TUMOR PROBLEM IN THE LIGHT OF RESEARCHES ON PLANT TUMORS AND GALLS AND ITS RELATION TO THE PROBLEM OF MUTATION

(A CRITICAL REVIEW FROM BIOPHYSICAL, BIOCHEMICAL AND CYTOGENETICAL POINT OF VIEW)

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Extensive work done on the morphology, cytology and physiology of cancer, its transplantations and investigations in tissue cultures as well as on tumor production by various agents have trown much light in many ways on the nature of cancer, but have not solved as yet the cancer problem.

This problem is at the present time not merely a subject for pathology but rather for general biology or more exactly, for all biological sciences. Pathologists, physiologists, biochemists, histologists, and genecticists are engaged in cancer research on human, animal and plant organism, since tumorical malformations occur on all of them. The pathologists, in studying the origin of the cancer looked for a long time for parasitic cause of the cancerous proliferations. Although in some cases such were found, in most of the cases, however, parasites seems not to be present.

Plant galls, no matter by what kind of parasites they have been produced, and what morphological and histological structure they have, represent proliferations with a cytological structure similar to that of animal and human cancer. This is especially true for the galls caused by *Bacterium tumefaciens*. But we know lately of *non-parasitic* tumors that appear in certain plant hybrids (KOSTOFF 1930, 1931) and correspond exceedingly well with the spontaneous tumors in animals and men. Thus in both the animal and plant kingdom parasitic and nonparasitic tumorical proliferations have been observed.

It is often questioned whether one may apply the term "cancer" for the plant tumors. Many investigators (JENSSEN, SMITH, LEVINE, STAPP etc.) who have shown the similarities of the plant tumors to animal cancer, are inclined to apply the term "cancer" to the tumorous proliferations in plants too. Many of the human pathologists, however, are definitely against such an unification. But the existance of spontaneous non-parasitic tumors in plants similar to those in animals speaks in favor of the former view. The similarities in the cytological structures of these tumors in both kingdom is especially conspicuous. We cannot say, of course, that an animal or human cancer is identical with a non-parasitic spontaneous tumor in plant hybrids, or with the tumors in plants obtained by various agents (SMITH 1917, KENDALL 1930, KOSTOFF 1931, KOSTOFF and KENDALL 1933), since we cannot say that an animal cell is identical with a plant cells, for there are some

differences in the cell structures and functions. But we can say that they are *similar* and that they behave *similarly* as far as the most of the general structures and the principal functions of the cells are concerned. The differences between the cells of the normal tissues in plants and the cells in the plant tumors are very similar to the differences between the cells of the normal animal or human tissues and the cancerous ones. In this sense one must understand the term "cancer" when applied for the tumorous proliferations in plants.

The non-parasitic tumors on hybrids and those experimentally produced by various agents are of greater interest for the *cancerologists* and for the geneticists than the parasitic plant tumors. The latter, however, supply very valuable data for generalization of certain *cytological* constitutions and *physiological* phenomena occuring in both parasitic and non-parasitic tumors.

In certain *Nicotiana*, *Crepis*, and other species hybrids in plants spontaneous appearance of fasciations or tumorous proliferations have been occasionally observed, but most thoroughly studied are those in *Nicotiana* (KOSTOFF 1930, 1930/1931, 1931). Not all of the hybrids resulting from the *Nicotiana* species crosses manifested equally tumors or other malformations to the same extent. For example, progeny of certain cross combinations represented by *N. glauca* × *N. Langsdorffii*, *N. paniculata* × *N. Langsdorffii*, *N. Tabacum* var. *wigandoides* × *N. Sanderae*, *N. rustica* var. *humilis* × *N. Sanderae*, *N. rustica* var. *humilis* × *N. alata*, *N. glauca* × *N. longiflora* etc. manifested cancerous proliferations so frequently and in such abundance, during their development that very many of the plants died before reaching the maturity, while progeny of other crosses represented by *N. Tabacum* × *N. glauca*, *N. glauca* × *N. Sanderae*, *N. Sanderae* × *N. Langsdorffii*, *N. alata* × *N. Langsdorffii*, *N. tabacum* × *N. paniculata*, *N. paniculata* × *N. glauca* etc. never, or very rarely, displayed any evidence of such abnormal proliferations. The hybrids forming tumors show the symptoms not equally in form, size, and extention. Some hybrids form tumors on the roots, others on the roots and on the stems, and third even on the leaves. The tumorous proliferations grade between large tumors without differentiated roots, shoots or leaves to formation of fasciated organs (KOSTOFF 1930, 1930/31, 1931). The nature of the hybrid tumors in plants reminds very much that of the malignant tumors in animals and man.

When branches of *N. glauca* × *N. Langsdorffii* hybrids that formed tumors were grafted on the parental plants *N. glauca* and *N. Langsdorffii*, tumors appeared only all over the hybrid parts only, but not on the stocks that represented pure species of the parental plants. The same phenomenon was observed when branches of *N. glauca* and *N. Langsdorffii* were grafted on the F_1 hybrids (*N. glauca* × *N. Langsdorffii*). In such cases the stocks formed tumors but not the scions, that represented pure species: *N. glauca* and *N. Langsdorffii*. The histological and the cytological studies of the tumors showed that there is no unicellular or multicellular parasites in them. The attempts of a parasitologists to isolate some parasites were unsuccessfull. Consequently such tumors formed by certain hybrids can be treated as non-parasitic ones; and the hybrids that formed tumors in Boston, reacted in the same way in Sofia and in Leningrad, i. e. they formed tumors in all these geographical localities, during all seasons in what ever environmental conditions they might be. In other words the cause for the tumor formation

in the plant hybrids is not external but internal. In the species hybrids we have brought together two genotypes differing somehow in genetic constitutions. These two different genetic contributions have reacted mutually in some way resulting in some definite processes giving rise to the formation of tumorous proliferations. Would it be possible to determine in some ways these kind of reactions, or at least some of these reactions involved? If we take extracts of the maternal and paternal plants and put them together, is there any kind of reactions that occurs *in vitro* which we can detect? We know that between extracts of certain species a precipitin reaction or a lytic reaction occurs (KOSTOFF 1929, CHESTER 1932). What would be the reaction between the parental extracts of the hybrids that formed tumors? The results of such experiments are given in table 1.

