foci does not necessarily guarantee the patient relief, for he may suffer from cytost arising from another cause, or from the cytost released by the infection, which is not removed from his body when the focus is cleaned up.

In order to ascertain the possibility of causing in animals a condition similar to that of arthritis in man, cytost preparations were injected into the bone marrow of cats. This was accomplished by trephining small openings in the proximal end of the tibia and the distal end of the femur. One eighth of a cubic centimeter of a 10% solution of an active cat tissue ash was then aseptically injected directly into the bone marrow through each of the two openings. As a control, other cats were injected similarly with corresponding quantities of horse and lion cytost. The cats receiving the cat cytost in this way developed a marked lameness, with stiff and sensitive joints, which lasted for several days. The animals injected with horse cytost showed no ill effects, while those which had been injected with lion cytost developed a slight lameness which lasted for two days only. (Turck, 1921.)

Further experiments showed that the injection of

homologous cytost intramuscularly directly adjacent to the sciatic or ulnar nerve resulted in the onset of a condition resembling neuritis and causing marked sensitivity and lameness, the duration of which in any individual case was found to depend upon the amount of cytost injected. The accompanying photograph (Fig. 16) shows a cat suffering from neuritis of the hind limbs induced by this means.

Microscopic examination of the affected nerves showed that the injections of cytost had caused congestion and extension of the vessels in the tissues surrounding the nerves and in the nerve sheath, although the nerve itself did not appear to be affected. This is shown in figure 17, wherein one may clearly discern three areas distinctly showing this fact. The onset of neuritis in the experimental animals is probably to be attributed to the stagnation caused by the cytost, which then causes extension of the vessels and consequent pressure upon the nerve, rather than to any direct effect upon the nervous tissue.

The results of these simple experiments indicate that cytost intoxication may actually be an important causative factor in the occurrence of analogous conditions in man. If this be the case, how is one to diagnose and treat patients suffering from this cause?

The diagnostic procedure is of necessity somewhat unsatisfactory. First, of course, one must examine the patient in order to ascertain the absence of any gross pathologies which may give rise to the cytost, and these must be cleared up by the usual medical and surgical procedures. The writer has found that intramuscular injections of homologous cytost at the site where patients complain of pain frequently leads to a marked

Figure 15.

Photograph of a cat with complete paralysis of the hind quarters caused by the intraspinal injection of a solution of homologous tissue ash as described in the text.

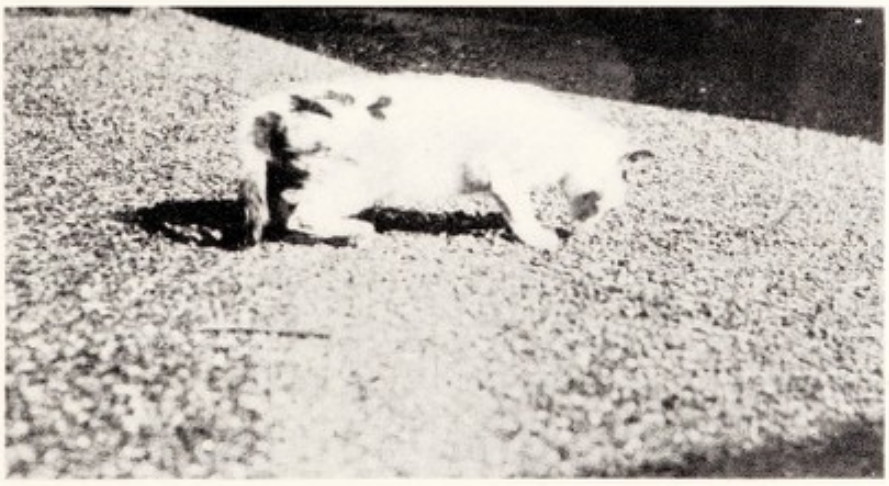

Figure 16.

Photograph of a cat with "neutritis" of the hind limbs resulting from intramuscular injections of cytosa directly over the sciatic nerve.

