Thymonucleic Acid in Tumors*†

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INTRODUCTION

The vital activities of cells are controlled to a great extent by complex conjugated proteins, nucleoproteins, which consist of one or more protein molecules combined with nucleic acids. These nucleic acids are esters of phosphoric acid and glucosides that consist of a pentose sugar (ribose or deoxyribose) and a cyclic derivative (adenine, guanine, cytosine, thymine, or uracil). The deoxyribonucleic, or thymonucleic acid, is found in the nuclear chromatin and the ribonucleic, or yeast nucleic acid, chiefly in the cytoplasm of plant and animal cells (5, 15, 65, 89, 90, 94).

Only a satisfactory beginning has been made toward attaining a complete understanding of the nucleoproteins. With new research methods progress will be more rapid, and the present views will probably be altered according to subsequent findings. Following the increasing recognition of the importance of nucleic acids in normal cells, evidence is accumulating to suggest that there is a disturbance of the normal balance of nucleic acids in some tumors. This paper will summarize the pertinent results of the investigations of thymonucleic acid in normal cells, and the evidence that it may play a significant role in tumors. Although the evidence is still inadequate to determine whether the observed changes in nucleic acid represent a primary cause, a contributing cause, or a result of neoplasia, it is hoped that the present summation, tentative though some of the views must be, will serve as a stimulus to increased discussion and investigation.

Certain aspects of nucleic acids will not be taken up in this paper. Their chemical and physical properties have been described by Levene and Bass (76) and by others (20, 48, 49, 65, 67, 99, 104). Physiological effects of the injection of nucleic acid have been described, such as an initial leukopenia followed by leukocytosis (50, 55, 59, 93, 97) and a lowering of the alkaline reserve and increase in inorganic phosphorus and sugar of the blood (55).

Extracts of cells that contain nucleic acids and nucleotides have a growth-promoting effect on other cells. Tennant, Stern, and Liebow (123) reported a stimulating effect upon the growth of mouse heart fibroblasts in vitro by various nucleic acids, including thymus thymonucleinates. The extraction of embryonic and adult tissues yields a substance that promotes the growth of tissues in vitro (47, 57, 58, 119, 122). These extracts contain varying proportions of the ribose and deoxyribose types of nucleic acids. Usually the ribonucleic acid has more stimulating action than the deoxyribose type. Saha and Ghosh (97) concluded that nucleic acid had no growth-promoting value in rats.

The growth-stimulating action of material from injured yeast cells has been attributed by Looibourow (79, 80) to adenine nucleotides rather than yeast adenyl acid or yeast nucleic acid. However, using similar material, other workers (37) conclude that the nucleosides, adenosine and guanosine, alone or combined with each other or with yeast adenyl acid, are important active constituents. Davidson (45) recently reviewed the subject of wound hormones and suggested that ribonucleic acid may be an important constituent of some of these substances that cause proliferation of cells. The possible relationship to neoplasia of such wound hormones, containing nucleic acids or their constituents, is an interesting sub-

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ject for speculation, but one that lacks substantial experimental evidence.

The Role of Thymonucleic Acid in Normal Cells

According to generally accepted ideas, nucleoproteins control the hereditary and vital functions of living cells. The most important nucleoprotein within the nucleus is the deoxyribose tetranucleotide, which is combined with basic proteins of the protamine and histone type (23, 89). Although the nucleic acids of a nucleoprotein are usually one of two types, the protein may vary from one species to another and may be relatively specific. The thymonucleic acid has important roles in controlling heredity, in mitosis, in polymerization of nucleoproteins, and in the formation of proteins and of ribonucleic acid of the nucleolus and cytoplasm. In leukemic blood cells the thymonucleic acid comprises 40 per cent of the chromatin complex (36).

Recently Stedman and Stedman (108-111) have presented evidence that a protein, which they have named chromosomin, is the most important constituent of chromosomes and the chemical basis of inheritance. They suggest that nucleic acid is united to this protein. The views of Stedman and Stedman that the Feulgen reaction is not a specific stain for thymonucleic acid, and that this acid is a more important constituent of the nuclear sap than of the chromosomes, have been severely criticized (3, 19, 26). It is asserted that Stedman and Stedman have misconstrued the work of other investigators and presented insufficient proof to substantiate their own statements. The possibility of the existence of a protein such as chromosomin may be admitted, but a final evaluation of its significance must await the publication of additional evidence.