Table 1

Tumors formed on the stems of the hybrids	Precipitin reaction between the extracts
N. glauca × *N. Langsdorffii* . . . . . +++	+++
N. paniculata × *N. Langsdorffii* . . . . . +++	++
N. rustica × *N. Langsdorffii* . . . . . ++	++
N. Tabacum × *N. Sanderae* . . . . . ++	trace to +
N. rustica × *N. Sanderae* . . . . . +	+
N. rustica × *N. alata* . . . . . +	—
N. glauca × *N. Sanderae* . . . . . −−	trace
N. Langsdorffii × *N. Sanderae* . . . . . −	—
N. Tabacum × *N. glauca* . . . . . —	trace (occasionally)

It is obvious from the table that the greater the mutual reaction is between the extracts of the parental plants the larger the tumors are, that are formed on the stems of the hybrids mentioned. This is a *tendency*, because there is not an absolute correlation. Besides, the data given in the table vary in some ways. Even if one tests more cases, there may be found occasionally certain disharmonies too.

It would be interesting to know what kind of substances participate in the precipitin reactions. But on this subject our knowledge is very limited. We only know that in some cases investigated, protein and calcium oxalate reactions take place, we also know that some other perhaps are involved too, but we do not know yet what kind of substances they are (KOSTOFF 1931, 1932, CHESTER 1932).

If the precipitations *in vitro* can serve as an indication for tumor formations it should be possible then to produce tumors in plants by chemicals which precipitate plant extracts. Such experiments were performed with a series of chemicals of definite concentrations, by injecting them in the hollow stem of *Ricinus communis*. The chemicals used were: Lactic, acid, formic acid, succinic acid, asparagin, urea, formalin, alcohol, ammonia water, tar water, anilin water, ether water, arsenic acid, $Zn(NO_3)_2$, $(NH_3)_2CO_3$, extract from hot pepper, extract from *Ricinus*, normal rabbit serum, immune rabbit serum, top water, destilled water, etc. (KOSTOFF 1931, KOSTOFF and KENDALL 1933). Some have been injected in stems of *Pisum* and *Lupinus* (KENDALL

1930). The data obtained from these experiments show as a similar *tendency* as in the former experiments, namely the greater the precipitation of the *Ricinus* extracts by the chemicals applied, the larger the tumors were formed in the hollow stems of *Ricinus* following the injections of the chemicals mentioned. It would be too dogmatic to believe, however, that the tumors formed on the hybrids and those induced by various chemicals represent exclusively a direct sequence of the precipitin reactions found since precipitation can be accompanied by lysis, or some other kind of reactions. Here we shall note that lysis occurs also independently of the precipitation. Mutual reactions between the extracts *in vitro* and perhaps between the parental contributions *in vivo* lead obviously to certain *changes of some substances*, a phenomenon the significance of which we shall discuss later. But here we shall only note that if we treat this phenomenon from a strictly genetical point of view, we must admit that some genetical factors must be responsible for these reactions as well as for the tumor formation in hybrids. This is in accordance with the general principles of genetics whereby factors are responsible for all processes and morphological characters of the genotypes, although the crossing experiments carried out until now cannot convince us that mendelian factors are involved for the tumor formation in hybrids. It would be of interest to note for the further discussions that although the F_1 hybrid *N. glauca* × *N. Langsdorffii* forms very large tumors, the trigenomal back crosses (*N. glauca* × *N. Langsdorffii*) × *N. Langsdorffii* form chiefly fasciations and relatively small tumors instead large tumors, i. e. they represent a transitional stage between the normal type and the hybrids which form tumors.

Before discribing the histology and the cytology of the tumors formed on the hybrids and those artificially induced by various agents we shall briefly consider one more kind of tumorous proliferation that the plants form following the attack of various parasites, i. e. the plant galls. At the present time we have much richer literature on the plant galls than on the experimentally produced tumors and tumors formed on the hybrids. MALPIGHI (1686) was one of the earliest investigators who made extensive cecidological studies. BEYERINCKs classical investigations on cecidology (1883) have lately been continued by many investigators. I shall recall here only the names of KÜSTER, NĚMEC, SMITH, MAGNUS, WEIDEL, ROSEN, etc. (for literature see KÜSTER 1930, and KOSTOFF and KENDALL 1929). The galls differ very greatly from the morphological point of view. Their histological structure with the few exceptions of the highly organized ones like the *Cynipid* galls on *Quercus*, for example, however, does not represent very great diversities. The cytology of all the galls is relatively similar. Their cytology is also similar to the cytology of the hybrid tumors and the tumors experimentally obtained. They even have many cytological characters in common with the human cancer.