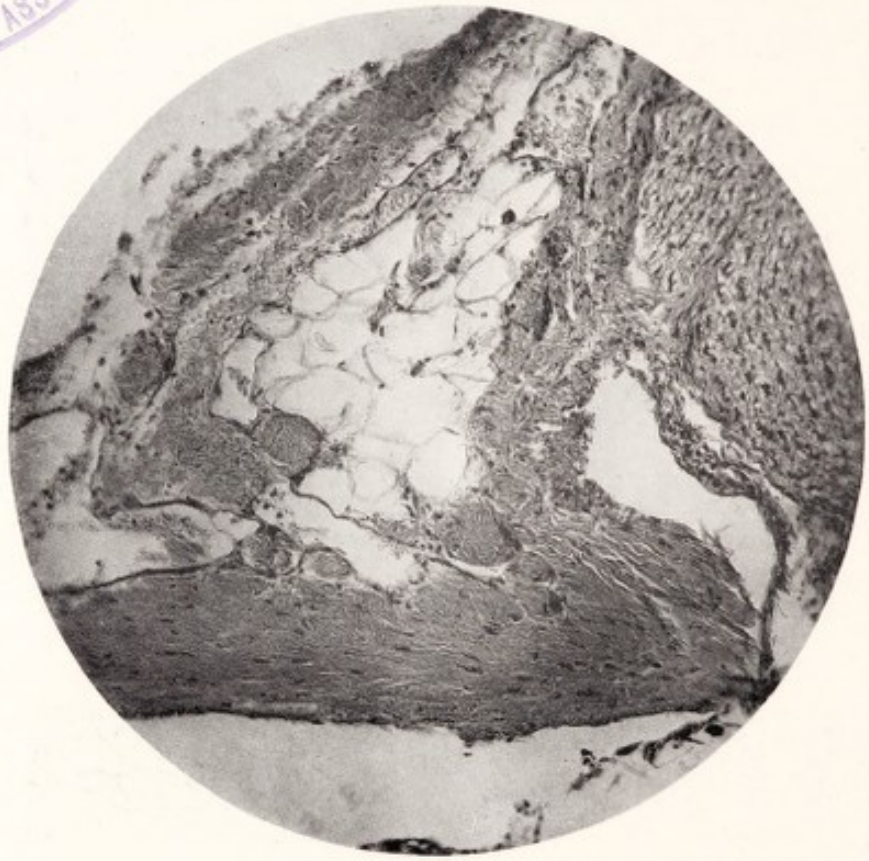

Figure 17.

Section showing sciatic nerve. Taken from the limb of a cat which had received an intramuscular injection of homologous cytost adjacent to the nerve a few days previously. Note the congestion of the tissues about the nerve, and in the sheath, which gives rise to pressure on the nerve.

increase in pain and sensitiveness. For this purpose we have employed a saline extract of the gray ash of human muscle. When heterologous cytost is injected similarly the only additional pain experienced is that which normally follows the intramuscular injection of almost any non-irritating solution.

The fact that injections of homologous cytost markedly accentuate the pain and soreness for which patients seek treatment is of diagnostic significance, for it indicates that the patient's tissues are hypersensitive to cytost. This may be due to either of two causes: his tissues may be naturally hypersensitive—i.e., his resistance to cytost is low; or because of an unduly large amount of cytost already present, the additional quantity introduced in the injection may raise its concentration in the tissue fluids to an abnormally high degree. In either case the end result is the same. The writer believes that when an individual is found to be hypersensitive to cytost the conclusion is warranted that such an individual is suffering from cytost intoxication.

Now the question arises as to how one is to overcome this condition,—that is, can we offer the patient any relief from the effects of cytost upon his tissues?

The answer to this question is to be found in the results of our laboratory experiments on animals. It may be recalled that we were able to obtain evidence that an animal's resistance to cytost may be materially raised by a properly regulated series of injections of homologous cytost. Because of this it appears logical that one should be able to do the same with humans. Elsewhere the writer has described the treatment of various diseases by this means.

As cytost obtained from human tissues is not readily obtainable, we have taken advantage of the fact that an animal may be caused to liberate cytost at will by the simple expedient of causing a small localized tissue damage by the subcutaneous injection of chloroform or ether at selected sites. In the author's experiments upon patients it has been customary to inject 0.25 cc. of chloroform at one or two points, usually in the lumbar region. This causes the patient no inconvenience, and the small quantity injected causes but little tissue damage, and consequently the release of but a small quantity of cytost. Several such injections spaced several days apart apparently have the same effect as similar injections of human cytost.

Following such treatment a number of striking "cures" have been obtained both in acute and chronic cases of migraine, arthritis, rheumatism, etc. Some of these have been described elsewhere (Turck, 1919, 1921, 1922), and we shall not discuss them here, for the writer well recognizes that general conclusions cannot be drawn from the results obtained with individual patients.

Strictly speaking, one can determine the utility of a given method of treatment only when it is applied under more or less constant supervision to a sufficiently large number of patients to allow the deduction of statistically valid conclusions.

Through the courtesy of L. E. Feldman, director of the Russian Red Cross (old organization) in Bulgaria, such an opportunity was offered at the Hospital and Dispensary in Sofia and the Asylum for old people and chronic invalids in Shipka. All diagnoses, treatment, and observations were carried out by the

physicians associated with these institutions for a period of several years. The writer's only connection with this practical experiment consisted in outlining the method of treatment described in the preceding pages, and supplying human cytost at the beginning of the venture. From time to time reports were received from the Russian Red Cross physicians, which presented evidence that the cytost treatment was actually found to be of benefit.

Typical of these reports is one from Dr. R. Berzin of Sofia, under the date of July 20, 1929, from which we quote: "During the last three years I have had more than 100 patients treated with cytost and anticytost. The first group (79 patients) were suffering from arthritis, especially from arthritis chronica deformans and polyarthritis chronica, 48 of them were men, 31 women, age from 35–68 years. The majority of them received the treatment in the Hospital of the Russian Red Cross, only a few were treated in their homes. Nearly all of the patients had been bedridden for weeks or months. Several of them had been invalids from two to four years. Everyone had been treated with mineral baths, etc., for years without any lasting results.

"Our treatment was very simple: We gave our patients only cytost and anticytost alternatively with an interval of 10 days, and nothing more. The dose varied from 1–5 cc. of anticytost and 0.4–0.5 cc. of cytost each time. The total dose was from 10–50 cc. of anticytost and 2–3 cc. of cytost.

"The results were excellent. We had no complications at all, neither local nor general disturbances. On the contrary the patients affirmed that they felt

much better, that not only affected joints were cured, but their very essence of life, their vitality, was increased in a very considerable degree."