Some cells seem to contain chiefly pentonucleic acids of the ribose type and very little deoxyribonucleic acid. This is especially noticeable in some ova (17) and in protozoa. In such cells the nucleoproteins may undergo cyclic variations in which, at some stages, the deoxyribonucleic acid is at a minimum. Thus Meglitsch (87) has shown that in the life cycle of the protozoan Endamoeba blattae there are two types of cyclic variation in the amount of thymonucleic acid, one associated with division of the trophic amebae and the other with encystment.

Chromosomes and genes.—The chromosomes are enormously extensible protein fibers to which is attached deoxyribonucleic acid at certain specific points, the genes (42, 101).

There are two types of regions in the chromosomes: the active genes, or euchromatic regions, and the inactive, or heterochromatic, regions. The inert chromatin of the heterochromatonic regions retains an extra charge of nucleic acid between metaphases. Underlying the cycle of mitosis and cell division is a concomitant cycle of attachment and detachment of this nucleic acid to and from the chromosomes, which accompanies the coiling and uncoiling of their protein framework. According to Caspersson (27) the maximum attachment corresponds with the maximum spiralization of the chromosomes at the metaphase. On the other hand, they are uncoiled and relatively free from thymonucleic acid within the resting nucleus.

At the end of mitosis the chromosomes give up their nucleic acid charge and secrete nucleoli, which dissolve at the initiation of the next mitosis when the nucleic acid content of the chromosomes is increased. Most nucleoli contain only ribonucleic acid, although Eckert and Cooper (56) and Koller (74) have reported deoxyribonucleic acid in the nucleoli of some tumor cells.

By using ultraviolet spectroscopy in combination with other methods (21, 23), Caspersson (24, 27) has been able to differentiate qualitatively and quantitatively between the 2 nucleic acids or their nucleotides, which have a high maximum absorption located at 2,600 A, and the proteins, which have a lower maximum absorption at 2,750 to 2,900 A. He has provisionally identified a histone and a globulin type of protein. The heterochromatin and nucleolus both show a high content of histone, whereas the euchromatin between the heterochromatic regions contains proteins of the globulin type. In the chromosomes at metaphase these higher globulin proteins are absent, and only histones and thymonucleic acid are recognizable. Caspersson believes that the active genes in the resting nucleus are producing large molecules of globulin while the inactive ones are producing smaller molecules of the histone type, which are less specific in their interactions. Thus the difference between activity and inertness of chromatin is a difference between high and low specificity of proteins as well as high and low content of nucleic acid. The interphase chromosome is dominated by the characteristics of the protein, and the mitotic chromosome by the nucleoprotein.

The similarity of content of the heterochromatin and nucleolus suggests that the heterochromatin secretes the nucleolus or a precursor of it. This belief is supported by the common observation that the size of nucleoli is proportionally greater in cells which, with the exception of nerve cells, are concerned with the most rapid protein production, and smallest in cells where no protein is being formed. Thus it is significant that ribonucleotide increases in concentration as cells produce increased amounts of proteins. The ribonucleic acids are constructed near
the nuclear membrane where the nuclear and cytoplasmic surfaces meet (Caspersson and Schultz, 31).

**Cytoplasmic nucleic acids.**—Because of the interrelationships of desoxyribo- and ribonucleic acids it is not possible to consider the nucleoproteins of the nucleus exclusive of the nucleic acids of the cytoplasm. The nucleoproteins important in cytoplasmic synthesis are influenced by genetic changes in the chromosomes (101). An increase of heterochromatin in the nucleus increases the concentration of cytoplasmic nucleic acids (Caspersson and Schultz, 29).

Yeast contains about 5 to 10 per cent and some bacteria 15 to 20 per cent of their dry weight of ribonucleic acid (Mirsy, 88). The pancreas contains 10 per cent of its dry weight of ribonucleic acid, which is more than is found in any other animal tissues.