The morphology histology and the cytology of the hybrid tumors reminds one very much of the morphology, histology and cytology of the crown galls caused by *Bacterium tumefaciens*. The tumors experimentally obtained by various agents remind us also in many ways of some bacterial galls, although they have no bacteria present. Their cytology is very similar to the cytology of galls caused by various parasites. Since we are here chiefly interested in the structure of the hybrid tumors and partly of the tumors experimentally obtained, we shall consider the histology and the cytology of

the plant galls only as much as they offer similar conditions. The hybrid tumors, and those experimentally obtained, represent more or less a mass of parenchymatous tissue. Abnormally differentiated cells, as some parenchymatous-cells with secondary thickening, are scattered about in the tumors but they form a definite structure in the highly organized galls, as for example in the *Cynipid* galls on *Quercus*, where the "nutrative zone", "sclerified zone", and "parenchyma zone" can be found consistently (BEYERINCK 1883, WEIDEL 1911, COSENS 1912, KOSTOFF and KENDALL 1929). In tumors, as well as in galls an abundant accumulation of starch has been found. Occasionally mineral crystals and tanin have been observed. The cells in galls and tumors are often very hypertrophied and in certain regions very rich with cytoplasm. The vacuolation is not the same as in the normal tissue. In the morbid tissue the vacuoles are more numerous in the cytoplasm. The nucleus behaves differently. It is hypertrophied in many instances, and usually stains deeper with iron-alum-haematoxylin than the nuclei of the normal tissues, the chromatin having more or less a reticular structure (vd. nodules, nematode galls).

In some galls as well as in the necrotic regions of hybrid tumors the nuclear membrane gradually disappears; the chromatin mass showing an organization resembling prophasal structure, stains very deeply with iron-alum-haematoxylin. Mitosis in galls and tumors deserves greater attention. Sometimes the chromosomes divide in the metaphase but do not separate and form one nucleus with double chromosome number. The anaphasal process also seems to occur often abnormally, because the chromosomes sometimes divide and begin to separate, but remain very near the equator and never reach the poles of the mitotic spindle. When they are situated very close to the equator, they form one single nucleus with double chromosome number; when they are situated more distantly, but still relatively close to the equator without reaching the poles, two nuclei are formed, but a cell wall does not appear between them. Thus binucleate cells originate in tumors and galls. If the subsequent mitotic process occurs with a similar abnormality, tetranucleate cells originate. In galls and tumors polyploid and polynucleate cells often occur. The mitotic processes in them show some other abnormalities too. In some dividing cells during the late anaphase single or group of chromosomes lag on the spindle. Such anomalies lead often to unequal distribution of the chromosomes to both poles and to formation of nuclei with abnormal chromosome numbers, i. e., chromosomal aberrant cells. Sometimes the chromosomes on the spindle form a third micronucleus with few chromosomes. When many chromosomes are spread all over the spindles, sometimes 8-like nucleus are formed with double chromosome number, i. e. all the chromosomes of both poles and those of the spindle are included in a single nucleus. When such nuclei with 8-like shape were first found by the earlier investigators they were interpreted as amitotic nuclear division in the galls. It seems that a similar phenomenon occurs in the human cancer. Sometimes another kind of phenomenon occurs that gives an illusion of amitotic nuclear division in tumors and especially in galls (Nematode galls. KOSTOFF and KENDALL 1930). In the nematode galls polynucleate cells occur very often. There are cells that sometimes contain more than 30 nuclei. By the advance of the parasitic attack the nuclear membrane of the numerous nuclei begin to disappear. The chromatin masses of the single nuclei, which stain very

deeply with iron-alum-haematoxylin at such a phase begin to fuse and form larger and larger amoeboid nuclei with numerous nucleolei. When the chromatin masses of two nuclei just touch and begin to fuse into one, the shape of the chromatin masses of these two nuclei is an 8-like one and remind one very much of an amitotic figure. The chromatin masses thus formed have numerous nucleolei as already mentioned. We do not know yet the role of the nucleolus. Some investigators are inclined to accept it as having some relation to the chromosomes and the hereditary substances, others assume, that it represents merely metabolic products. DE MOL pointed out that the nucleolei increase in number in the polyploid cells. In the tumor and in gall cells they are definitely many more in number than in the normal tissues, but whether the polyploidy or the abnormal metabolic processes are responsible for their increase, we cannot tell at the present time.

The chromosomes in galls and in tumors are more or less contracted, when compared with the chromosomes of the normal tissues. Often instead of appearing as small rods, they have an ovoid and even spherical shape that reminds one of the shape of the chromosomes in the pollen mother cells and in the embryo sac mother cells. Very typical chromosome contractions have been observed in the *Cynipid* galls on *Quercus* (KOSTOFF and KENDALL 1929) and in the necrotic region of the tumors on the hybrids *N. glauca* × *N. Langsdorffii* (KOSTOFF 1930). In fact chromosome contraction seems to be a general phenomenon when mitosis proceeds under abnormal conditions. Various chemical, grafting, wounding, low temperatures, etc., also cause a contraction of the chromosomes (NĚMEC, SAKAMURA, KOSTOFF, KENDALL, DELAUNAY, SARANA). Consequently, the length of the chromosomes can be influenced by the environmental conditions.

In conection with the morphology and cytology of galls and tumors we shall call attention to one phenomenon which has lately attracted more and more interest amongst geneticists. We know that some bacteria like *Bacterium tumefaciens*, *Rhizobium radicicola*, etc. are gall forming bacteria and that there are other bacteria that do not induce gall formation in plants under normal conditions. Lately NĚMEC (1928) succeeded in producing enormous callus proliferations by introducing *Bacterium coli*, *B. megatherium*, *B. mesentericum*, and *B. proteus* in plant traumas. Considering these facts and the facts that polyploid cells often occur in bacterial galls, we may advance the question whether the trauma, as such, is responsible for the polyploidy in wound calluses following decapitations in JORGENSENS experiments, from which calluses, tetraploid shoots have regenerated, or whether for this polyploidy some bacteria are responsible. We shall note here that neither WINKLER nor JÖRGENSEN kept the traumas sterile in their decapitation experiments, and that tetraploid shoots have been obtained from the region of tumors produced by *Bacterium tumefaciens* in tomato, where attempts have been made to avoid the influence of the trauma (KOSTOFF and KENDALL 1932). An exact experimental analysis on this subject is very desirable, since we do not now know definitely how much the trauma is responsible for inducing polyploidy in the decapitation experiments and how much the bacteria.