The author has received similar reports from other physicians who state that they have consistently found that patients suffering from arthritis are benefited by injections of cytost or chloroform. In some instances similar treatment has been found of distinct benefit in cases of arteriosclerosis, asthma bronchialis, neurasthenia, and various ailments which may be classed as senile changes. Further discussion of such cases would be out of place here, but will be reported elsewhere in the future.

Quite recently others have recognized the usefulness of tissue extracts in the treatment of disease. Thus Ludwig (1931) states that extracts of both heart and skeletal muscle are of distinct utility in the treatment of various cardiac troubles, particularly angina pectoris. Similarly Weiss (1931) found that in four cases of angina pectoris an immense improvement followed treatment with an extract of ox hearts. Weiss states that the cases which he treated seemed to experience a renewal of physical capacity and eventually lost all indications of pressure symptoms.

This is of particular interest since this observation apparently substantiates the writer's conclusions that, when properly administered, cytost actually brings about a general increase of physical well-being.

In our discussion of the stimulating effects of cytost upon animals it was pointed out that a properly regulated course of cytost injections gives rise to the observed beneficial effects, solely because it raises the animal's natural resistance towards its own cellular

toxins. There appears to be no reason why this concept should not be carried over to humans. Indeed the fact that patients suffering from various diseases of obscure origin are distinctly benefited by cytost or chloroform injections appears to substantiate this conclusion.

With this in mind we may briefly extend the present discussion of the possible application of our experimental researches to medicine. For example, let us consider infections of the respiratory tract. It may be recalled that respiratory disturbances of non-bacterial origin may be easily induced in animals by exposure to cytost-laden air. Once such a disturbance is produced, bacterial infection readily follows. On the other hand, it is by no means easy to induce respiratory infections in healthy animals not previously exposed to cytost. (Turck, 1919b.) It follows from this that exposure to cytost-laden air may be a predisposing cause of the onset of various respiratory diseases in humans.

It has frequently been stated that the incidence of respiratory infections is higher in urban communities than elsewhere. This in part is due to greater chances of contact with infected individuals and may in part be due to the fact that in congested areas the air actually contains human cytost sufficient to provoke a reaction in the sensitive respiratory endothelium of the less resistant members of the community. In favor of this concept is the fact, frequently remarked by Arctic and Antarctic explorers, that respiratory disturbances are exceedingly rare amongst the members of their expeditions. This is especially interesting since during the winter months

the members of such expeditions are closely crowded together in their small huts. It would seem that the absence of respiratory disease among the members of such expeditions is in part due to the fact that the air of the polar regions must be free of human cytost; hence the men are not exposed to this predisposing cause.

In this connection it is of interest to note that Dr. S. E. Jones, a physician who accompanied the Australasian Antarctic Expedition led by Sir Douglas Mawson, records in his medical report that an epidemic of influenza broke out on the ship and that the recovery of the convalescents was much more rapid after arrival in the Antarctic than on the ship. (See Mawson, 1916.) This is in agreement with the well known fact that patients suffering from various respiratory disturbances recover more rapidly in spacious country sanitoria than in congested city hospitals, a fact which suggests that removal from cytost laden air may be distinctly beneficial in the treatment of such conditions. Indeed, if possible, this would seem to be of value in the treatment of any type of disease in which cytost intoxication may be an important factor.

By analogy with our experiments with animals, it should be apparent that any measures which may increase an individual's resistance to cytost should be of value both in the prevention and the treatment of disease. In our discussion of so-called natural resistance it was pointed out that such factors as proper diet and adequate exercise seem to raise one's natural resistance by raising his resistance to cytost. Conversely, improper diet and lack of exercise tend to

decrease resistance to cytost and consequently are predisposing causes of disease.

During and following the Great War, large numbers of various European populations were forced to subsist upon rations little short of actual starvation. In consequence, it is not surprising that many Europeans, particularly children, developed many obscure and rather ill-defined ailments indicative of a general lack of body tone and diminished resistance comparable to that found in animals as a result of partial inanition. In animals, as we have shown, this leads to cytost intoxication and the degeneration of various tissues. It seems highly probable therefore that in many instances the poorly defined ailments of the war refugees may have been due to a similar cause.

In this same class we may place those who suffered shock from the severe wounds of war but did not fully regain their health upon recovery from the primary injuries. Such individuals were frequently found to be suffering from various ill defined chronic complaints whose very nature suggested that they were basically due to a more or less chronic cytost intoxication.

With this in mind the physicians of the Russian Red Cross have at the writer's suggestion treated such individuals with a view to increasing their resistance to cytost. In order to obtain comparative data such patients were divided into two groups. Both groups were well fed and housed and given whatever general medical treatment was indicated in particular cases. The members of one group were given injections of chloroform, such as have been described earlier in the chapter, with a view towards raising their resis-

tance to cytost, while the members of the other group did not receive such injections. According to the medical men who observed these individuals, those receiving the chloroform injections were, on the average, restored to health more rapidly than were the members of the control group. This experience again testifies to the fact that immunization to cytost is a very useful adjunct to common medical treatment.

The few facts relative to medicine which have been considered in this chapter have been presented in the hope that they may be of use to others who desire to make use of them. It must be remembered, however, that such treatment is not a panacea for all human ills, but is to be considered only as a supplement to the more common methods of medical treatment of proven value.

Chapter XIV

CONCLUSIONS

SINCE the major aspects of the writer's experiments have been discussed in the preceding pages in a more or less topical form, it seems advisable to review and integrate these investigations in such a way as to form a harmonious picture of the experimental findings and the theoretical conclusions deduced therefrom.

