[*From the* PROCEEDINGS OF THE ROYAL SOCIETY, B, VOL. 77, 1906.]

On the Occurrence of Heterotypical Mitoses in Cancer.

BY E. F. BASHFORD, M.D., AND J. A. MURRAY, M.B., B.Sc.

[*Reprinted from the* Proceedings of the Royal Society, B. Vol. 77]

On the Occurrence of Heterotypical Mitoses in Cancer.

By E. F. Bashford, M.D., and J. A. Murray, M.B., B.Sc.

(Communicated by J. Rose Bradford, M.D., F.R.S. Received November 2,—Read November 23, 1905.)

[Plates 5 and 6.]

The present paper refers to a communication* made to the Royal Society in January, 1904. In that paper and its expansion,† published later, we emphasised the significance of the zoological distribution of cancer; we discussed the unique features of the processes responsible for the experimental transmission of carcinoma from one animal to another and the limitations to its successful attainment: we also published a series of figures depicting the characters of the nuclei of cancer cells during division, in the malignant new growths of fishes and mammals. We shall give a different explanation of the mitoses we figured in our earlier communications as resembling the heterotypical mitoses of reproductive tissue. We have found that those mitoses may be interpreted as somatic mitoses with longitudinally split chromosomes. Their apparent heterotypical form is thus due to variations in the development of the achromatic figure, the peculiar form of the chromosomes and their mode of attachment to the spindle.

Our figures of heterotypical mitoses in cancer confirmed the observations of Farmer, Moore and Walker, communicated‡ to the Royal Society at the preceding meeting, but we dissociated ourselves from their conclusions on the diagnostic value and the significance of the phenomenon. The amount of chromatin entering into the equatorial plate of the dividing cells of human cancer had long been known to be subject to diminution (von Hansemann,§ 1893), but the presence of heterotypical mitoses appeared to throw a new light on its occurrence and meaning.

We have pointed out that the characteristic changes accompanying the heterotypical mitosis in the reproductive tissues are absent from cancer cells undergoing what we regarded as this form of division, and that the want of correspondence extends to the stages which precede and follow it.|| We have

* 'Roy. Soc. Proc.,' vol. 73.

† First Scientific Report, Cancer Research Fund.

‡ 'Roy. Soc. Proc.,' vol. 72.

§ 'Studien über die Spezificität, den Altruismus und die Anaplasie der Zellen,' Berlin, 1893, etc.

|| *Loc. cit.*, and 'Lancet,' April 1, 1905.

also illustrated some of the appearances simulating bivalent chromosomes, but in reality conforming to the type met with in ordinary (somatic) karyokinetic cell-division.* In what follows we shall illustrate other sources of error on the basis of a renewed analysis of the preparations from which the figures of heterotypical mitosis in our previous papers were made and by other figures not yet published.

In the sexual cells of animals the heterotypical mitosis is preceded by a stage known as the "synapsis." In it the chromatin filament is split longitudinally and gathered into a rosette at one part of the nucleus, the nucleolus lying to one side and usually flattened against the nuclear membrane. In this stage the chromosomes are believed to unite in pairs, thus giving rise in the equatorial plate of the heterotypical mitosis to bivalent chromosomes, half as numerous as those characteristic of ordinary somatic cells. The examination of many sporadic and transplanted malignant new growths failed to reveal a corresponding sequence in their nuclei. We therefore undertook a renewed analysis of the preparations of the stages in cell-division already figured from transplanted mouse tumours, and of other preparations resembling them, to determine whether or not their identification as heterotypical were justified. We shall confine our statements mainly to five transplantable mouse tumours because they permit of control observations with varying methods of preservation and staining in a manner not possible with material from sporadic new growths; but our remarks apply also to the figures we have published from sporadic tumours of the trout and cat.

