THE CHROMOSOME-NUMBER IN CANCER TISSUE
OF MAN, OF RODENT, OF BIRD AND IN
CROWN GALL TISSUE OF PLANTS

MICHAEL LEVINE

(Laboratory Division, Montefiore Hospital, New York City)

In a comparative study of plant overgrowths induced by
Bacterium tumefaciens Sm. and T. and human and animal cancer,
the present writer (Levine, 1925) stressed the facts that in the
former growths the rate of cell division was greatly increased
but the nature of the division was apparently of normal type.
In malignant growths of man and animals, cell division was
rapid too; and aberrant types of mitoses occurred that were not
found in the plant overgrowths. Further stress was laid upon
the presence of cells with nuclei of normal size and behavior
together with cells of giant size. These enclosed either single
giant nuclei, or multiple nuclei of smaller size, or simple lobed
giant nuclei, or multilobed giant nuclei in the same tissue mass
of animal, or human cancer. It was further contended that these
aberrant cells divided mitotically and that their rate of division
and size played an important function in the rate of growth of the
tumors.

Some of these types of cells had already been observed by von
Hansemann (1890–1891), Galeotti (1893), Krompecher (1895),
Trambusti (1897), Nedjelsky (1900), Bashford and Murray
(1904), and others, but the attention given them was centered
on their diagnostic significance in cancer. Cytologically, the
giant cells in cancer were generally associated with amitotic divi-
sions and it was believed that they represented stages preceding
necrosis. No attention was given to the behavior of their
chromosomes.

The question of the chromosome-number in cancer tissue or
crown gall tissue was not seriously considered until a few years
ago. This may be attributed to the fact that the number of
chromosomes in normal tissues of man and the various laboratory animals used for cancer study was not definitely established. The generally accepted belief that amitosis was the common method of division in cancer and the inconstancy of the numbers of chromosomes observed in various cells of malignant growths deterred further study of the question.

Von Hansemann (1890–1891) in attempting to establish a means of recognizing malignant growths in man, called attention to aberrant mitotic divisions in this tissue. He counted chromosomes in these cells and figured as many as forty chromosomes in large cells and as few as seven in small cells. He believed that the difference in the number of chromosomes in the various cells he observed was due to an asymmetrical division of the nucleus.

Farmer, Moore, and Walker (1903–1906) compared cytologically, various types of human carcinoma and normal tissues. They reported that the somatic number of chromosomes in man is thirty-two. They found cells in carcinoma tissue with sixteen chromosomes and believed that reduction divisions occurred in this tissue. They further advanced the notion that these cells with the reduced number of chromosomes fused with leucocytes and so initiated the cancerous growth. They figured what they believed a leucocytic inclusion undergoing nuclear division simultaneously with the large nucleus of the host cell.

Further study of Farmer, Moore, and Walker's material was made by Walker and Whittingham (1911) who, while contending the accuracy of the work of their associates, counted as many as seventy chromosomes in some of the cells studied although they held to the belief that the greatest number of cells contained sixteen chromosomes and the cells that had thirty-two chromosomes were next in numerical importance. Dividing nuclei were found which showed less than sixteen chromosomes.

While it was not the purpose of my study to do more than to compare the cytological phenomena observed in the so-called plant cancer and human carcinoma, I figured some stages in cell division in the epithelioma studied (see Levine 1925, Figs. 10, 12, 13, 16, and 17) which show clearly small cells with small
numbers of chromosomes and giant cells with chromosomes far in excess of the now generally accepted somatic number of chromosomes in man. Since these cells form part of the subject of this paper I shall refer to them again.

Winge (1927) was the first to call attention to the chromosome-numbers in the cells of the crown gall of the beet. In 1917 he reported nine and eighteen chromosomes as the reduced and somatic chromosome-numbers in the normal beet. My early studies of the crown gall of the beet (see Fig. 19—a late anaphase in which eighteen chromosomes are to be seen at each pole) confirm this observation. Winge (1927) studied spontaneous crown galls of the beet and galls induced by implanting crown gall tissue of the beet into healthy beet roots. His studies led him to the conclusion that there is a similarity between animal cancer and the crown gall of the plant. I am calling attention to this conclusion and refer the reader to an early study which I have made in collaboration with Doctor Isaac Levin (Levin and Levine 1920) and a more recent study on the transplantable crown gall tissue (Levine 1928, 1929).

Winge points out that in the crown gall cells of the beet tetraploidy occurs; that is, the number of chromosomes found equals the haploid or the diploid number of chromosomes multiplied a definite number of times. He reported that in the nuclear division of large cells of the crown gall of the beet, thirty six chromosomes may be counted in the polar view of the equatorial plate stage. He observed that some cells contained 72 chromosomes, and others as many as 135 or possibly 144 chromosomes. Binucleate cells are not uncommon in this tissue. The cells which permitted a polyploid count were giant cells. Winge believes that tumors of animals or plants may be explained on the basis of tetraploidy.