Strictly speaking, the various topics which have been considered are the logical outgrowth of the writer's early investigations concerning the nature of so-called shock in animals. Thirty odd years ago this was considered to be of psychoneurogenic origin. The earliest investigations of the writer indicated that such was not the case, but rather that the train of physiological events termed shock are primarily due to the liberation from injured tissue of a toxic entity, first termed shock toxin, and later cytost.

Initially this concept arose from the following observations. In all cases of shock, regardless of the apparent cause, the onset of typical symptoms was accompanied by a marked stagnation of blood in the splanchnic area of the experimental animals. This was easily observed in animals whose abdomen was open during the course of an experiment, and in other

animals was indicated by the drop in surface temperature and blood pressure. Such reactions as these, which always accompany shock, were never found to follow immediately after the infliction of an injury, as would be expected if the shock were due to a nervous mechanism of any sort. In general, regardless of the nature and magnitude of the inflicted injury, shock does not supervene until approximately two hours following the injury. This fact of course suggested the intervention of some relatively slow chemical process, rather than a nervous one, and since the onset of shock is the direct outcome of the injury, such a chemical process must take place in the injured tissues.

This conclusion was substantiated by three lines of experimental evidence which have been discussed in detail in the early chapters of this monograph. First, ligation of an injured limb above the site of an injury was found to prevent the onset of shock because it prevented the absorption of materials from the wound. If after several hours the ligature was loosened, then the various symptoms of shock rapidly appeared, thus indicating that the shock was due to the passage of substances from the site of injury to the splanchnic area. Secondly, histological examination of the injured tissues disclosed the fact that, following injury of any sort, the affected tissue cells undergo autolysis. Such a process requires a definite time, and it is this interval which must elapse between the infliction of the injury and the onset of shock. From this observation, it was deduced that the primary cause of shock was an endocellular toxin liberated by the tissues incident to their autolysis.

The validity of this conclusion was subsequently

confirmed by the observation that severe shock and death could be induced in intact animals by the injection of aqueous extracts of homologous tissues which had been permitted to undergo sterile autolysis *in vitro,* while aqueous extracts of freshly excised tissue, when injected similarly, did not cause the animals any apparent inconvenience.

These results clearly substantiated the author's conclusion that shock was of toxic origin, and, since the World War, others, to whom reference has already been made, have reached essentially similar conclusions.

Since the extract of autolyzed tissue proved to be such a highly toxic substance when introduced into the circulation of an animal, it became of interest to investigate the effects of sublethal quantities of cytost. So far as could be determined, the injection in a single dose of a minute quantity of cytost does not as a rule lead to any distinct or definite reaction. However, when such injections were repeated every few days for a protracted period of time, it was found that temporarily the injected animals gained in weight more rapidly than control animals of the same age under the same conditions. Furthermore, as far as could be judged from external observation, during the period of rapid gain in weight the injected animals appeared to be more active and in better health than the controls. As the injections were continued, the animals lost this advantage, and after a period during which they lost considerable weight, they rapidly became ill, and died.

At autopsy, such animals were found to have suffered marked degenerative changes in the organs of

endodermal origin, the lungs, liver, kidneys, etc. Thus, by the long continued action of sublethal quantities of cytost, it was found that one may induce chronic pathological changes in the same tissues, and that they respond most rapidly to the action of cytost during shock. The rate at which such degenerations may be induced was found to depend upon both the dosage of cytost employed in the experiments, and the frequency of the injections.

It is of especial interest to note that the degenerative conditions which can be induced by the often repeated injection of cytost are similar to those which are of the utmost importance in pathology, and whose cause is frequently the most obscure. The writer is of the opinion that such conditions arise most frequently from the action of cytost derived from injured tissues. As is well known, the kidney involvements which sometimes occur in individuals who have been badly burnt are commonly attributed to "tissue toxins," and this is in harmony with the writer's contention.

From the investigations which we have discussed earlier, it should be clear that since any injury, traumatic, chemical or bacterial, may result in the liberation of cytost which will affect the visceral organs in greater or lesser degree, if long continued, this may eventually lead to pathological conditions of apparently obscure cause. It follows, therefore, that a mild more or less chronic injury may finally result in such pathological conditions at a distance from the actual site of injury.

With this in mind, one may easily trace the possible connection between foci of infection and disease of obscure origin, such as nephritis, or arthritis. That

such a connection exists has been established with a high degree of probability by clinical observers, but the actual details of the relationship have not been satisfactorily established. In our discussion of the relation of bacterial infection to cytost intoxication, it was pointed out that bacterial infection, although localized in a particular area, may cause the liberation of considerable quantities of cytost in much the same manner as would any other localized tissue damage.

If such a condition persists for some time, the animal is continually subjected to a concentration of cytost analogous to that which occurs when sublethal quantities of this substance are injected at frequent intervals during a protracted period of time. In consequence, the cytost liberated by the activities of the bacteria in foci of infection may be expected to bring about a degeneration of the tissues of endodermal origin in much the same manner as was found to be the case in our experiments. The writer feels confident that an investigation of this mechanism would yield results of considerable interest in the interpretation of some disease processes.