Von Hansemann† has combated the statements on the presence of heterotypical mitoses in malignant growths, and ascribes the appearances figured to clumping of the chromosomes, and to pathological abnormalities in their form. He adheres to his conclusion that the numerical diminution is not an exact halving of the normal number, but is irregular, and due to (1) asymmetrical mitosis, and (2) casting out and degeneration of chromosomes. Häcker reproduced three of our figures in a paper in which he admitted the striking similarity to heterotypical mitosis, but suggested that adhesion of the chromosomes in pairs,‡ together with longitudinal splitting of the couples

* *Loc. cit.*

† 'Biolog. Centralb.,' vol. 24, 1904; vol. 25, 1905; 'Verhandl. physiol. Ges.,' Berlin, 1904.

‡ Häcker uses the term "heterotypical" in a purely morphological sense, and embraces in it the mitoses of the cells destined to give rise to the sexual products of Ascaris and the Copepods. In the latter he has shown that exposure to ether ('Anat. Anzeiger,' 1900) may cause all the cells of the developing egg to exhibit this modification (viz., cohesion of the chromosomes in pairs). In the strict sense of the word, the term "heterotype" is

thus produced, might account for the phenomena encountered in cancer. The appearances in some preparations are explicable in the manner suggested by von Hansemann,* but all the forms of cell-division resembling the heterotypical mitoses of reproductive tissue cannot be accounted for in this manner.

Some of the nuclear divisions previously figured have been found on re-examination to be due to an artificial grouping together of distinct chromosomes. Fig. 1, reproducing at higher magnification fig. 3 of our Royal Society paper, and fig. 27 of the First Scientific Report of the Imperial Cancer Research Fund, affords an example of this source of confusion. The chromosomes seem to be bivalent, *i.e.*, to have the form of rings and loops. This mitosis is not completely depicted in fig. 1, the remainder of the chromosomes being in the next section. The preparation has been carefully restained. The result is shown in figs. 2 and 3. The "rings" and "loops" resolve themselves into a larger number of ordinary short chromosomes, split longitudinally. The dense equatorial plate of the next section shows clearly the presence of many short chromosomes split longitudinally, and arranged in the manner described below. The mitosis is therefore somatic, and not heterotypical.

The ordinary scheme of karyokinetic cell-division presents, in its phase of equilibrium (amphiaster or equatorial plate), a series of V-shaped loops with limbs of equal length, arranged around a central spindle and all lying in a plane at right angles to its axis. This arrangement is by no means universal. Some of the deviations are of great importance to a proper understanding of what occurs in cancer. Frequently the limbs of the V-shaped loops are of unequal length. When this is the case the attraction fibres are relatively few in number and attached only to the apex of the V and its immediate vicinity, and therefore nearer one end of each chromosome than the other.

applied to the form of mitosis characteristic of the first ripening division of the spermatocytes of amphibia, as first described by Fleming. In the Salamander, ring-shaped chromosomes, half as numerous as the longitudinally split chromosomes of somatic mitoses, are stretched out into elongated ellipses upon the spindle, giving rise to a barrel-shaped figure. Chromosomes of similar form are associated with their numerical reduction in many animals and plants, but it must be borne in mind that different forms of chromosomes occur in the corresponding mitoses of some animals. In Ascaris, *e.g.*, both ripening divisions appear to be effected by a longitudinal splitting of chromosomes arranged transversely on the spindle, and in others ring or loop chromosomes are never formed. It was, of course, conceivable that reduction-divisions might occur in cancer by means of chromosomes unlike those in the reproductive tissues of the same animal. The frequency of cells with diminished numbers of chromosomes led us to examine many tumours for evidence of their occurrence, but without result.

* 'Biolog. Centralb.,' vol. 24, 1904.

As a consequence the longer limb does not come to a position of equilibrium in the equatorial plane, but may take up one inclined to the axis of the achromatic figure. When the attraction fibres are attached to the chromosomes in this manner in a cell of elongated form, the longer limbs may even come to lie parallel to the spindle axis, and nuclear divisions closely resembling heterotypical mitoses may result. In such mitoses pairs of distinct chromosomes whose longer limbs lie on opposite sides of the equator, while their apices are closely opposed, simulate bivalent chromosomes, *cf.* figs. 5 to 10. In polar view the apparent halving of the chromosome number due to superposition, and the unusual vertical extension of the free longer limbs, are even more deceptive; unless the longitudinal splitting of the chromosomes is very clear, the resemblance to heterotypical mitoses may be almost perfect. During the separation of the daughter chromosomes such nuclear divisions are especially deceptive, because the longer limbs adhere for some time after separation of the apices and short limbs. Barrel-shaped forms result, in which the crowding together of the chromosomes renders their enumeration impossible, but at the same time conveys the impression of a diminution in their number.