In a paper presented before the American Phytopathological Society (Levine 1929), I confirmed the observations of Winge on the number of chromosomes in the neoplasia of the beet on material artificially produced by inoculations with the crown gall organism. In addition, I extended the observations to the crown

1 Haploid, reduced number x; diploid, somatic number 2 x; triploid, 3 x; tetraploid, 4 x; pentaploid, 5 x; polyploid, several or many x.
gall of a species of tobacco, *Nicotiana glutinosa*, in which the haploid number of chromosomes is 12 as shown by Goodspeed (1923–1924) and also by material grown in my garden. Examination of cells of the mouse tumor 180 and human epithelioma were also reported in which polyploid cells were found.

Margaret K. Lewis and Jane Lockwood (1929) reported the discovery of a tetraploid number of chromosomes (84) in the malignant cells of the Walker rat sarcoma No. 1. These studies were made on cells grown in culture. The tetraploid cells were characterized as large cells and not very abundant. The origin of the tetraploid cells, the authors believe, is by simultaneous divisions of two nuclei in the same cell. The binucleate condition, these authors believe, is the result of cell fusion. They further contend that fragmentation of chromosomes or asymmetrical division of the nucleus cannot be used as a basis for explanation of the origin of tetraploidy.

This study was soon followed by three papers on similar topics, one by Heiberg and Kemp (1929) on the number of chromosomes in the carcinoma cells in man, another by Goldschmidt and Fischer (1929) on the chromosomes in the cells of Ehrlich’s mouse adenocarcinoma and breast tumors of mice, and the third by Hirschfeld and Klee-Rawidowicz (1929) on Jensen rat sarcoma grown in vitro.

Heiberg and Kemp used biopsy material in which the giant cells were found to have 94–96 (tetraploid number) chromosomes. Other cells in the same tissue showed over 100 chromosomes. Cells with normal numbers of chromosomes 47–48 were observed; still other cells showed 23 chromosomes (haploid count).

Goldschmidt and Fischer studied cells grown by the tissue culture method. Their observations led them to conclude that most of the mitoses are normal since only two abnormal divisions were seen. In the majority of the cells in division 32–36 chromosomes were observed while some cells had 24–28 chromosomes. The very large cells observed have as many as 80 chromosomes (tetraploid number). Hirschfeld and Klee-Rawidowicz studied the cytology of the Jensen rat sarcoma and report subdiploid,
diploid, tetraploid and polyploid number of chromosomes in the cells of this tissue. Their polyploid cells show 89 chromosomes, which is slightly above the number expected for the tetraploid cell in this species.

**MATERIALS AND METHODS**

At present I am reporting the chromosome-number in cells of the Rous chicken sarcoma No. 1, of tumors on white mice produced by tar-painting, and of the Jensen rat sarcoma. I am also adding further data on the cytology of human epithelioma, mouse tumor 180 on the white mouse, and the plant overgrowths on the common garden beet (*Beta vulgaris*) and the tobacco (*Nicotiana glutinosa*). The tissues used in this study were uniformly fixed in a number of agents of which Flemming's weak and medium solutions and Bouin's solution were especially valuable. Carnoy's mixture gave very clear figures. The animal tissues were all taken in living state, immediately after biopsy or after the animals had been sacrificed. A large number of preparations of the human epithelioma, mouse 180, and the Jensen rat sarcoma were previously fixed and were found to be suitable for this study. The preparations stained by the Flemming's triple method had not lost their brilliancy or luster of color. Many new preparations of similar materials have been made in the past two years. The tissue of the Rous chicken sarcoma used represents a metastatic tumor in the lung found twenty-three days after inoculating the breast muscles with the Rous chicken virus. For this material I am indebted to Doctor James B. Murphy who has permitted me to fix this tissue from animals used in his experiments. I am also indebted to Doctor Francis C. Wood, who has kindly furnished mouse tumor 180 and the Jensen rat sarcoma tissues which were grown in my laboratory for a number of years. The mouse tar tumors were developed by painting ether extracts of tar on the backs of several hundred mice. These tumors were taken five to six months after the paintings were started. The crown galls on the beet were induced by inoculations with *B. tumefaciens*. The organism was introduced into the beet at the crown so that the tumors
were formed primarily at the upper part of the roots. Crown gall tissue on Nicotiana glutinosa has not been produced on this species of tobacco up to the present time. The petioles of the leaves were found to respond very rapidly to inoculations with the Smith-Townsend organism and overgrowths the size of lima beans were formed within a month. I am indebted to Professor H. T. Goodspeed for pedigreed seeds of this species of plant and to Mr. Kenneth Boynton who developed the seeds at the New York Botanical Gardens to a stage where I could inoculate them. A few plants of N. Langsdorfi, it is interesting to note, were also inoculated but produced no crown galls after this treatment.

The materials studied were imbedded in paraffin and sectioned 5-7-10 μ. Heidenhain's iron alum hematoxylin stain, and the safranin, gentian violet, and orange G. stains of Flemming were used. Several hundred cells especially selected were photographed and drawn under the same magnification so as to enable me to compare them with greater care. The Zeiss 2mm. objective and number 10 K. ocular were used.