After we described briefly the cytology of the tumors and galls we want to point out *similarities with the cytology of the animal and human cancer*. While in plant tumors and galls we have an abundant accumulation of starch

in the animal and human cancer there is an accumulation of glycogene (animal carbohydrate) (SOKOLOFF, ROBERT). It is of interest also to note the cellular and nuclear hypertrophy in animal and plant tumors and plant galls. One of the greatest interests here, however, is the inconsistency of the chromosome number in tumors (animal and plant), since it has been used for basis by many biologists for constructing working hypothesis to explain the aetiology and the nature of the tumors. The cytology of the animal tumors in this respect seems to be very similar if not identical with the cytology of the hybrid tumors and galls and in plants and especially with the cytology of the crown gall in plants caused by *Bacterium tumefaciens*. According to LEVINE, WINGE, KEMP, etc., mitosis in cancer is irregular, and the irregularities lead to formation of cells with abnormal chromosomal constitution (polyploidy and heteroploidy as well as polynucleate cells). Sometimes even micronuclei are formed.

Before discussing the existing hypothesis aiming to interprete the aetiology and the nature of animal and plant tumors we shall mention some important facts that we know at the present time about tumors. There are chiefly *physiological* and *biochemical* ones. On this subject we have good summaries (LAWIN 1928, MELOWAN 1932, etc.), therefore we shall not go into details here but we cannot avoid mentioning the more important statments that we shall need for the later discussions. Thus, for example, permeability, temperature, pH-value, lipoid content, $\frac{K}{Ca}$-quotient, etc. in tumors and in normal tissues are not the same. An important phenomenon in tumors is the anaerobic glycolysis (WARBURG 1924). Rous sarcom, for example, forms lactic acid in aerobic conditions 8% and in anaerobic conditions 12% of the tumor weight per hour. Another important point is that X-rays, one of the best cancer producing agent in animals, causes abnormal mitosis and meiosis in both plant and animal kingdoms. According to KOMURO it produces tumors in plants too. Tar, anilin, and arsenic acid (As_2O_3) are also agents that induce tumors in the animal and plant kingdoms; these agents also precipitate plant extracts to varying degrees. Tar, one of the best producers of tumors in plants and animals contains water soluble substances, which strongly precipitate rabbit serum and agglutinate slightly rabbit red blood corpuscles (KOSTOFF 1931). All chemicals used for producing tumors in plants (KOSTOFF 1931) stop the cytoplasmic streaming in *Petunia* trichomes. Other phenomena that deserve mentioning here are: 1. the increase in cytoplasmic viscosity in hybrid tumors and in cynipid galls (KOSTOFF and KENDALL 1930), and the increase of permeability in plant tissues under the activity of certain substances (MAGISTRIS and SCHÄFFER 1929, GELLHORN 1929, etc.).

Finally we shall mention that *spontaneous tumors on hybrid organisms* occur not only in plants but also in animals. POLL (1920) reported such observation in birds and KOSSWIG (1929) in fishes. *Spontaneous tumors in men and in plant hybrids appear more frequently in older age while galls are usually formed on young plant organs.* One more fact will be mentioned, namely, that tumorical proliferations are formed in the calluses of heterogeneous grafts in plants (KOSTOFF 1928, 1930/31) and that precipitation and lytic reactions occur between the extracts of many species of various genera in plants (KOSTOFF 1929, CHESTER 1932).

Until now we have reported facts observed by various investigators which must be systematized in attempting to discuss the hypothetical interpretations of the *aetiology of tumors.*

At the present time we have numerous hypothesis for the aetiology of spontaneous non-parasitic tumors. They are many and no one seems to give a satisfactory explanation of all that we know about cancer. VIRCHOW's hypothesis which attributes the aetiology of tumors to irritation by various agents, is very popular among the physicians, but the biologists consider it for too narrow, because it cannot explain all phenomena conected with tumors. Genecticists looked for mendelian factors, and Miss SLYE and LEO LOEB believe they have found a single mendelian factor that causes cancer. LITTLE's (1928) investigations and discussions showed, however, that the matter is not so simple in reality. Another hypothesis was offered by BOVERI. He believed that the cause for tumors rested in an abnormal chromosome complex. This hypothesis have been derived from the fact that cells with abnormal chromosome constitutions (various chromosome number) have often been found in cancer. It is interesting or rather curious that METCALF, using BOVERI's definition of cancer as an abnormal chromosomal complex of the nucleus, suggested, and no doubt quite logically, that there might be a case of cancer (?) in *Protozoa* (*Opalianoptigon*) too. Wrong postulates lead to wrong conclusions. BOVERI's hypothesis was lately renewed by WINGE (1927, 1930). WINGE studied the cytology of the crown gall in beet and the cytology of mous tumors and found polyploid cells and cells with various chromosome number. In his first publication he interpreted cancerous proliferations by polyploidy. The growth of the tetraploid (or generally speaking of the polyploid) regions is faster because they have twice as many genes for growth as the normal diploid tissue. In his last publication he is more conservative and pays some attention to abnormal physiological conditions too. Another theory that deserves greater attention, is BAUER'S mutation theory of tumors (1928). On this subject BAUER wrote a little book: "Mutationstheorie der Geschwulstentstehung". According to BAUER the tumors are somatic mutations and the cause for them can be diverse. Thus X-rays, that are one of the best agent for producing mutations are also one of the best agents for producing tumors experimentally. Under the term "mutation" BAUER understands gene mutations and chromosomal aberrations.