A much more serious source of error results from individual differences in the size of the chromosomes in one and the same nucleus. Montgomery* and Sutton† have drawn attention to this phenomenon in the sexual cells of invertebrates. We have found it to be present also in many vertebrate mitoses. When the chromosomes attain the position of equilibrium in the equatorial plate the smaller take up a position nearer the axis of the central spindle than the larger or more massive ones. Seen in profile the larger may then completely screen the smaller from view, and lead to an underestimate of the chromosome number.

Normally, nuclear division takes place by means of bipolar mitosis distributing the halves of the chromosomes to two daughter nuclei. An interesting abnormality results, when, from any cause, this segregation of the daughter elements does not take place. This may either result from the centrosome, remaining single or, if after division of the centrosome, one only become attached to the chromosomes by the attraction fibres. The chromosomes then remain in one group, but the daughter elements separate slightly from each other before combining to form one large nucleus, containing twice as many chromosomes as the mother nucleus. This form of mitosis, known as a "monaster" (from the presence of only one active attraction sphere) has been

* T. H. Montgomery, 'Proc. Acad. Nat. Sc., Philadelphia,' 1901; 'Biol. Bull.,' vol. 4, 1903.

† W. S. Sutton, 'Biol. Bull.,' vol. 4, 1902.

studied and described in detail by Th.* and M.† Boveri in invertebrates: figs. 13, 14, and 15, from a squamous cell carcinoma of the tongue (human), represent a cell in this condition. The nuclear membrane has disappeared, but there is no trace of radiations or centrosomes to be seen. The chromosomes are arranged as a hollow sphere around a central clear area, and for the most part consist of two parallel daughter elements in different stages of separation. Some chromosomes in which the separation of the daughter elements is least advanced have a horse-shoe shape. In others, the daughter elements are parallel and widely separated. In a few, the separation is incomplete, the ends only remain apposed, giving the appearance of rings. In fig. 13 this cell has a striking resemblance to a heterotypical mitosis such as occurs in testis. Its cytoplasm is clear and voluminous, and there are no intercellular bridges between it and the surrounding epithelial cells. The number of chromosomes in the cell, however, is not diminished, but amounts to 45 to 50 when both sections are examined. Such a mitosis, instead of reducing the number to half, really results in the original number of chromosomes being doubled, because the separated halves of the chromosomes combine again to form a single nucleus.

After elimination of these sources of confusion, there remain other apparent heterotypical mitoses which cannot be explained in any of these ways. The chromosomes present an irregular contour and become drawn out like a viscid fluid in the later stages of separation. Individual chromosomes cannot be made out. The achromatic spindle develops at such a rate that the evolution of the chromosomes cannot keep pace with it, and they are drawn towards the spindle and stretched upon it before they have completely contracted and condensed.

All these abnormalities may occur in nuclei possessing the usual number of chromosomes. They also occur in cells with a greater or lesser number associated with the presence of multipolar and asymmetrical mitoses in other cells. In such cases there is no evidence warranting the assumption that the diminution in the number of the chromosomes is due solely to a nuclear division effecting a reduction comparable to that of the sexual cells.

Galleotti‡ and von Hansemann§ have shown that nuclei with diminished numbers of chromosomes (hypo-chromatic) may arise from larger ones by asymmetrical mitosis, in which entire chromosomes pass to one daughter

* Th. Boveri, 'Sitz.-ber. Phys.-med. Ges.,' Würzburg, 1897; 'Zellenstudien,' vol. 4, Jena, 1901.

† M. Boveri, 'Jen. Zeitschr. f. Naturwiss,' vol. 37, 1903.

‡ 'Ziegler's Beitr.,' vol. 14, 1893.