THE CHROMOSOME-NUMBERS IN NORMAL HUMAN AND ANIMAL TISSUES

Before undertaking the study of the chromosome-number in tumor tissue of the animals and plants upon which I am reporting, it was found very desirable to investigate, wherever possible, the normal number of chromosomes in the forms under observation. Possible variations in the number of chromosomes may occur without giving any recognizable evidence in the soma. This applies particularly to laboratory animals. In plants this is particularly true since various species of the same genus are known to have different chromosome-numbers.

The chromosome-numbers in normal tissue of man are generally accepted as 47–48 (von Winiwarter (1912), Painter (1923), von Winiwarter and Oguma (1926), Kemp (1928), and Evans and Swezy (1929)) in the somatic tissue and 23–24 in the spermocytes. The spermatogenesis of the mouse has been studied extensively and the most recent studies of Painter (1926), Cox (1927), Minouchi (1928), and my own observations on material
used especially in connection with some radium investigations unpublished, show that there are 40 chromosomes in the somatic cells while the haploid number observed in the spermatocytes is 20. The white rat shows a slightly larger number of chromosomes than the mouse. Some of my studies on testes of rats show a haploid number of 21 chromosomes in the primary spermatocytes while in the spermatogonia 42 chromosomes were counted in a large number of cells. These observations are in accord with the findings already described for these animals by Painter (1926), Swezy (1927), and Minouchi (1928).

The determination of the chromosome-number in the bird has been fraught with considerable difficulty. Varying numbers have been observed in embryonic tissue of the chick and in the spermatogenesis of the pigeon and chicken (see Harvey 1920). Harper (1904) in a study of the fertilization and early development of the pigeon's egg found that the haploid number of chromosomes is 8. Guyer (1909-1916) in a study of the spermatogenesis of the chicken (Plymouth Rock and Rhode Island Red), the domestic guinea, and embryonic tissues of the fowl found the diploid number 16, while in the spermatocytes he found 8-9 chromosomes. In an endeavor to study the methods of fixing avian tissues, Hance (1925) figured 34-39 chromosomes in chick embryos. In a later study (Hance 1926) a variation in the number of chromosomes is definitely reported although the author believes that probably 35 to 40 chromosomes occur in the metaphase stage in somatic division. Hance contends that it is difficult to determine the exact number owing to the minuteness of the smaller chromosomes.

Cole, Painter, and Zeimet (1928) studied the chromosome constitution of individuals resulting from crosses between the pigeon and the dove. Sixty chromosomes were counted, of which number 6 large chromosomes appeared in the male and 5 large chromosomes were seen in the female. Oguma (1929) found as many as 61-62 chromosomes in young embryo testes of the pigeon, while in the secondary spermatocytes he counted 31.

In connection with the present study, I examined cytologically
the testes of the Plymouth Rock cockerel. The chromosomes in the division of the spermatocytes are fused as shown by Guyer, but when they were destained considerably, I was enabled to observe 8 or 9 rounded bodies in polar views of the equatorial plate stages. Smaller numbers, possibly 4 or 5, were observed in massed form in the secondary spermatocytes.

I also verified the chromosome number in the two plant forms studied. The forms of *N. glutinosa* showed consistently 12 haploid chromosomes in the reduction divisions of the pollen mother cell, and 24 (diploid) in the vegetative tissue in the flower. This is in conformity with Goodspeed's (1923–1924) observations on this species. The chromosome numbers in the beet were likewise studied. The chromosomes, in the reduction divisions, are fused and massed very much like those in the spermatogenesis of the cockerel. The vegetative tissues are clearer and confirm the opinion of Winge (1917) that 9 and 18 chromosomes are the haploid and diploid numbers respectively.

THE CHROMOSOME-NUMBERS IN HUMAN AND ANIMAL TUMORS

Paraffin section of human epithelioma, the Rous chicken sarcoma No. 1, tar tumor on the white mice, mouse tumor 180, and the Jensen rat sarcoma, all show uniformly a mixture of cells of various sizes. The human tissue, which came from a section of the lip of an old man suffering from the disease for a number of years, showed the most diversified cells in form and size. Some of these I have already described (1925). It may be of interest to mention again at this time that at least two categories of cells occur in this tissue. The small and semi-giant cell and nuclear structures of apparently normal appearance may be placed in one group, and in the second category may be placed the giant cells with many nuclei or giant nuclei of varied form.

The cells in the first group are the more common, and appear to resemble most nearly the normal somatic cells. The small cells are diploid, as I am pointing out below. The semi-giant cells which approximate the tetraploid condition are apparently large uninucleate cells. Karyokinesis is generally normal although multipolar spindles may be formed.
The giant cells are not of uniform size. The size of these cells is proportionate to the size and number of the nuclei present. The multilobulate nuclei are generally the largest. This is true for all the animal tumors studied, although there is considerable difference in the size of the cells among the different species of animals. In this group of cells, found especially in the human carcinoma and the mouse tumors studied, the chromosome-number is more than tetraploid and in some cases the chromatic bodies are so numerous as to make an actual count impossible.