Reading his booklet one gets the impression that he puts greater weight on chromosomal aberrations than on gene mutations. When he speaks of gene mutations he is inclined to accept mutation of many genes, i. e. of gene complexes. Using Bridges scheme for non-disjunction he attempts to show how chromosomal aberrations and cells with non-viable constitution may originate and calls it a scheme of non-disjunction of tumor chromosomes. BAUER does not believe in inheritance of cancer. He writes: "Es gibt sonach *keine 'Krebsvererbung'* im wissenschaftlichen Sinne, d. h. keine Übertragung der Erkrankung selbst auf dem Wege über mendelnde Gene, sondern biologisch stellt sich die Frage dar als *Vererbung von Gewebsminderwertigkeiten die bei hinzukommenden äußeren Faktoren die Geschwulstentstehung wesentlich begünstigen.*"

Considering the activity of various irritating substances BAUER admits the conclusion by BORST formulated in the following way: "Nachdem durch die allerverschiedensten Reizstoffe mechanischer, chemischer, aktinischer, para-

sitärer Art experimentell Krebs erzeugt worden ist, sollte man doch endlich von dem Gedanken einer Spezifität der Reize abkommen" (BORST 1924).

We cannot go here into details in discussing BAUER'S hypothesis, but we shall note that BAUER advances in many places in his book interesting ideas. There are places, however, where he goes too far into speculations. His hypothesis has one weak point which is at the same time the weak point of BOVERI'S and WINGE'S hypothesis too. They all forget that chromosomal aberration and polyploidy have not always for sequence a tumor. We know so many organisms that represent chromosomal chimeras and chromosomal aberrants, and others that have 3, 4, 6, etc. whole genomes without forming any tumors. If the tumors have for cause simply abnormal chromosome number, then everywhere this phenomenon occurs tumors must be expected. The occurance of polyploid cells and cells with abnormal chromosome number in tumors can be treated rather as a sequence of the conditions which cause tumors, than as a cause for the tumor formation, in other words the chromosomal aberration and polyploidy in tumors is more resonably to assume for a secondary phenomenon than for a primary cause. Such a conclusion seems to me more probable, if one keeps in mind that in tumors a great many of the cells, even the majority of the cells have neither aberrant nor polyploid chromosomal constitution, but a normal diploid one. But what then can be the primary cause of the tumors and of the chromosomal aberration and polyploidy in tumors? The experimental biology does not give us a definite answer to this question, therefore, we must attempt to look for a working hypothesis. It is more difficult, of course, to define what a tumor is and how it originates, than what it is possible to be and what it may not be in reality. Tumor seems not to be a somatic mutation as BAUER is inclined to believe, even if we call each chromosomal aberration a mutation. Tumors have cells with normal diploid chromosomal constitution as well as with various number of chromosomes. Man, for example, has normally 48 somatic (diploid) chromosomes. LEVINE found in human tumors 23—24, 47—48, 94—96, and ca. 200 chromosomes. We found cells with 21, 28—30, and 42 chromosomes in spontaneous tumors of *Nicotiana glauca* × *Langsdorffii* hybrids, that have normally 21 somatic chromosomes. Cells with various chromosome numbers were found by WINGE in mouse tumors. But which chromosome constitution of all mentioned in man, for example, can be accepted as specific for tumors? In tumors non-disjunction occurs. Some cells have for example 2n+a and 2n−a chromosomal constitution, other types of aberrants may be 2n+b and 2n−b, here being involved another chromosome, &c. Which of these aberrant cells have the specific genic balance of tumor? The investigations show that all of them must have it, since all of them have been found together in tumors. But that means that tumor cells have not a definite specific genic balance.

Let us disregard for a moment the chromosomal aberrations in tumors and consider the possibility of occurance of gene mutations. Can we explain tumor by postulating a gene mutation or mutations of groups of genes? But before answering this question, we must first consider whether it would, in this case be logical to postulate a gene mutation, or gene mutations. From the genetic literature we know definitely that somatic mutations do not occur as frequently as tumors do, especially in men. *If tumors were mutations then every F_1-hybrid of the crosses N. glauca × N. Langsdorffii and N. paniculata* ×

N. Langsdorffii, for example, must represent somatic mutations because every one plant of these two cross combinations (especially the first one) forms spontaneous non-parasitic tumors (in older age), at the end of the florescence period or even earlier. More over, if we postulate that every tumor is a somatic mutation, then we must admit, what our genetical scepticism does not allow us, that in every one single F_1 (*N. glauca* × *N. Langsdorffii*) plant, for instance, somatic mutations occur at ca. 200 or more than 200 places, since more than 200 tumors are often formed all over the plant. A metastasis by transmission of tumorous cells in plants cannot occur as some cancerologists believe it occurs in animals and men, because of the different anatomical structure of the plant organism. Even in human organisms a metastasis due to the transmission of tumorous cells by blood circulation does not seem very plausible, since the blood, according to the serologists must contain "Abwehrfermente". A transmission of the tumorous cells by the lymphatic system seems more possible, although not very probable.

Formally, of course, neither we could demonstrate experimentally, for example, that in each plant of the cross combinations *glauca* × *Langsdorffii* and *paniculata* × *Langsdorffii*, the tumors, that appear, do not represent mutations, nor can BAUER give us an experimental proof that all these numerous tumors are mutations. But the geneticists know quite well approximately how often somatic mutations occur.