§ *Loc. cit.*

cell, because they are only attached to one or other attraction sphere, and also by "casting out of chromatin." "Casting out of chromatin" is merely an exaggeration of what occurs in asymmetrical mitosis; in it some chromosomes remain unattached to either attraction-sphere, and therefore fail to be included in either daughter nucleus. Krompecher* and we ourselves† have shown that multipolar mitoses may also lead to a diminution in the number of chromosomes. We stated that nuclei with diminished and half the somatic number of chromosomes occur without it being possible to determine whether the diminution has been effected by asymmetrical mitosis, casting out of chromatin, multipolar or heterotypical mitosis.

We have given our reasons for now believing that the mitoses we formerly assumed confirmed the occurrence of a heterotypical reducing division in cancer, are, in reality, somatic mitoses. Although we do not presume to explain in the above manner all the figures which may be brought forward resembling that form of nuclear division, we submit that the occurrence of heterotypical mitoses in cancer requires further proof. Multipolar mitosis and other irregular forms of cell-division occur in cancer, but they do not supervene upon heterotypical mitosis. They are entirely independent of its presence, and, of themselves, suffice to account for the diminutions frequently occurring in the number of chromosomes in cancer throughout the vertebrates.

DESCRIPTION OF PLATES.

[Plates 5 and 6.]

Fig. 1.—Apparent heterotypical mitosis. Transplanted carcinoma of mouse. Analysis of ring, loop, and bivalent chromosomes (heterotypical). Replica of fig. 3 of Royal Society paper, and of fig. 27, First Scientific Report, 1904. × 3000/1.

Fig. 2.—Same section as fig. 1. Analysis after restaining, showing how a fortuitous association of short somatic (longitudinally split) chromosomes gives the appearance of bivalent elements. × 3000/1.

Fig. 3.—Partial analysis of the remainder of the mitosis, of which part only is shown in figs. 1 and 2. Longitudinally split chromosomes with limbs of unequal length lying at various angles to the spindle axis. × 3000/1.

Fig. 4.—Diagram of a somatic amphiaster, in which longitudinally split V-shaped chromosomes, with limbs of unequal length, are apparently arranged parallel to the spindle axis. Adjacent chromosomes, with their longer limbs on opposite sides of the equator, if regarded as together forming one chromosome, would convert such a mitosis into a heterotype with half the somatic number of chromosomes arranged longitudinally on the spindle, *e.g.*, figs. 3, 5, 6, 7, 8, 9, and 10.

Figs. 5 and 6.—Apparent heterotypical mitosis. Fig. 5, replica of fig. 4, Royal Society paper, and of fig. 26 in First Scientific Report, 1904. Transplanted carcinoma of

* 'Centralb. f. Path. u. path. Anat.,' vol. 13, 1902.

† *Loc. cit.*

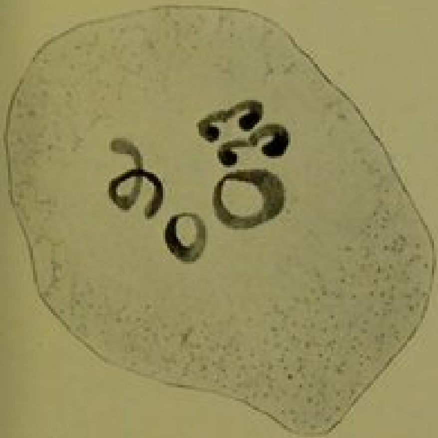

FIG. 1.

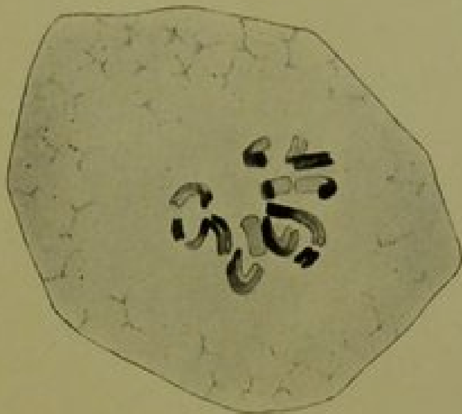

FIG. 2.