I have found both groups of cells in the human and animal tumors studied. The giant group is more common in the human cancer, mouse tumors, and chicken sarcoma. I brought the diploid and tetraploid cells together for the purpose of presenting more clearly these giant cells in which the numbers of chromosomes are almost too numerous to be counted.

I have searched my material especially for dividing nuclei in which the chromosomes were so distributed as to make the count of their number as easy as possible. While large numbers of nuclei in division have been observed, the polar view of the equatorial plate stages with chromosomes in the same plane are not abundant.

The evidence is rather clear from these studies that the apparently normal small cells in human epithelioma and the animal tumors studied show a count which varies, depending upon the excellence of the preparation. In the human epithelioma 43–48 chromosomes have been frequently counted. In this tissue I have been able with great facility to count 47–48 chromosomes (diploid number) by carefully plotting and drawing the units of each cell. The dividing nuclei in metaphase stage show a uniform layer of chromosomes when viewed from the polar end of the spindle. The chromosomes are rod-shaped or slightly curved; V-shaped bodies are occasionally found also. In other preparations of this tissue, such as I have figured previously (Fig. 12, 1925), 44–48 chromosomes may be seen in the late anaphase stages with 22–24 chromosomes near each pole. In other cells of apparently slightly larger size, I have been able to count
67–70 chromosome units and as many as 94–96 in still other cells. The latter cells are tetraploids and are similar to those reported by Heiberg and Kemp (1929). These cells appear frequently in my preparations. In the group of small cells tripolar and tetrapolar spindles are of common occurrence. In some of these cells 48 chromosomes have been counted. These are oriented in the metaphase stage within three poles (see Fig. 13, 1925). In larger cells as many as 96 chromosomes have been counted in equatorial plate stage arranged in a tetrapolar spindle. Tripolar spindles occur quite frequently in the cells of the mouse tumors.

It has been possible by means of serial sections of these tumor tissues to study the chromosomes of the tetraploid cells with considerable ease. When the chromosomes of these nuclei are arranged on a bipolar spindle they are distinct and readily counted, although some appear to have been cut. Serial sections of human cancer cells have simplified the matter of counting the chromosomes. I have been able to count with considerable ease 96–106 chromosomes. Taking into consideration the possibility of cutting some of the chromosome units, the number is clearly tetraploid. Larger numbers of chromosomes than the tetraploid have not been observed in this group of cells. They are more numerous in the other types of cells which I am describing below. The semi-giant cells that have the tetraploid numbers of chromosomes are larger than those that I have called of small size. They appear generally to have normal bipolar spindles and seem to originate in uninucleate cells.

The small diploid cells in the Rous chicken sarcoma show two groups of chromosomes. There are large chromosomes, approximately 16–18 in number, with as many or more minute granular bodies. These smaller bodies are quite distinct but are extremely difficult to count because of their size and location. The staining capacities of these granules are the same as the chromosome units. They take a deep gentian-violet color with Flemming's triple stain. The large chromosomes are of several lengths. Most of them are rod-shaped but some are wide V-shaped bodies. These seem to be grouped in pairs although
I have not attempted to match nor to measure them. At the anaphase stage the chromosomes become relatively short and almost globular in form and approximate 32–36 in number. Half of them move in the direction of the opposite poles. I have been unable to trace the small granular bodies in division although the sizes of the chromosomes as they approach the poles are somewhat larger than the granular bodies seen in the polar view in metaphase. Few tetraploid cells that permitted accurate counts have been found. Giant cells with a polyploid number of chromosomes have been observed more frequently. I am describing these cells below.

The small and apparently normal cells of the mouse tumor produced by tar painting show a diploid number (40) of chromosomes most frequently. The chromosomes in both the mouse tumors studied are short rod-shaped bodies. Cells with the tetraploid (80) numbers have been found and the chromosomes have been carefully counted. Counts of the chromosomes in these cells were made which were below the normal tetraploid number. While the numbers of chromosomes fluctuated, they most frequently approached the tetraploid number. The tetraploid cells are comparatively large and are matched only in size by the uninucleate giant cells. The nuclear behavior in division in this tumor resembles very much the types of division found in human epithelioma. Teutschländer and Schuster (1926) found few atypical divisions in this tar tumor tissue of mice.

Chromosome-numbers in the cells of the mouse tumor 180 have already been mentioned (Levine 1929). The diploid number (40) appears commonly and the larger or semi-giant cells show the tetraploid number. These numbers are not uniformly 40 or 80 but considerable variation is found similar to that described by Goldschmidt and Fischer (1929) for mouse adenocarcinoma. Frequently chromosomes in the tetraploid cells become globular in shape and appear to be early stages in chromosome disintegration.