Since we cannot explain tumor by chromosomal aberration and by gene mutation or mutations, is there something else that can *indicate where to look for the cause of tumors?* It seems to me that such an indication can be seen in the investigations carried out during the last five years, where attempts have been made in producing gene mutations by various agents. The source is not new. The results obtained from such experiments were the stimulus for BAUER'S mutation theory of tumors too. We shall only consider another side of such experiments. By X-raying various plants and animals many investigators produced lethals, deformations and finally chromosomal aberrations and viable gene mutations. The first two seem to occur more frequently than the last two. Under deformations we understand all non-hereditable anomalies appearing after the treatment of plants and animals by various agents. Deformations, chromosomal aberrations, and gene mutations may have one and the same inductor, therefore they may occasionally occur simultaneously but not necessarily so. From the developmental point of view, deformations result following inactivation of the formative substances (SPEMANN'S organizers).

In plants we know that X-rays cause chiefly deformations, chromosomal aberrations but less gene mutations. Similarly seems to act various other agents such as radium rays, certain chemicals, etc., but perhaps, not so effecively as the X-rays. Most of the E. STEIN'S "Radiomorphose" belong to the group "deformations". *Antirrhinum* radiomorphoses represent all kind of fasciations in which tumorous malformations occasionally occur. Deformations can be obtained by various chemicals too. We produced deformations in cereals by $CuSO_4$. Copper sulphate precipitates the extracts from the cereals. Some of the STUBBE'S so called mutations may also be deformations. The chemicals that gave the best effect in STUBBE'S experiments precipitate proteins and extracts from *Antirrhinum* as shown in table 2.

Table 2

Chemicals	Precipitation of			% Mutations in P after STUBBE from treated	
	egg albumin from duck 1 : 10 in dest. water	Rabbit serum	Extract from Antirrhinum majus	Seeds	Seedlings
$CuSO_4$	++++	++++	++++	26.66	
$AgNO_3$	++++	++++	++++	21.05	
Potassium ferocyanide	—	—	trace	8.88	
Ironchlorid	++	++++	++++	—	18.75
Ironalum	++++	++++	++++	9.09	
Potassium bichromate	0 to trace	++	trace to +	35.71	
KJ	—	—	trace	15.15	

After these brief notes we shall attempt to give a characteristic of tumors on the basis of the morphological, histological, cytogenetical, and physiological investigations.

A tumor represents unorganized mass of frequently dividing cells, some of which expand and die, others expand continuing to divide. The tumor cells originate, from the differentiated as well as from undifferentiated cells or cell. A differentiated or undifferentiated cell, with a definite hereditary constitution, from a genetical point of view, should produce something very definite, instead of a mass of more or less identical cells, like those in tumors. This is especially true for the plants. In older *glauca* × *Langsdorffii* hybrids, for example, the growing points or the buds continue to develope further into tumors. Evidently something has happened within the cells from which tumors originate. It was stated that the agents which induce tumor formations act on the cell substances and provoke some changes *in vitro* and perhaps *in vivo*. *In vitro* they cause precipitation, agglutination and lysis of plant extracts, serum, or red blood corpuscles. *In vivo* some of the agents that have been studied by various investigators may act on the cytoplasm and on the cell nucleus increasing the permeability, changing the cytoplasmic viscosity and causing reversible (or in certain cases irreversible too) gelations in the cytoplasm and in the nucleus. We mentioned also that the nuclei in galls and tumors stain somehow deeper with iron alum haematoxylin than the nuclei of the normal cells. This means that in the gall and tumor nuclei some changes have occured and the nuclei react differently in comparison with the normal tissues. We shall recall here that the geneticists (most of them) are inclined to assume the nucleus particularly the chromosomes as a locus of the hereditary unites, consequently the changes that occur in the nucleus may affect the genes and especially the formative substances, i. e. the gene products. Since the genes (the hereditary unites) are something not very labile and not so readily changeable, the changes in the formative substances seems to be more effective. *Various kinds of modifications occur more frequently than mutations.* If a specified cell with definite genetical constitution is attacked by certain chemicals or other agents which have the ability

to change the specific proteins of the cell by a process of precipitation, dissolution or even destruction, without killing it, such a cell will be despecialized, degraded or despecified, it will turn into a "*REDUCT*". Many of the formative substances in the reduct are destroyed or changed. The same may occasionally happen with certain hereditary unites, though relatively seldom. The despecification process can progress in degrees, in other words the destruction and the change of the formative substances (and occasionally of certain hereditary unites) qualitatively and quantitatively depend, generally speaking on the nature, intensity, and continuity of the acting reductor (reducing agent) as well as on the tissue and the species to which the cell belongs. At the same time we must note that the living tissues react, in certain instances, in a similar way to a group of agents. If the most primitive property of a reduct — the ability to divide — is not destroyed, then it will continue further to divide and produce undifferentiated tissue. Changes and destructions of formative substances and occasionally of hereditary units in plant and animal cells have been lately produced in many plants and animals by various agents. Such changes in plants result in the formation of defective organs and fasciations (and occasionally gene mutations). The appearance of defective organs follows the despecification processes. When the despecification of the cells caused by X-rays, for instance, is carried out further, the cells loose their differentiation properties and instead of defective organs or fasciation tumors (KOMURO 1928) are formed with histological and cytological structure characteristic of a cancer. Similar phenomena seem to occur in the species crosses. The mutual precipitation potency between the parental extracts of the hybrid *N. glauca* × *N. Langsdorffii* is somehow greater than between the parental extracts of the hybrid *N. Tabacum* × *N. Sanderae*. The former hybrid forms typical tumors without any tendency of differentiation of organs as well as such with small deformed leaves all over the plant organism (roots, stems, shoots, leaves), after the florescence period, while the latter hybrid forms, though not so frequently, defective organs and tumorous malformations in an old age with numerous deformed leaves, but it has not been observed to form just tumors without deformed leaves or shoots. In the hybrid *N. glauca* × *N. Langsdorffii* the despecification must be more advanced, since the precipitation between their parental extracts is greater than in the hybrid *N. Tabacum* × *N. Sanderae*. The back crosses (*N. glauca* × *N. Langsdorffii*) × *N. Langsdorffii* manifested also less despecification than the hybrid *N. glauca* × *N. Langsdorffii*, the back cross forming chiefly fasciations and occasionally tumorous malformations with deformed leaves and leafy shoots. These examples also show that there are varying degrees in the despecification processes.