Rapidly dividing cells have high concentrations of desoxyribo- and especially of ribonucleic acid, whereas the absorption band of mature cells shows that they consist chiefly of proteins. In the cytoplasm of cells in the process of oogenesis—a cytoplasm in which many divisions are to occur—Brachet (16) has shown that the ribonucleic acids decrease while the desoxyribonucleic acids increase in amount. The distribution of ribonucleic acids in cytoplasm parallels the synthetic activities that are proceeding there. The basophilic staining of glands and embryonic tissues has been attributed to the high concentration of nucleic acids associated with rapid protein synthesis.

There is some evidence that the cytoplasmic nucleic acids have a higher rate of phosphorus turnover than the nucleic acids of the nucleus. In the resting state, the continuous turnover of nucleic acids is very slow, whereas synthesis takes place rapidly during growth (Brues, Tracy, and Cohn, 18). Although the relative amounts of nucleic acids may be considerably increased in embryonic tissues, the observations of Davidson and Waymouth (46) indicate that the amount of ribonucleic acid relative to the amount of thymonucleic acid remains the same, or may be slightly greater, in the adult as compared with that in the embryo.

Claude (32-35) has used a differential centrifugation technic to isolate particulate components of normal tissues and tumors. These small cytoplasmic bodies, which Claude considers as mitochondria, are aggregations of ribonucleoproteins associated with various proportions of phospholipids. The possible role of mitochondria in the synthesis or storage of ribonucleic acid is not understood. Because of their wide distribution, the presence of ribonucleoproteins in tumors is of significance only if it is demonstrated that their amounts of configuration are unusual.

**Protein synthesis.**—The nucleus is the center of protein synthesis in the cell, and the nucleic acids are the essential agents of this synthesis. The desoxyribonucleic acids may be important not only for the formation of a chain structure by their own polymerization, but also for their actual synthesis of the fibrous proteins of the chromosomes (Schultz, 100).

Caspersson (27) has developed the hypothesis that the process of protein synthesis proceeds by way of the histones in the nucleolus, which diffuse through the nuclear membrane forming the ribonucleic acids concerned with the synthesis of cytoplasmic proteins. Thus the special heterochromatic regions concerned with the formation of the nucleolus are the major producers of histones.

The ribo- and desoxyribonucleic acids are similar. They seem to bear a reciprocal relationship to each other, and can probably be formed interchangeably from each other during the cyclic variations of nucleic acid within the cell. However, the desoxyribose radical apparently gives its nucleotides a flatness to which they owe their capacity for polymerization. In the native state the desoxyribonucleic acids may form polymers with a molecular weight of as much as 1,000,000 (Signer, Caspersson, and Hammarsten, 104). These desoxyribonucleotides form columns of plates, which Schmidt (99) has shown by polarized light to lie crosswise to the protein thread. According to Caspersson this orientation is somewhat less precise. These plates agree in spacing with the location of the side chains on the extended polypeptide chain of the chromosome. Thus the possibility for chain formation exists in both the protein and in the prosthetic nucleic acid group (Schultz, 100). Because of this capacity thymonucleic acid is considered essential in the reproduction of the chromomere. Such a system of polypeptide chains, with their numerous and varied side chains and their ability to change shape by intramolecular folding, would appear to be well suited to the genetic task of bearing the patterns of life (Astbury, 1).

**Bacteria and filterable viruses.**—Bacteria contain nucleic acids of the ribose, desoxyribose, and, in the case of tubercle bacillus, perhaps a third type (76, p. 277). Of the total nucleic acid in streptococci, 10 to 30 per cent is of the desoxyribose type and the remainder of the ribose type. Two to 6 per cent of the dry weight of bacteria is thymonucleic acid (Sevag, Smolens, and Luckman, 102). Henry and Stacey (68) have suggested that the gram-positive material in bacteria is a high-molecular complex formed by the combination of a reduced basic protein substrate with magnesium ribonucleate.