In the Jensen rat sarcoma the small cells in division permit of a count of 44 chromosomes, although the most common number of
chromosomes is 42, which represents the diploid number. Cells with a small number of chromosomes (hyperhaploid 26–27) have been observed and counted. In this tissue as in the mouse tumors the chromosomes take a short, straight, rod-like form. Many of the chromosomes in equatorial plate stage show a longitudinal splitting in preparation for division. This splitting is not found in all chromosomes and suggests the possibility that some of these bodies do not divide. The chromosomes in equatorial plate stages are arrayed in circular fashion about a clear area. Occasionally a space appears as if one or several chromosomes were missing. This observation is in accord with that of Goldschmidt and Fischer (1929) for the mouse cancer. The number of chromosomes in these cases however is not always below 42, although some counts showed that some of them appeared to be missing. Tetraploid cells have been observed in this tissue. These cells are similar to those described by Lewis and Lockwood (1929) for the Walker rat sarcoma. The giant cells in this tumor are not large in comparison with the giant cells of the other tumors studied. I have been able to count approximately 100-105 chromosomes in these cells.

Late stages in the mitotic division of nuclei have been observed in the animal tissues studied. In the late anaphase or early telophase stages the chromosomes are frequently fused beyond the recognition of the individual units, yet the mass of chromosomes at each pole is uniform in size and the resulting daughter nuclei have apparently the same number of chromosomes. Small cells and cells that are presumably tetraploids have been observed in division by the normal process of animal cell cleavage. In many cells the late telophase stages are in evidence without any indication of the beginning of cell division. Asymmetrical divisions have not been observed.

**POLYPLOYDY IN HUMAN AND ANIMAL TUMORS**

The chromosome-numbers in the giant cells of human and animal tumors are the most interesting of all those studied. I am now referring to the numerous chromosome divisions which occur in large units of cytoplasm in these tissues. The number
of chromosomes exceeds the tetraploid count. On the basis of size of the cytoplasmic mass, I believe these cells are to be associated with the multinucleate and multilobulate giant cells which are common in the cancer tissues studied. These cells are found abundantly in my preparations of human carcinoma, tar tumor of mice, and mouse tumor 180. They are present in the Rous chicken sarcoma although in my limited number of fixations of this tissue I have found a small number. Lobulate nuclei were not commonly seen in this tissue. In the Jensen rat sarcoma lobulate nuclei and uninucleate giant cells are found. The number of chromosomes in these cells is 84 (tetraploid), although 100-105 chromosomes (polyploid) have been counted in some cells as mentioned above.

It is of interest to note that the recent studies report (Heiberg and Kemp 1929) approximately 96–100 chromosomes in human carcinoma cells as the largest number of chromosomes found in this tissue. Goldschmidt and Fischer (1929) found a few tetraploid cells in mouse breast cancer and Ehrlich's mouse adenocarcinoma. From tissue culture material, Lewis and Lockwood, and Hirschfeld and Klee-Rawidowicz report tetraploid cells in the Walker rat sarcoma and the Jensen rat sarcoma respectively. It seems evident that these reports are based only on the study of small and semi-giant cells with diploid and tetraploid number of chromosomes. No larger number of chromosomes above the tetraploid seems to have been recognized.

In the epithelioma of the human, the mouse tar tumors and the mouse tumor 180, the Rous chicken sarcoma and the Jensen rat sarcoma, the number of chromosomes approaches the number in excess of the tetraploid and in most cases in excess of the octaploid number for the species. The number of chromosomes in the cells of the human carcinoma is so large as to defy accurate count. It may be noted again that these tissues were serially sectioned and I have been able to trace as many as six sections of a single cell in mitotic division. The chromosomes in the cells were plotted and an effort was made to count them. The shape and size of the chromosomes in these giant cells are typical of the species and are similar to those described for the diploid or
tetraploid cells already mentioned above. I am led to believe that these cells arise from giant multinucleate or lobulate cells. Giant cells with four to five nuclei of different sizes in late prophase stages have been observed in my preparations. The nuclei progress in division at a uniform rate of speed. A small number of these cells has been found in which each nucleus, small and large, presents a segmented spireme. While counting the segments of the spireme presents considerable difficulties, yet over one hundred distinct bodies have been recognized in some of the mouse tumor cells. These figures are infrequent but indicate clearly that the multinucleate cell may undergo mitotic division.

The disappearance of the nuclear membranes frequently occurs before the spireme has completely segmented or before there is evidence of the appearance of spindle fibres. In such cases a number of well formed typical chromosomes together with long segments of the spireme band are seen lying free in the cytoplasm. Further development of this type of cell results in the simultaneous formation of a number of spindle figures. The division figures of two or more nuclei mingle so as to form a complex structure when viewed in one plane. Other nuclei may form independent division figures in the same cytoplasmic mass similar to that figured by Farmer, Walker, and Moore (1906).