A reduct might often result from several causes, i. e. several factors can be involved in the degradation process. The hybrid tumors represent the best example in this respect. Young hybrids of the cross *N. glauca* × *Langsdorffii* do not form typical tumors, but occasionally only defective organs and whichs broom like malformations (various fasciations) while the older hybrids of the same cross form typical tumors, less frequently such with abnormal small leaves. This phenomenon is apparently in a close connection with the protoplasmic hysteresis (RŮŽIČKA 1932). The proteins (the specific ones too) being colloids gradually approach their isoelectric point (IEP) in older organisms, where their precipitation is much easier than at a younger age. Wounding can be con-

sidered as another agent that might be involved in the despecification process. The typical tumors in the hybrid plants, begin very often from a wound, no matter whether it is caused by an insect, by taking off of some leaves, from *the scars where old leaves have droped*, punctures by needles, or some other kind of slight injury. The activity of the wound seems to be similar to that of the tumor forming agents. BÜNNING (1926) found that wounding of plants induces a coagulation of the cytoplasm near the wounded cells. Wounding induces irregular cell division too (KOSTOFF and KENDALL 1931). In animals, merely frequent woundings induce tumorous formations.

We pointed out very briefly how a reduct is formed, but a tumor will not be formed if the reduct does not divide continuously. Reducts that produce tumors will divide continuously and frequently only when in favorable growth conditions, i. e. favorable for cell division, cell expansion, and abundant supply of nutritions. When a cell begins to divide (prophase) its cytoplasmic viscosity (CV) seems to increase. CV in plant tumors of *Nicotiana* hybrids is somehow increased (KOSTOFF 1930, 1932) and the tumor cells (at least in some regions) divide continuously. The agents or generally speaking the conditions that change a cell into a reduct seem to be responsible (at least at the beginning of tumor formation) for the increase of the CV. Increasing of CV in cells means, so to say, creating biophysical conditions for prophase, i. e. favorable conditions for cell division. In normal conditions CV increases during the prophase, obviously partly, on account of a decrease of the viscosity in the nucleus. The dispersion medium of the cytoplasm enters into the nucleus and the nucleus grows in volume. Wounding and various chemical and physical agents increase the permeability of the tissues treated (BÜNNING 1926, MAGISTRIS and SCHÄFER 1929, GELLHORN 1929, &c.) and induce cell division. Obviously the increase of the permeability facilitates not only the accumulation of nutrition, necessary for the growth, but perhaps also the entrance of the dispersion medium from the cytoplasm into the nucleus and brings about the biophysical state of the prophase. Such phenomena are to be expected, at least at the beginning of tumor formation when all of the despecification agents are present, inducing not only the despecification process but involving also the process of a relatively frequent cell division. Later the increase of the permeability and the CV is kept by the special metabolic processes in the tumors, no matter whether the despecification agents are present, or not, whether the tumor is transplanted or not. I shall only mention here of the anaerobic glycolysis in tumors investigated chiefly by WARBURG (1926). He also found that: "In bezug auf die anaerobe Glykolyse — zum mindesten in bezug auf die Größenordnung — besteht also kein Unterschied zwischen gutartigen und bösartigen Tumoren". WARBURG and his students also stated: "daß zwischen gutartigen und bösartigen Tumoren keine prinzipiellen, sondern nur graduelle Unterschiede bestehen" (WARBURG, POSENER, and NEGELEIN 1924) due apparently to the degree of the despecification. Other investigations show that the "Sauerstoffmangel führt zu einer reversiblen Permeabilitätssteigerung" (GELLHORN 1929, v. further liter.), and to a reversible gelation of the nucleus (NASSONOV 1930, 1932, ALEXANDROV 1932).

The insufficient supply of oxygen in tumors is due, perhaps, in a great extent, if not exclusively to the bad organization of the tumor cells for a normal blood circulation in animals and sap exchange in plant tumors. The reducts have lost a great deal of their formative properties, the normal capillaries are often injured, while *new normal* ones are not reproduced in the new cancerous

tissue. Similar conditions are created in the plant tumors too. Plant tumors represent usually a mass of parenchymatous and meristematic cells. The little badly organized vascular tissue represent parenchyma cells with abnormal secondary thikening, slightly expressed. They are short and resemble only slightly to the normal ones.

The increased permeability in tumors insures an abundant food supply. The circulation, however seems to be quite inadequate to prevent the anaerobic glycolysis. The expanded tumor cells are stores of an abundant food supply of proteins and carbohydrates (SOKOLOFF 1926, KOSTOFF 1930, KENDALL 1930). The expansion of the tumor cells—the reducts—is not only insured but even stimulated. Cells attacked by various agents or such that are in pathological conditions and have sufficient food supply expand more than the cells growing in normal conditions. I shall mention here the cells filled with bacteria in the nodules of the leguminous plants, which are 3—4 times (and even more) larger than the normal ones. The cells in the affected region of the galls (KOSTOFF and KENDALL 1929, 1930, KENDALL 1930, &c.) are likewise hypertrophied. In tumors the despecification agents, as well as the special metabolic processes, and finally the proteolytic agents, acting on the tumor tissue, when such are present, can act at the same time as agents stimulating the expansion of the cells. In hybrid tumors as well as in experimentally obtained tumors in *Ricinus* the parenchyma cells are somehow much larger than the normal ones away from the tumorous proliferation.