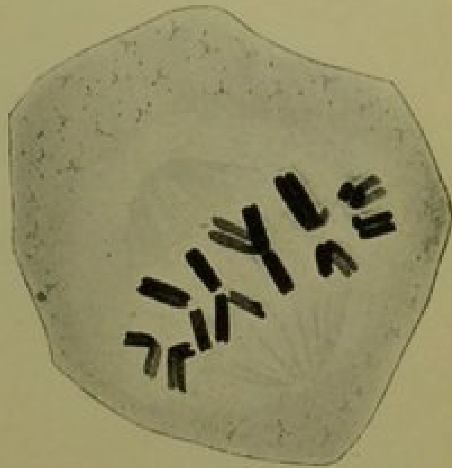

FIG. 3.

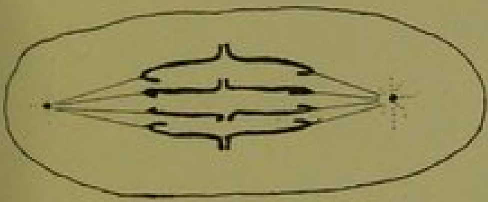

FIG. 4.

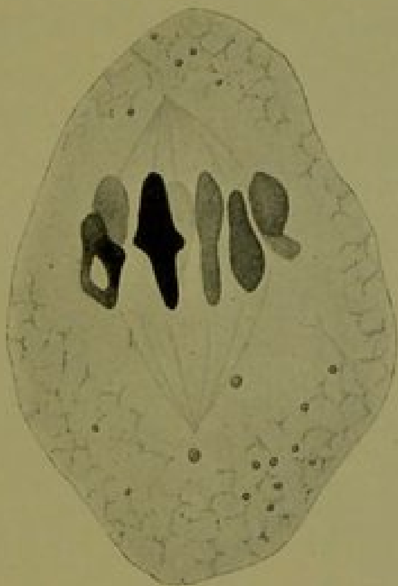

FIG. 6.

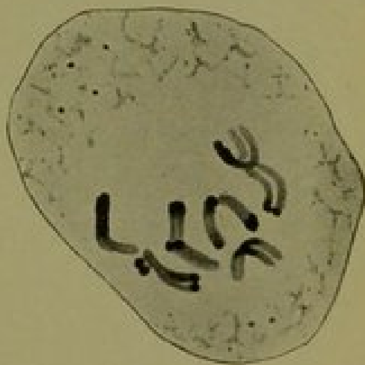

FIG. 7.

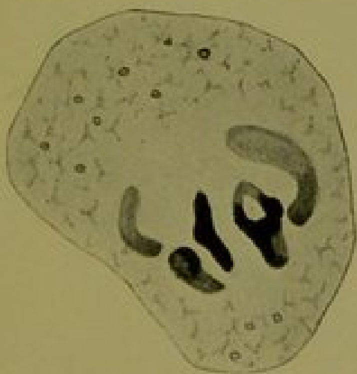

FIG. 5.

FIG. 8.