The work of Avery and his co-workers (2) gives some interesting information on the role of thymonucleic acid in bacteria and in heredity. The most striking example of inheritable and specific alterations
in cell structures that can be experimentally induced and reproduced among bacteria is the transformation of specific types of pneumococci. A biologically active fraction was isolated in highly purified form from type III pneumococcus that was capable of inducing the transformation of unencapsulated R variants of pneumococcal type II into encapsulated cells of the same specific type as that of the heat-killed bacteria from which the inducing material was removed. The material consisted of a highly polymerized viscous form of desoxyribonucleic acid. The alterations produced were predictable, type specific, and transmissible in series. The type III capsular substance that produced were predictable, type specific, and transmissible in series. The type III capsular substance that is evolved by the nucleic acid is a non-nitrogenous polysaccharide and quite different from thymonucleic acid. This work with desoxyribonucleic acid is one of the first successful attempts to induce, by chemical means, predictable and specific changes that are transmissible thereafter in series as a hereditary character.

Chemical analyses of both plant and animal viruses show that they all contain some nucleoprotein, and some of the smaller viruses are pure nucleoprotein (Stanley, 105, 106). The nucleoproteins of the plant viruses are of the ribose type (75, 107). Loring (81, 82) found evidence that the pentosenucleic acid of tobacco mosaic virus differed from the usual yeast nucleic acid. Tobacco ringspot virus is 40 per cent nucleic acid and 60 per cent protein.

The findings in animal viruses are variable. These, in contrast to plant viruses, usually contain some lipid or phospholipid. The desoxyribose type of nucleic acid has been found in some animal viruses. According to Hoagland, Lavin, Smadel, and Rivers (70) the elementary bodies of vaccinia contain at least 5.6 per cent thymonucleic acid and possibly a small amount of ribonucleic acid. Psittacosis virus also contains thymonucleic acid. The rabbit papilloma virus contains 8.7 per cent of thymonucleic acid, 6.5 per cent carbohydrate, and 1.5 per cent lipid, with no evidence of ribonucleic acid (118). The virus of the eastern strain of equine encephalomyelitis is a complex of high molecular weight consisting of 54 per cent lipids, and ribonucleoprotein, of which 10 per cent was ribonucleic acid (103, 120). Nucleic acid of the desoxyribose type has been identified in swine influenza virus (121).

Stanley believes that the viruses have essentially the structure of genes but are further adapted for independent existence. However, the genes themselves contain chiefly the desoxyribose type of nucleic acid and have little if any ribonucleic acid. Both genes and viruses mutate (101). The suggestion has been made by Pollister and Mirsky (94) that certain viruses may be related to the self-duplicating bodies of the nucleus, the genes, which contain desoxyribo-

nucleoproteins, and that other viruses may be related to the self-duplicating bodies in the cytoplasm such as the chloroplasts and mitochondria, which contain ribonucleoproteins (35). The presence of nucleic acids in viruses and phages and the augmentation of nucleic acids on the chromosomes at the time of reproduction of the genes support the idea that it seems to be characteristic of self-producing substances to have nucleic acids in their molecules, at least during the time of their reproduction.

The Role of Thymonucleic Acid in Neoplastic Cells

Experimental cancer research has shown that the concept of "the cause of cancer" is now obsolete. Because of the complex and variable nature of neoplasia a single cancer may be attributable to several factors. Histologically identical cancer may be elicited by several quite different etiologic agents. Under controlled laboratory conditions the Shope papilloma virus, any one of several synthetic carcinogens, and ultraviolet light may produce epidermoid carcinomas of the skin that are indistinguishable. Mammary tumor-inciting substances, estrogenic stimulation, and hereditary constitution may contribute simultaneously as causes of a mammary cancer in a mouse.

The causes of cancer may be divided into the extracellular and the intracellular. In the study of extracellular causes oncologists have made considerable progress. It is recognized that factors such as chemical carcinogenic agents, heredity, hormones, milk factors, viruses, physical trauma, and precancerous conditions may be contributory causes of cancer in the lower animals and in man. In the study of intracellular causes, however, progress has been much less satisfactory. The nature of the intracellular change that characterizes neoplasia is unknown, and although a knowledge of the extracellular causes may permit us to prevent and to cure cancer, it will be necessary to know the intracellular causes in order ultimately to understand and control it.