The presence of large numbers of chromosome masses such as appear in some of the cells of the human epithelioma and the mouse tumors, indicates the division of a multilobulate nucleus or many nuclei lying in close proximity to each other. It appears that at the time of nuclear division the chromosomes of the various nuclei mingle giving the appearance of unity to the mass although their arrangement is aberrant. In the division of the giant cells spindle fibres are not well developed. In the chicken tumor where the giant cells show in metaphase approximately 90 chromosomes there is only a faint indication of spindle fibres, while in the human and mouse tissues the cytoplasm is generally devoid of these structures. The small cells and semi-giant cells mentioned above, show well developed spindles and an orderly orientation of the chromosomes. While the chromosomes in the
giant cells assume the size and shape characteristic of the species, they are frequently longitudinally split as if dividing irrespective of the development of the spindle. There appears to be unlimited proliferation of the chromosomes regardless of nuclear formation. This excessive duplication of chromosomes may be associated with the necrosis of the cell. In some of these cells of the mouse tar tumor it seems that at first only some of the chromosomes disintegrate. These become rounded and take a more or less diffused stain with the Flemming's triple method. This indicates possibly the beginning of a necrotic process which will ultimately involve the entire cell. Is the material liberated taken up by other cells? Do these substances stimulate other cells to divide? Oschmann (1914) in the study of the aquatic worm, Tubifex bavaricus, found that many cells and their nuclei may fuse to form the egg cell. Some nuclei degenerate so as to facilitate their absorption by the developing egg nucleus. Schneider (1917) found in Deilephila euphorbiae, a sphinx moth, that the chromatic material of the nurse cells serves as food for the development of the egg. The solution of the chromatin of the nurse cell is followed by its transfer to the cell body of the egg.

There is an abundance of evidence to show that some small clusters of chromosomes become separated from the larger groups and become invested by a nuclear membrane. This gives rise to cells with a much smaller number of chromosomes (subhaploid) than is typical for the species. As indicated above, chromosomes occasionally lag on the spindle and fail to be included in the daughter nuclei. Such evidence appears to be rather abundant in the tar tumors of mice and in the human carcinoma. How these cells are cut out of the large cytoplasmic mass that constitutes the bodies of these giant cells is not very clear. The reconstruction of these chromosomes into the various types of nuclei mentioned above has not been followed. It seems possible to suppose that large clusters of chromosomes lying close to each other may become surrounded by a membrane and thus form a single giant nucleus. Then again the chromosomes may be arranged in irregular clusters so that the mem-
brane formed is lobulate in outline. The third possibility that presents itself is that the clusters of chromosomes lie so far apart as to bring about the development of a membrane around each cluster giving rise to a multinucleate structure.

THE CHROMOSOME-NUMBER IN CROWN GALL TISSUE

As I have already stated above, the chromosome-number is here reported on two species of plants. The common garden beet (*Beta vulgaris*) is especially favorable for study because of its rapid response to the crown gall organism and because of its relatively small number of chromosomes. The flowers of these plants are readily produced by planting mature roots. Inoculations with *B. tumefaciens* made into the roots at the same time produces a crown gall. This permits the study of the chromosome-number in the pathological cells of the gall and the reproductive cells in the flower of the same plant.

The chromosomes in the cells of the vegetative tissues in the flower show 18 chromosomes in polar view of the equatorial plate stage. This number appears constant and I have been unable to find any aberrant nuclear phenomena in this tissue. In the reduction division of the pollen mother cell, I have already indicated the presence of normal reduction divisions in which the haploid number (9) can be made out. Young crown gall tissue presents a large number of actively dividing cells (Levine, 1925). Back from the periphery of the growth, large binucleate cells may be observed. Multinucleate cells are not frequent although, as I have pointed out in my early publications, three nuclei and even four nuclei may occur in one cell. These cells are not common. Nuclear structures which seem to arise from nuclear fusion have already been mentioned (Levine, 1925).

I have studied and counted the chromosomes in these large cells and found a rather definite number of chromosomes of which the haploid number forms a constant multiple.

The tetraploid cells are larger than the diploid cells. The chromosomes appear to be somewhat larger although they are identical in shape with those found in the normal vegetative cells. Slightly curved rod-shaped bodies are the most common
forms found. The tetraploid cells are more numerous than the octaploid cells, but the most common cells in young crown galls are those with the diploid number of chromosomes. Winge (1927) found tetraploid cells most frequently in the crown gall tissues he investigated. The diploid cell, he believes, results from a reduction division of a tetraploid cell.

In one preparation in which I found a tetraploid cell the chromosomes were changed in form. Each chromosome took on a globular shape instead of a rod form and each body was divided into 2 or 4 smaller parts. That is, each chromosome was divided into two or four smaller units. The significance of this premature division of the chromosomes is not clear, except that it may indicate the possibility of division of the chromosomes without nuclear formation.

In the tobacco, *Nicotiana glutinosa*, my attention was directed especially to dividing giant cells in the crown gall tissue. I also studied the germ cells and the dividing vegetative cells of the flower of the same plant upon which crown gall tissue had been induced. Goodspeed (1923) has already pointed out that this species has 12 chromosomes as the reduced or haploid number. This number I also found in the pollen mother cells of the plants I studied. The chromosomes in the metaphase stage are most frequently globular in shape. The normal cells in the vegetative portion of the flower show 24 (diploid) chromosomes. The chromosomes are U shaped or hook shaped and may be readily counted in polar views of the metaphase stage. The chromosomes in the cells of the crown tissue on the petioles and stems were carefully studied. Cells with the diploid (24), tetraploid (48), and octaploid numbers (96) were found in the same section. The cells in each case become larger as the number of chromosomes increases. The chromosomes on the other hand seemed to be approximately the same size. I am using photographs and drawings made under similar magnification for this comparison. The chromosomes in this tissue are rod-like although the majority of them are slightly curved as in the premeiotic or somatic divisions of normal tissue.