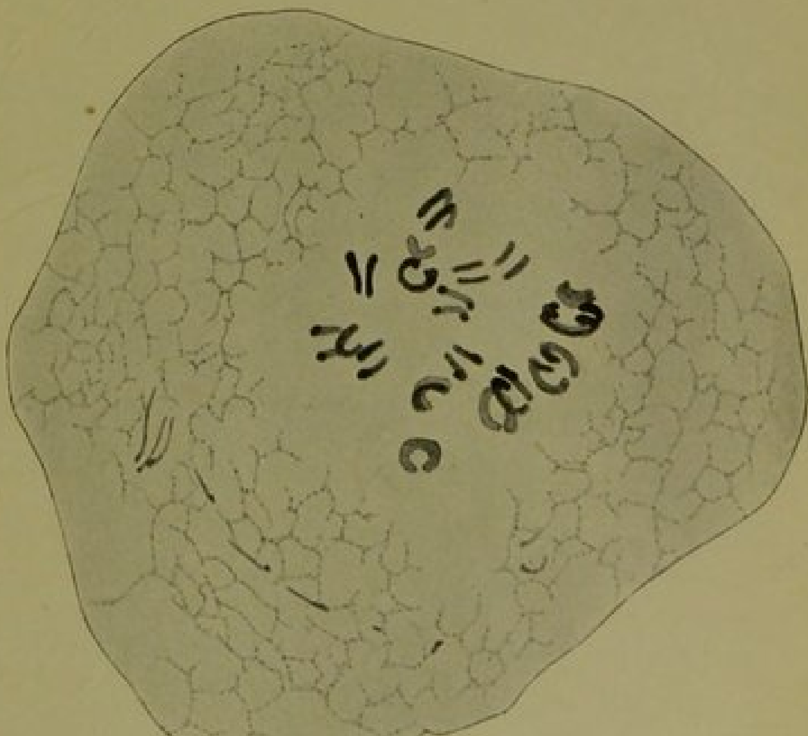

FIG. 15.

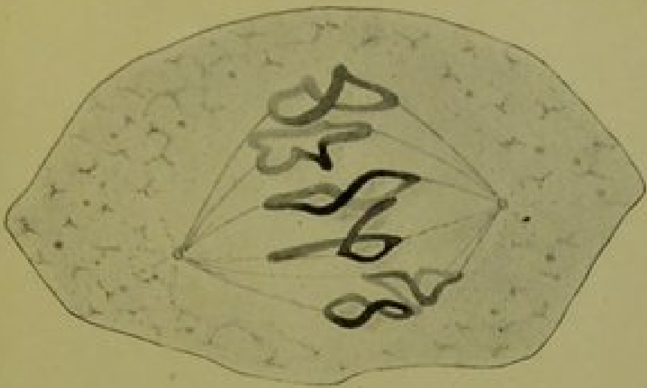

FIG. 9.

FIG. 10.

FIG. 11.

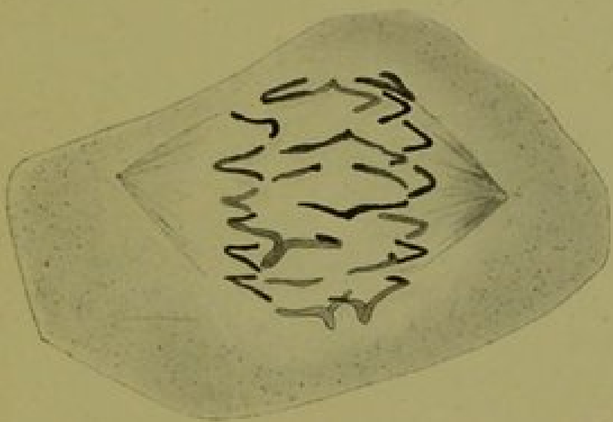

FIG. 12.

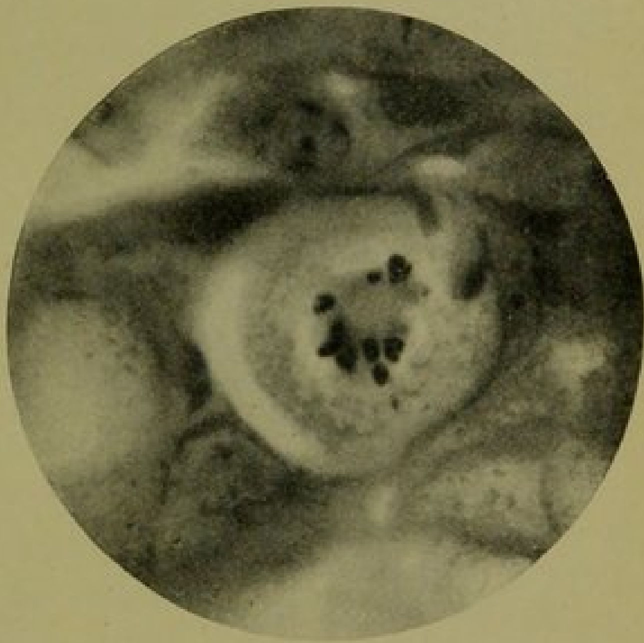

FIG. 13.