When a large number of animals is injected with equal quantities of the same extract of autolyzed tissue, it is found that they react to the injection in varying degrees. If the quantity is sufficiently great, the larger proportion of the animals develops shock and dies. Certain other members of the group develop a severe shock, and recover, apparently none the worse for their experience, while a lesser number reacts but slightly to the injection. This, of course, is a common finding in all types of biological experimentation, and

is commonly ascribed to individual variations in resistance or susceptibility.

These variations in resistance to cytost injections, however, are especially interesting since they represent a resistance to a product of the animal's own cells, and suggest that, other things being equal, an animal's health and well-being may depend upon his resistance to the toxic products resulting from the activities of his body cells.

This line of thought led to two series of investigations concerned respectively with the toxic effects of sublethal quantities of cytost, and the stimulation of natural resistance to that substance. These have been discussed at length in previous chapters, where it was shown that, depending upon both the quantity of cytost injected, and the number of injections, an animal may be caused to suffer an actual decline in weight and health, or stimulated so that it gains in weight and general appearance.

The latter result appears to depend upon a general stimulation of the metabolism of the body cells of the animal, as well as the production of an increased tolerance for cytost. The apparent stimulation of metabolism may really be due to the latter, for it is conceivable that in the case of an animal having a low resistance to cytost, the accumulation of the latter in its body fluids may impair the functioning of the body cells so that their activities are below normal. If, on the other hand, the animal's resistance to cytost is raised by a course of immunizing injections, such impairment of function is largely removed and the growth of the body cells assumes a more normal course. This aspect of the problem is one worthy of

further investigation, since it should lead to a better understanding of bodily function, both in health and disease. This seems all the more certain, since, as we have shown, a number of pathological conditions of admittedly obscure origin can be easily induced by a series of sublethal injections of homologous cytost.

These effects of cytost on the intact animal are but the expression of the integrated effects upon the individual tissue cells. This follows from our experiments on tissue cultures and upon the growth of paramecia, where, it may be recalled, small quantities of homologous cytost were found to exert a distinctly stimulating effect upon the cells, while the characteristic toxic action of cytost became manifest if its concentration in the media was too high.

These two actions of cytost may seem paradoxical, but since they are attested to by the results of literally hundreds of experiments, they cannot be questioned.

Indeed, nature seems to have endowed all biological systems with apparently paradoxical reactions to baffle the investigator. Thus, the life of the majority of animal cells is adapted to a definite partial pressure of oygen; oxygen tensions less than or greater than the optimum lead to anomalous activity frequently resulting in death. The same is true of the ionic balance of salts in the fluid media surrounding such cells.

It is not illogical, therefore, to postulate that a certain optimal concentration of cytost is compatible with the normal functioning of cells, whereas when this optimum is exceeded in one direction or the other anomalous behavior results. When such anomalous behavior becomes excessive, a pathological condition ensues.

Care must be taken not to press such analogies too far, for while isolated cells or tissues are unable to control such factors as oxygen tension, they are, in a measure, able to control the cytost content of their immediate environment, since, as we have seen, cytost is an endocellular autosynthetic product. On the other hand, our experimental results have shown that the liberation of cytost in quantities sufficient to affect the cells in one way or another is dependent upon an injury of some sort; that is, although the living cell contains cytost or its immediate precursor, mobilization of this material in quantity sufficiently large to cause an observable alteration in the activity of cells does not take place until an injury of some sort occurs. It should be noted in this connection that we use the term injury in a very general sense, including any response to various physical and chemical agencies which we have discussed previously. It follows therefore that so-called "mild stimulation" as well as physical destruction falls in this category.

Since such injury provokes the liberation of cytost to a greater or lesser degree, and since, as our experiments have shown, an optimal quantity of cytost in the surrounding media appears to lead to maximal cellular activity, we are enabled to see a definite relationship between cellular activity and alterations in the physical environment. Assuming that such an environment is well adapted as regards oxygen, sources of nutriment, and so forth, the activity of a cell, tissue, organ, or organism is to a considerable degree conditioned by alterations in any environmental factor which may lead to a liberation of cytost.

Let us first consider the beneficial action of such

environmental factors as temperature and sunlight. In all likelihood such environmental agencies affect an organism in manifold ways and it is difficult to subject their effects upon an organism to complete analysis. Nevertheless the writer's experience has convinced him that in part the action of such agencies depends upon their ability to cause the liberation of small quantities of cytost from the exposed cells and thus effect a general stimulation of the body.

Because of this conviction the writer (Turck, 1899) long ago recommended the introduction of hot water into the colon and stomach as a means of increasing the activity of the body cells in general. This method was introduced before the writer's cytost theory was formulated as clearly as it now is, but the results obtained by this method of treatment were in complete harmony with what we should expect on the basis of this theory. Sluggish individuals subjected to such treatment consistently manifest an increased activity following the brief exposure of their gastro-intestinal tube to temperatures considerably higher than that normal to the body.

Biologists at large are inclined to attribute the response of an organism to changes in temperature solely to the effects of temperature upon the rate of the chemical reactions taking place within the cells. Within certain limits this view is undoubtedly correct, but when temperatures are attained which cause cellular injury we know from our experiments with burns that considerable quantities of cytost are liberated. Such being the case, it does not seem unreasonable to assume that even at temperatures considerably below that necessary to cause rapid cellular disinte-

gration, cytost may be liberated from the exposed cells in sufficient quantity to evoke a response in neighboring cells.