My preparations lead me to disagree with Winge (1927) who
believes that the crown gall tissue arises from stimulated tetraploid cells. The majority of the cells in the crown gall of the beet and tobacco are diploid and the polyploid cells are much less numerous in the newly formed tissue.

Aberrant types of nuclear divisions involving diploid or polyploid cells have not been observed in my crown gall studies. Tripolar, tetrapolar or aberrant multipolar spindles resulting from simultaneous but independent divisions of the two or more nuclei in the same cell have not been seen. It must be mentioned that the material investigated was actively growing crown gall tissue, instead of matured galls which have proven unsatisfactory for sectioning. Cell inclusions such as are commonly seen in animal cancer are absent in the crown gall tissues studied.

ORIGIN OF POLYPLOID CELLS IN HUMAN AND ANIMAL CANCER AND CROWN GALL

The origin of heteroploid and polyploid cells in neoplasia of animals and plants has been studied from material imbedded in paraffin and from preparations grown by the various tissue culture methods. At this time it is only possible for me to indicate the methods which seem to ensue in these tissues as studied from paraffin sections briefly described above. I am recording here also some of the methods other than that I observed in my material.
1. Cell fusion.
2. Asymmetrical divisions.
4. Chromosomes which fail to divide but which move to one or the other pole.
5. Lagging chromosomes.
6. Proliferation of chromosomes without nuclear division.

I have been unable to find evidence for cell fusion either in animal or plant tissues to account for the origin of polyploidy. Lewis and Lockwood (1929) from their tissue culture study of Walker rat sarcoma contend that cell fusions occur. However, the number of repeated chromosomes reported would indicate
bicellular fusion. No evidence of cell fusion has been found in crown gall tissue. Cell fusion has however, been observed in plants, resulting in duplication of the chromosome-numbers. In the pollen mother cells of various hybrids of wheat, Gaines and Aase (1926) have made this observation, while Karpechenko (1927) has made similar observations for hybrids resulting from the cross between the radish and the cabbage. Gaines and Aase have figured fusions of adjacent microsporocytes after which giant spindles are formed. These show an aberrant distribution of chromosomes which resembles in a measure the giant cells observed in human and tar tumors of mice.

Von Hansemann (1890) contended that asymmetrical division and monopolar spindles characterize cancerous tissue. He figured late telophase stages in which two unequal masses of chromosomes appear at each pole. These figures fail to appear in my preparations of animal and plant material. It appears from my sections that such figures represent parts of oblique sections of dividing nuclei. Stout and Susa (1929) in a cytological study of the root tips of the daylily contend that the chromosome units in some cells may be increased by asymmetrical divisions. This type of division results in cells with large and small numbers of chromosomes. The subsequent division of these nuclei with failure of cell division brings about heteroploid or subtetraploid cells.

Nuclear division without cell division is a well known phenomenon. Gerassimow (1904) showed that dividing nuclei in Spirogyra cells subjected to low temperature completed their division; the cell however fails to divide. The nuclei in many cases fuse ultimately to form giant nuclei. Nčmec (1904–1910) subjected the roots of Pisum sativum to chloral hydrate and brought about giant cells with polyploid chromosome numbers. Winkler (1916) observed tetraploid cells in hybrids produced by grafting. Winkler explains that the most likely method of originating tetraploid cells is by fusion of two normal nuclei in a cell.

Some nuclei in the human and animal cancer cells and the crown gall tissue of plants divide without being separated by
cell division. These nuclei consist of equal masses of chromosomes as seen in the telophase stage and two equal daughter nuclei are formed which remain together in the undivided cytoplasm. These nuclei fuse in the animal and plant overgrowths to form a tetraploid nucleus. Trinucleate or tetranucleate cells presumptively fuse into one single nucleus before division. The assumption that the chromosomes of these nuclei appear on a single spindle appears possible.

The failure of some chromosomes to divide on the spindle appears to be well established in the spermatogenesis of certain animals and plants. In dividing cells of human and animal cancer this apparently occurs for the slight irregularities of the number of chromosomes is quite frequent and can be explained on this assumption. The actual demonstration is more difficult because of the relatively large number of chromosomes involved. I have mentioned above that in the polar view of the mouse and rat tumors, longitudinally split chromosomes appear while other univalents appear to be unchanged. The latter chromosomes may move to the pole without division. This contention is amply supported by the studies on the spermatogenesis of the butterfly crosses of Federley (1913) and in the microsporogenesis of the various haploids, hybrids, and other plants. Gates (1913), Rosenberg (1917, 1927, 1930), Hance (1918), Belling and Blakeslee (1923), Chipman and Goodspeed (1927), Karpechenko (1927), Matsuda (1928), Stout and Susa (1929), Davis and Kulkarni (1930), and many others have shown heteroploid and polyploid cells may arise by the irregular distribution of chromosomes, by the failure of the chromosomes to segregate, the failure of the first division to occur, the division of the chromosomes in the second (homeotypic) division, the association of two nuclei and the gathering of two diploid sets of chromosomes through the fusion of the heterotypic spindles, and fragmentation of chromosomes.