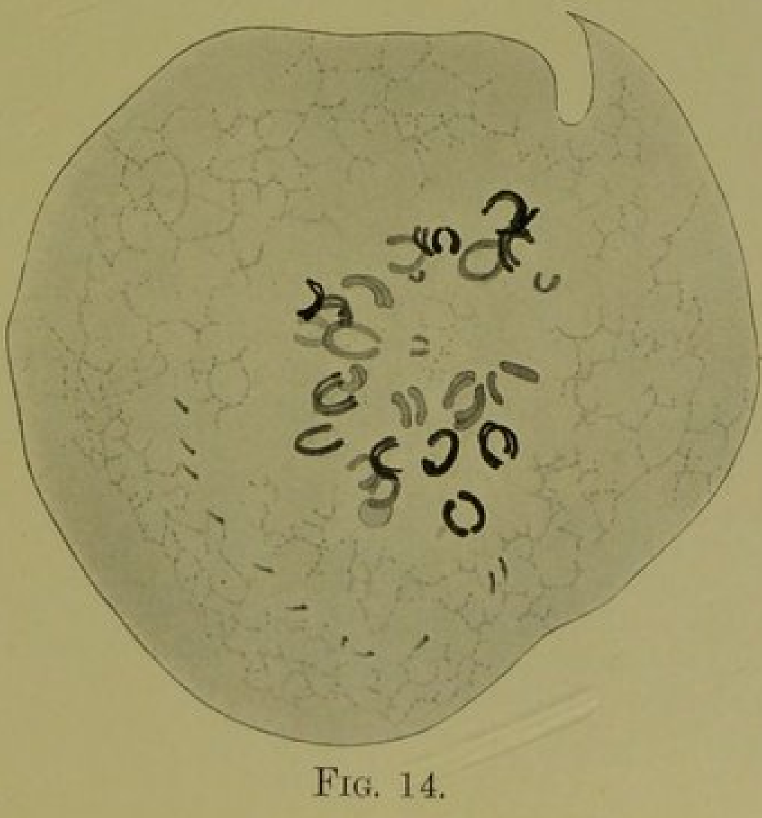

FIG. 14.

mouse. Chromosomes arranged longitudinally on the spindle. The mitosis is contained in two consecutive sections. × 3000/1.

Figs. 7 and 8.—Same sections as figs. 5 and 6. Result of analysis after restaining. Longitudinally split chromosomes with unequal limbs projecting above and below the equatorial plane. × 3000/1 of diagram, fig. 4.

Fig. 9.—Apparent heterotypical mitosis. Transplanted carcinoma of mouse. Loop and figure-of-8 chromosomes arranged longitudinally on the spindle. × 3000/1.

Fig. 10.—Analysis of the same preparation as fig. 9, showing the slight differences in interpretation sufficient to make this mitosis conform to the somatic type. The loop chromosome in the middle of the equatorial plate consists of two distinct V-shaped chromosomes with unequal limbs projecting above and below the equator. The attraction fibres are attached to the apices, and not to the ends of the long limbs as would be the case in a true heterotype. × 3000/1.

Fig. 11.—Diagram of a somatic metaphase in which the limbs of the chromosomes are of unequal length. The longer limbs still cohere after separation of the apices and shorter limbs. The barrel-shaped figure thus produced resembles a heterotype, especially when the compressed form of the cytoplasm crowds the chromosomes together.

Fig. 12.—Shows the detailed analysis of the mitosis at the upper part of fig. 20, Plate 7 Second Scientific Report, 1905. It illustrates the mode of separation of daughter chromosomes with unequal limbs, as represented diagrammatically in fig. 11. Transplanted carcinoma of mouse. × 3000/1.

Fig. 13.—Microphotograph (untouched) of "monaster" mitosis from squamous-celled carcinoma of the tongue (man). Shows ring and U-shaped chromosomes. × 1000/1.

Fig. 14.—Analysis of same section as fig. 13. Partial separation of the daughter chromosomes accounts for the presence of ring and U-shaped chromosomes. No centrosomes or achromatic figure visible. × 3000/1.

Fig. 15.—Remainder of same cell in next section. Shows large number of chromosomes of ring and U-shape, along with others in which the widely separated daughter-rods are parallel to each other. × 3000/1.

Harrison and Sons, Printers in Ordinary to His Majesty, St. Martin's Lane.