Much the same argument obtains in the case of sunlight, whose therapeutic value has been definitely recognized since the time of Hippocrates. Today it is common knowledge that the ultra-violet portion of the spectrum of sunlight is of importance in connection with the synthesis of the antirachitic vitamin, but the beneficial effects of exposure to sunlight cannot be attributed to this fact alone.

Experiments have shown that the penetration of sunlight into the skin is limited; hence the systemic effects which follow prolonged exposure to such radiation must be due to the absorption of cellular products liberated from the radiated area. That such products, including cytost, are liberated is evident, since prolonged exposure to sunlight frequently leads to a severe sunburn and the eventual onset of symptoms typical of cytost intoxication. It seems highly probable that even brief exposures to sunlight must likewise result in the release of cytost, for the characteristic erythema resulting from such appears to be identical with that which follows the release of cytost by any other mild localized injury, such as has been discussed in the previous text.

Upon extending this line of thought, it is readily conceived that the magnitude of the cytost released under the influence of such environmental agencies must vary in some quantitative manner with the intensity of such injury. Thus when one unaccustomed to intense sunlight is foolish enough to expose a considerable area to the sun's rays for a protracted period,

the quantity of cytost liberated may greatly exceed the quantity sufficient for healthy stimulation of the tissues, and a mild toxic action may become apparent some hours afterwards. In some degree the severity of such a toxemia is found roughly to parallel the extent of the tissue injury caused by the sunlight. Of course individuals vary in this respect. Some active persons can contract a markedly destructive and painful sunburn without any apparent general ill effects. Presumably such individuals, through previous activities, have developed a resistance to cytost comparable to that which follows a series of immunizing injections.

The essential point to be gleaned from the foregoing is that cytost occupies a unique rôle in the activity of cells, for through its action such activity is closely determined by alterations in the environment. Of course the response of cells to all conceivable environmental modifications cannot be dependent solely upon the action of cytost, but the evidence which the writer has accumulated points strongly to the fact that many changes in cellular activity are uniquely controlled by the liberation of cytost under the influence of environmental factors. This is a point not generally appreciated by workers in the biological sciences at the present time, and the writer is convinced that the adoption of this view may do much to effect a rational explanation of many apparently uncorrelated biological facts.

It is the writer's earnest hope that other investigators will attempt to repeat and extend his observations. Certain of our researches, although prosecuted to the fullest extent which available facilities permitted, still

require re-investigation and extension. Thus, the experiments upon immunity towards cytost and the relationship of such immunity to natural resistance and breeding might well be extended to larger numbers of animals and particularly to species other than those used in our laboratory.

The writer has not carried out any experiments with invertebrate forms other than protozoans, but there is every reason to suppose that in such animals cytost must play a rôle similar to that in higher animals. Indeed, Child (1915) has presented some evidence that this is so in the case of planaria, for he states (page 66) : "There is no doubt that a relation exists between the general metabolic condition of organisms and their susceptibility to a large number of substances which act as poisons, i.e., which in one way or another make metabolism impossible." He further points out that the action of such poisons resembles that of "certain soluble products of metabolism," and in so doing comes close to the writer's concept that the toxic action of many cellular poisons is largely due to their ability to cause the liberation of cytost. Throughout his book Child stresses the possible relationship between the "soluble products of metabolism and the activity of the animal." Thus (page 188) : "Fatigue, the decrease in rate of metabolism which follows continued stimulation, is generally believed to be due to the accumulation of toxic products of metabolism." And, again: "Various metabolic intoxications are probably very similar in character . . ."

In the chapter on the possible rôle of cytost in the activities of the plant, we have presented evidence that endocellular products which are liberated as a

result of injury are of importance in determining the response of plants to their environment. Most of this evidence gleaned from the writings of others should be reinvestigated, using extracts of plant tissues in a manner similar to that which we have employed in our animal experiments.

If by such methods it can be established definitely that cytost is of equal importance in the plant and animal worlds, we shall have at our disposal a deeper appreciation of an important factor in the physiological activity of living systems.

Our experiments have shown cytost to be a substance of remarkable thermostability, but we have failed to identify it as a distinct chemical substance. This is a biochemical problem which still awaits solution.

So far we have been unable to find any chemical test for the presence of cytost in a tissue extract. The only means of detecting this substance remains the physiological reactions described in the text. For laboratory purposes the induction of shock and death in animals, and the localized reaction in the lungs remain the most satisfactory methods for the assay of various cytost preparations. While these methods yield positive and unquestionable evidence for the presence of cytost, it may be desirable to expand them by the study of the action of cytost on isolated organs. Such a study may give rise to a quantitative method of assay better suited for routine laboratory experiments.

The development of such methods may be of extreme value in future investigations of the action of cytost and its relation to the physiology of the cell, both in health and disease. Certain of our experiments have shown that it is possible in young animals

to produce retrograde senescent changes by the frequent injection of cytost. This opens the way to the possibility of obtaining important information concerning the nature of senility, a problem of immense importance in biological science.

It has been shown that by an appropriate series of immunizing injections, one may raise an animal's resistance to cytost, and we have deduced that this is of importance in determining an animal's so-called natural resistance. This is of importance from both the theoretical and practical standpoints, and consequently is deserving of further investigation. In this connection we have postulated the existence of an anticytost whose formation within the animal protects the latter from the action of cytost. Since anticytost is an antibody which protects an animal from a toxin developed by his own cells, a new concept has been added to the science of immunology.