Lagging chromosomes or small clusters of them may be left on the spindle or extruded in the cytoplasm to form small nuclei. This condition has been frequently described in the formation of dwarf pollen grains in many plant hybrids. I have
already indicated their absence in crown gall tissue and their frequent occurrence in human and animal neoplasms. These small nuclei may disintegrate or regenerate into apparently normal nuclei by chromosome division without nuclear division.

The excessive duplication of chromosomes without spindle formation or nuclear reconstruction in human and animal tumors is of great interest. Up to the present the large number of chromosomes in human, mouse, and chicken tumors has not been reported. It appears, as I have already indicated, that the chromosomes proliferate by division and fail to separate to form parts of daughter nuclei until large numbers of these bodies are formed. This type of heteroploidy is common in human and mouse tumors. Metcalf (1928) in a study of *Protoopalina caudata* finds nuclear division without cell division. He also states that cells occur whose chromosomes have divided to give twice the normal number of chromosomes without division of either nucleus or cell body. The causes that bring about this behavior are not clear. The function is likewise obscure. I have already suggested that the division of these giant cytoplasmic masses into a number of smaller cells may be responsible for the rapid growth of the tumor.

The significance of polyploidy in tumor tissue is not clear. Any interpretation of this phenomenon which has as its basis the etiology of cancer does not seem plausible at present. It appears that the initial aberrant nuclear and chromosomal phenomena are responses of the cell to some chemical or physical influences. The subsequent behavior is determined by still unknown laws governing growth.

**SUMMARY**

A report is made of a study on the chromosome-number in epithelioma of man, in the Rous chicken sarcoma, in tar tumor of the mouse, in the Jensen rat sarcoma, and in mouse tumor 180. Chromosome studies of cells of crown galls of the beet, *Beta vulgaris*, and the tobacco, *Nicotiana glutinosa* are included.

1. The chromosome-number of the somatic cells of man is 47–48. Human carcinoma imbedded in paraffin and studied in
serial sections, shows cells which have 23–24 chromosomes and still other cells of smaller numbers which are apparently derived from larger masses of chromosomes. These cells may frequently be found included in a large cytoplasmic mass or giant cell. Tetraploid cells which show approximately 94–96 chromosomes appear among the semi-giant cells of this tissue. The nuclear division of giant cells shows larger numbers of chromosomes. Over 200 have been counted. Still other cells in the category of giant cells show a number of chromosomes so great as to defy accurate count.

2. The haploid chromosome-number in the fowl is apparently 8 to 9. In the pathological somatic tissue, 16 large chromosomes have been observed with an equal number of smaller granular bodies which have all the staining capacities of chromosomes and occupy the inner zone of the metaphase stage when looked at from the polar view. Polyploid cells have also been found in these tumors and as many as 90 chromosomes have been observed in this tissue.

3. The tar tumors of mice and mouse tumor 180 show comparatively similar stages of cell division found in the human epithelioma. The diploid number of chromosomes (40) may be counted in dividing apparently normal cells which occur in this tissue. Tetraploid groups of chromosome-units appear in these mouse tumors. Giant cells with large uncountable masses of chromosomes are frequently to be observed in these cells also.

4. The Jensen rat sarcoma with cells which behave normally shows 42 chromosomes (diploid). Tetraploid numbers of chromosomes (84) also appear in these cells. Lobulate nuclei of giant size, and giant cells with approximately 100–105 chromosomes have been counted in these cells.

5. The hypodiploid and heteroploid cells in the various tumors reported observed are due to the failure of chromosomes to reach the pole and to become included in the daughter nuclei. The failure of chromosomes to divide appears to be a potent factor in the chromosome-number of these cells. The tetraploid cells appear to arise from semi-giant nuclei which result from the failure of cell division to follow the division of the nucleus.
6. The giant cells which show numerous chromosomes appear to arise by repeated division of chromosomes without nuclear organization. The multinucleate condition and lobulate nuclei may have their origin in this type of cell.

7. The chromosome-number in the crown gall tissue of the beet (*Beta vulgaris*), and the tobacco (*N. glutinosa*) shows more frequently the diploid number of chromosomes. Lagging chromosomes may be seen similar to those found in normal tissue. The giant cells in both species show tetraploid numbers and octaploid numbers of chromosomes most frequently. The origin of giant nuclei appears to be due to nuclear fusions. The uninucleate cells give rise to binucleate, trinucleate, or tetrinucleate cells where nuclear division is not followed by cell division. Independent nuclear divisions in multinucleate cells have not been seen in my preparations.

A more extensive description of these data will follow, at which time numerous figures and plates will be presented.

REFERENCES