What is the nature of the intracellular changes produced by various extracellular carcinogenic factors such as viruses or chemical carcinogens, which, after a variable latent period, cause subsequent generations of cells to exhibit the characteristics of malignancy? Although the answer to this question is not yet known, several of the theories that have been advanced as explanations of the change will be examined briefly.

One of the more popular theories of etiology is the virus theory. That some tumors such as the Rous fowl sarcoma are elicited by a virus is generally accepted, but there is inadequate evidence at present that any large proportion of tumors are so caused. The proponents of the virus theory have received some
which apparently has many properties of a virus.

As already mentioned, the nucleic acids are probably the most important constituents of viruses. A desoxyribonucleic acid capable of inducing a cellular alteration transmissible to subsequent generations of bacteria has been discussed. It is possible that the nucleic acids of viruses might also induce transmissible intracellular changes characteristic of neoplasia. Potter (95), in his discussion of an enzyme-virus theory of carcinogenesis, mentions the possible significance of ribonucleoprotein in the production of cancer. However, the demonstration of a virus as an etiological factor of a tumor does not explain the nature of the intracellular change associated with cancer any better than the observation that synthetic carcinogens elicit neoplasia.

Rhoads (96) and his colleagues showed that a metabolite of p-dimethylaminoazobenzene poisoned the coenzyme I system of the normal liver cell, and that cancer cells may develop a resistance to this toxic agent. From such research an enzyme theory of etiology has been evolved, suggesting that because of an interference with its enzyme system a cell is forced to develop a new type of metabolism that is not subject to the usual regulatory mechanism of the organism. At present there is insufficient evidence to recognize the importance of such an enzymatic disturbance as a general cause of cancer, although this is one of the more interesting fields of investigation.

Loeb (78) has suggested that the intracellular cause of cancer is a series of progressive changes accompanied by the formation of excessive amounts of autocalytic growth substances. The evidence suggesting that nucleoproteins and their nucleic acids may be important in such autocalytic action will be presented in this review.

The significance of nucleic acids in the somatic mutation theory of etiology.—One theory that does attempt to explain the intracellular change in many tumors is that of somatic mutation. Its historical background has been reviewed by Bauer (4), and more recently by Berrill (6) and by Furth, Boon, and Kaliss (60). Boveri (14), who is usually credited with originating the idea that cancer is due to a definite abnormal chromosome complex, traced it to von Hansemann. Although the theory has been discussed by many writers, with but slight modification, conclusive evidence of a change within the chromosomes has not been presented. The best evidence has been advanced by Biesele and his associates (7-13), who found an increase in number and size of the chromosomes in many tumor cells. The investigations of Heston (69) on genetic factors of susceptibility of mice to pulmonary tumors is one piece of experimental evidence that lends support to the idea of the localization of susceptibility to cancer in a single definite gene. He found that heterozygous mice bearing the lethal A<sup>y</sup> gene for yellow coat color had a higher incidence of induced pulmonary tumors than control mice.

The objection is sometimes raised that mutations are believed to be relatively rare while cancer is a more common occurrence. However, conditions in the mammalian body may be extremely sensitive for the disclosure of a cancer cell. For example, if one of the billions of normal lymphocytes in the mammalian body assumes malignant properties, the organism will develop leukemia (88).

When Hollaender, Greenstein, and Jenrette (63, 71) exposed aqueous solutions of sodium thymonucleate to radiation of 2,537 A. they observed a loss of structural viscosity and streaming birefringence due to a progressive depolymerization of the thymonucleate. From the genetic and physiologic viewpoints, it is interesting to speculate on the processes involved in the effects of ultraviolet radiation. The wave length range near 2,600 A. is most effective for lethal action and gene mutation. The nucleic acids of the cells are readily affected by ultraviolet light. The chromosomal changes that result in mutations may be initiated by an alteration or breaking down of the nucleic acid macromolecules. Thus it has been demonstrated that the physical agent, ultraviolet light, which is known to be active in the production of genetic mutations in lower organisms and in eliciting cancer in mice, produces a breaking down of thymonucleate. Although the proof is not at hand, it is conceivable that some of the other factors that aid in carcinogenesis might also exert their action through the induction of a transmissible change in the nuclear proteins. A disturbance in either the nucleic acid or protein component would produce effects upon the other constituents of the nucleoprotein.