The writer is confident that the various types of experiments described in the text are capable of substantiation in the hands of other experimenters. It must be remembered, however, that animals vary markedly in their susceptibility to injury of any sort; but as we have seen, the gross effects which result from various types of injury have in common the liberation of the tissue substance which we have termed cytost. In consequence, the individual difference between animals may be largely explained as due to their difference in susceptibility to cytost; or, in other words, to put it crudely, to their available anticytost, with which they are able to combat the action of cytost itself. Bearing this in mind it is obvious that the experimenter working with a limited

number of animals may fail to obtain results comparable with those which we have described. However, if a sufficiently large number of animals is subjected to a given experimental procedure, it will be found that results similar to ours will be obtained in a sufficient majority of instances to warrant the conclusions which we have stated. This of course is a common experience in biological investigations of all kinds.

As an aid to any investigator who wishes to repeat or extend such experiments, we have appended a short selected list of procedures taken from our protocol books in the hope that these may prove of service to anyone who wishes to venture into this interesting field of investigation. These experiments have not been chosen because of the particular value of the results obtained, but rather as an illustration of the technique developed in our laboratory.

The writer fully appreciates that many of his deductions and concepts will meet the opposition usually accorded new theories. This is desirable, since such opposition frequently constitutes the impetus necessary to provoke experimental researches which will finally prove or disprove the validity of the concept.

APPENDIX

SOME REPRESENTATIVE EXPERIMENTS

THE experiments described below in the form of the actual protocols are representative of many which show the toxic nature of shock induced in various ways. These have been presented as a further illustration of some of the concepts discussed in the text. Experiments concerning the influence of cytost upon the growth, development, and breeding of animals have not been included because the descriptions of these in the text are believed to be sufficiently detailed to permit of a proper understanding of their technical details.

TRAUMATIC SHOCK

Cat, Male, Weight 4,300 grams, Temperature 39.5°. Room Temperature 21°. Ether anesthesia begun at 9.15 A.M.

9.30 A.M. Temperature 38.5°. Esmarch bandage applied so as to exert pressure on internal iliacs and abdominal aorta, thus keeping blood from lower extremities. Animal placed on radiator to keep warm.

Thigh and gluteal muscles severely bruised by means of bone forceps. Toe cut, no bleeding indicated blocking of circulation effective.
10.15 A.M. Temperature (esophageal and gastric) 36°. Temperature taken in this fashion because of interference with circulation.
11.00 A.M. Intragastric temperature 35.5°.
11.20 A.M. Intragastric temperature 35°. Bandage removed, and the bruised tissue was occasionally massaged to reëstablish circulation.
11.40 A.M. Intragastric temperature 34°. Anesthetic discontinued.
1.55 P.M. Intragastric temperature 35°. Limbs massaged. Onset of shock.
3.00 P.M. Temperature 32.5. Mucous membranes of mouth and nose were pale and cold.
4.00 P.M. Intragastric temperature, 32°, rectal temperature 32°.
6.00 P.M. Rectal temperature 30°. Cat died shortly afterwards. Immediate autopsy. Hepatic lobules well marked. Congestion of pyloric region. Lower small intestines and colon pale. Histological examination of liver showed stagnation in portal veins with marked distension of the portal capillaries and well marked periportal changes in the parenchyma of the liver.

Cat, male, weight 2,523 grams, rectal temperature 40° C.

9.00 P.M. Under anesthesia, the upper and inner sides of the left leg were shaved. Muscle tissue taken from the mangled leg of another cat which had been in profound shock was aseptically inserted between the muscles through incisions made through the

shaved area of the subject. This was accomplished by separating the leg muscles with the blunt end of a knife and forceps in order to prevent laceration and hemorrhage. After insertion of the mangled tissue (40 grams) from the other cat, the wounds were closed by suture. Anesthetic discontinued at *9.30 P.M.*

9.30 P.M. Rectal temperature 38°.

10.15 P.M. Rectal temperature 37°.

11.15 P.M. Rectal temperature 37°. Beginning symptoms of shock indicated by gradual onset of inactivity and weakness.

Following day. 5 *A.M.* Rectal temperature 36.5°.

12.00 M. 35°. Animal very weak, apathetic, reflexes nearly abolished, heart weak, respiration shallow, general pallor and coldness.

Third day. *6.30 P.M.* Rectal temperature 34°. Profound shock, died at 8. P.M. Immediate autopsy disclosed the usual shock picture. Congestion and edema of the liver, stomach, upper part of small intestines and pallor of lower splanchnic area. Microscopic examination of sections showed marked venous engorgement with extravasated cells in the submucous tissue (Zona Transformans) of the pyloric end of stomach; active venous stasis and periportal extravasation in the portal zone of the liver; peripulmonary arterial congestion in the lungs; and straight tubular venous stasis, hemorrhagic areas and agglutination in the glomerular region of the kidney.

Rabbit, male, weight, 1,400 grams, temperature 40°. Anesthesia commenced at 9.15 A.M. Left leg tied off above the great trochanter. Leg mangled and badly bruised with bone forceps.

10.15 A.M. Anesthetic stopped. Ligature released. Animal depressed so that no anesthesia needed.
11.00 A.M. Difficult shallow breathing, temperature 35°. Rapid heart.
12.45. Exit. Immediate autopsy showed liver, pyloric end of stomach and small intestines markedly congested.