The irregular cell division in tumors, the appearance of polyploidy, heteroploidy, binucleation, multinucleation, micronuclei, giant cells, abundant storing of food, and other caryological diversities described, seems to me are rather a result of the despecification processes and the phenomena involved with them (abnormal metabolic processes, change of CV and permeability, &c.) than a cause for tumor formation as some investigators are inclined to believe.

The cancerologists are using the genetical investigations on inducing deformations and increasing the mutation rate by various agents to throw light on some phenomena in tumors. At the same time the tumor problem gives us some indications, if not definite statments about the intimate nature of the mutation processes, at least, where to look for the latter. It seems to me that the geneticists can learn something from the cancerologists and cytophysiologists, deriving methods for attacking the mutation problem and collecting data which give some indications how to attack it. In this respect I shall only call attention to the cytophysiological investigations on the reversible gelation of the cytoplasm and nuclear substances induced by various agents, to the deviations of all biophysical and biochemical processes not only in a single cell but in the whole tissue under the activity of the tumor producing agents, to the agents increasing the mutation rate, and finally to the results given in table 2. STUBBE (1930—1932) used various chemicals and other agents for inducing mutations and produced in many instances deformations and an increase of the mutation rate. The chemicals which gave the best effect in STUBBE's experiments precipitate the proteins and the extracts of *Antirrhinum*, the plant with which STUBBE worked as shown in table 2. Some of these indications may be mere coincidences, but even so, the problem seems to me to deserve an attack from such a point of view too. Considering the indications given in table 2, we may suppose that the salts of the heavy metals and all the chemicals which precipitate in certain ways the proteins or change them

somehow, can be effective agents for producing deformations and perhaps increase the rate of mutations. The question, however, of why when two cells with identical genetic constitution are exposed to the activity of one and the same agent with equal (if this really is as it seems to be) intensity (or quantity) and duration, one of them mutates and the other does not, will remain open, it seems, for a long time.

We pointed out possible processes indicated by the reactions observed *in vitro* in the tumor formations in the hybrid plants and in the tumor formations following the application of certain tumor forming agents, namely the degradation processes from which the reduct results. The discussion of these phenomena raise the question as to whether the spontaneous human tumors are also to be interpreted by some precipitation, agglutination or lytic phenomena *in vivo.* or generally speaking, by some degradation processes. From the works of LANDSTEINER and others we are aquainted with the four human blood groups: O, A, B, and AB. The agglutination reaction between the sera and the red blood corpuscles of these groups is given in table 3. "+"—indicating agglutination and "—"—non.

Table 3

Red blood corpuscles	SERA			
	O	A	B	AB
O	—	—	—	—
A	+	—	+	—
B	+	+	—	—
AB	+	+	+	—

According to these data the greatest reaction between sera and red blood corpuscles *in vivo* might be expected in the progeny of a combination (of parental contributions) A × B (A as well as B can be either mother or father). Thus if the biophysical and biochemical processes in human are comparable with those in plants and animals, one might expect an appearance of spontaneous cancer most frequently and in the earliest age in the progeny of a A × B combination, less in those of A × O, B × O, AB × O, A × AB, and B × AB; and rarely or not at all (if one takes in consideration only the blood groups) in those of the combinations O × O, A × A, B × B, and AB × AB, —a promissing prospect for human cancer prophilaxis!(?)

We must note that we do not have sufficient observations on the correlation between the inheritance of the blood groups and the appearance of human tumors, which would allow us a definite conclusion upon this subject. Even the inheritance of the human blood groups seems to be not so simple as it was at one time thought. The recent discovery of subgroups will undoubtedly complicate a correlative investigation between the blood groups and the appearance of human cancer.

The clinical statments that cancer appears more frequently in older individuals than in younger, and that agglutinins in the blood of new born children cannot be demonstrated, are in favor to the idea advanced. When one considers the summation of the despecification factors, as they were outlined in plants, one can expect some deviations in appearance of human cancer

in the above mentioned blood group combinations. A very important summative factor (component) seems to be the protoplasmic hysteresis (RŮŽIČKA). The proteins (colloids) can be more easily precipitated in older age than in younger, consequently the despecification (degradation) processes may occur more easily in an advanced (older) age. Many surgeons even have the opinion that "every body would have cancer if he lives long enough", i. e. every body would have cancer if he reached the age when his proteins (colloids) approach the isoelectric point and can be very easily despecified (precipitated) by some internal or external agents (wound, chemicals, temperature changes, &c.)

When the both factors: 1) blood group combination and 2) protoplasmic hysteresis are favorably combined for tumor formation in certain individuals, any third sumative factor involved, which acts as a despecificator (despecification factor) can induce tumor in the place of its activity. Such places usually are: the alimentary track, where the food with the various chemicals (often such that can cause changes in the proteins of the living cells) and with various temperature reaches the stomach and later passes through; the urogenital organs, where traumatism is often involved, and where most of the catabolic substances pass through; the liver, which is the regulative organ of many poisons of the organism, some of which can cause despecification by precipitating or destroying the proteins (or the formative substances in general) of the surrounding cells, &c.

We are convinced that the data we are reporting here and considering in the critical discussion are insufficient to solve the cancer problem or to throw much light on the problem of mutation, but they give some indications and directions for further experimental work on both problems.

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