SHOCK PRODUCED BY BURNS

A rabbit (No. 1) under ether anesthesia was burned over the back by means of a hot iron. The burned area was immediately removed and grafted onto the back of another rabbit (No. 2) and the normal skin of the latter was transferred to No. 1, from which the burned tissue had been removed. Rabbit No. 2 showed signs of shock about two hours after the insertion of the burned tissue graft, and died in shock after 68 hours, while rabbit No. 1 showed but slight signs of shock and made an uneventful recovery from its wound.

A cat was slightly burned along the spinal region, the burned skin dissected out and transplanted to a second cat. The latter developed shock after four days, probably due to delayed absorption. At autopsy the post-mortem findings were typical of shock. This experiment illustrates the necessity of keeping experimental animals under observation for several days before passing judgment on the outcome of an experiment.

"SHELL SHOCK"

8.15 A.M. A guinea pig was wrapped in a small cloth bag and the latter bound to a stand placed within a few centimeters of the revolving arm of a centrifuge

making 10.000 r.p.m., for ½ minute. Animal appeared depressed with slight symptoms of shock. Observed for 10 minutes and then placed in proximity to centrifuge arm for another half minute. Well developed shock, eyes dull, fur staring.
8.45 A.M. Temperature 35°. Marked trembling.
1.05 P.M. Temperature 36°.
3.45 P.M. Animal in crazed condition, began to eat feces and chewed frantically at steel forceps under jar.
6.00 P.M. Autopsied. Upper part of intestines and stomach markedly congested and hemorrhagic. Liver showed extensive small hemorrhagic areas. Kidney and spleen hemorrhagic. Brain and spinal cord findings were practically negative although the membranes were congested.

A guinea pig, weight 775 gms., placed in proximity to centrifuge arm as above described for two ½ minute periods spaced 5 minutes apart. Slight symptoms of shock from which the animal recovered. Although kept upon a normal diet this animal lost weight. 10 days after the experiment it weighed 649 grams (loss 126 grams). Was dull and listless with dull, rough coat.

This experiment offers evidence of cytost intoxication following an injury of a sort which leaves no definite external indication of its nature.

EXPERIMENTS WITH AUTOLYZED TISSUE

For experiments such as those described below, sterile autolyzed tissue extracts are easily prepared as follows: The desired tissue is removed aseptically

and transferred to a sterile vessel which in turn is placed in a covered desiccator containing a small vessel of chloroform. The vapor of the latter serves to inhibit any bacterial growth which might otherwise take place. The dessicator and its contents are then placed in an incubator at 37.5° C. for twenty-four hours or longer in order that autolysis may proceed at a reasonable rate.

At the expiration of this time the autolysate may be diluted with a convenient volume of isotonic saline solution and centrifuged to remove suspended matter. This process need not be carried out aseptically, for, as stated in the text, the cytost extracts prepared in this fashion may be boiled and filtered through a Berkefeld filter without any loss of potency.

Before using such an extract for any particular experiment it is well to test its toxicity. This may be conveniently accomplished by determining the minimum lethal dose when injected intraperitoneally into guinea pigs, as described in Chapter XI, or by direct injection into the mesenteric vein of animals with opened abdomen. From the standpoint of general utility the former method is perhaps the most satisfactory.

Rabbit, weight 1,500 gms. Abdomen opened under ether anesthesia. One drop of homologus muscle autolysate (previously diluted with 10 volumes of saline) was injected into the liver. This caused a slight turgescent reaction. 1 cc. of the extract was then injected into the gastric branch of the right epiploic vein. This caused the onset of shock—the symptoms developing slowly in the same sequence as is found in traumatic shock. Upon removal of the anes-

thetic, the animal regained consciousness promptly, but severe shock soon supervened. The injection was made at 4.30 P.M. when the rectal temperature was 39.4°. Following the injection the temperature dropped regularly, reaching 34.1° at 8.45 P.M., when the animal died in shock.

Rabbit, weight 1,200 gms. *1:30 P.M.* Rectal temperature 39°. Injected 2.5 cc. of the autolysate into the marginal vein of the ear. Symptoms of shock began at 2.00 P.M., the animal appearing apathetic. *2.30.* Eyes dull, gums pale. *3.30.* Reflexes diminished, marked pallor of mucous membranes of mouth, nose, and conjunctivae, pulse, rapid and weak, labored breathing, rectal temperature 37°. Temperature 34° at *8.45 P.M.* Died *9.00.* Immediate autopsy disclosed marked congestion of stomach, duodenum, upper small intestines, and liver.

That extracts of charred tissues obtained from burns behave similarly to tissue autolysates is shown by the following experiments:

One half gram of charred tissue was dissected from the burned area on the back of a cat which had passed into shock as a result of a burn. This was ground in a mortar with 5 cc. of saline and filtered. 2 cc. of this extract were then injected into the jugular vein of a cat weighing 2,200 gms. Shock developed immediately following the injection, but the animal recovered after several hours.

One cc. of this extract was injected into the mesenteric vein of a cat weighing 2,500 grams. Death followed within 3 minutes.

Ten grams of rabbit muscle were charred in a crucible over a Bunsen flame. The black mass was

extracted with 10 cc. of sterile water, boiled for a few minutes and filtered. 5 cc. of this extract were injected into the ear vein of each of two rabbits weighing 1500 grams. Both developed immediate shock with diminished reflexes, nystagmus, respiratory and circulatory failure. One animal died after 2 hours in profound shock and the other after a lapse of six hours.

The experiments presented above are typical of literally hundreds of similar ones recorded in our laboratory notebooks.

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