According to Lockhart-Mummery (77), the only recognizable and important form of gene mutation occurring in a somatic cell would consist of, or be accompanied by, an increased rate of division as compared with the normal rate for that cell. In this way a colony of mutant cells, a tumor, would be produced among the normal cells.

Donovan and Woodhouse (51) have suggested that an unorthodox production of nucleotides or nucleic acids is the basis for the abnormal growth of tumors. Some of the details of the chemistry involved in their theory, such as an improbable stereochemical configuration and composition of nucleic acid molecules, have been justly criticized by Gulland, Barker, and Jordan (66).

The importance of the thymonucleic acids in the hereditary and vital functions of normal cells has
been discussed. If the somatic mutation theory is correct, it seems probable that such changes would involve the desoxribonucleotides of the nucleus. That there is a disturbance of nucleic acids in cancer has been suggested by the experimental observations to be discussed in the following sections.

**Chemical evidence of disturbance of nucleic acids in tumors.**—The results of chemical analyses of nucleoproteins by various workers are unsatisfactory in some instances, and contradictory in others. The reports of increased nucleic phosphorus and diminished lipid-phosphorus in malignant tumors, as compared with normal tissues, may be explained by the increased content of nuclei in malignant growths (113, p. 129). The few investigations in which there was a chemical isolation and quantitative analysis of nucleic acid from tumors did not give conclusive results. In nucleic acids isolated from metastases to the liver, the nitrogen content averaged 9.8 per cent and the phosphorus-nitrogen ratio was 1:1.2 to 1:1.0 as compared with a nitrogen content of 14 to 16 per cent and a phosphorus-nitrogen ratio of 1:1.76 in normal tissues (112, 125). Other investigators, including Klein and Beck (73), were unable to confirm a diminished nitrogen content of tumor nucleic acids.

Greenstein, Jenrette, and White (64) concluded on the basis of chemical analyses that the corresponding nucleoprotein fractions from rat liver and from transplanted hepatic tumors were nearly identical. The fact that the nucleoproteins are similar does not exclude the possibility that their configurations might be different.

Dounce (52-54) has extracted the desoxribonucleic acid from normal rat liver, rat hepatoma 31, and Walker rat carcinosarcoma 256 by chemical methods. He concluded that the nuclei of Walker tumors have nearly the same concentration of desoxribonucleic acid as the nuclei of normal rat liver, while the nuclei of hepatoma 31 appear to have a much lower desoxribonucleic acid content. His observations were based on the percentage of dry weight of nuclei and are not directly referable to such important biologic criteria as the mean amount of nucleic acid per unit volume of tissue, per unit volume of nucleus, or per nucleus. Variations in size and water content of tumor cells are important factors. The extraction of nucleic acid from tissue is a technical procedure that needs improvement before being generally accepted as a highly accurate quantitative procedure.

Qualitative color reactions revealed no change in the carbohydrate content of nucleic acids of tumors as compared with normal tissues (73, 125).

Masayama and Yokoyama (86), using a colorimetric macrochemical technic, reported an increase in thymonucleic acid in the livers of rats that had been fed $p$-dimethylaminoazobenzene. The nucleic acid was doubled after 30 days, and there was a sudden further increase as cancer developed.

Both the ribo- and desoxribonucleic acids occur in polymerized forms, with molecular weights estimated to be about 20,000 for the former and 1,000,000 for the latter (67, 81). In this form the yeast nucleic acid is relatively insoluble in acids, and the sodium salt of thymonucleic acid shows streaming birefringence and structural viscosity. When the nucleic acids are treated with tissue enzymes from various sources, these specific physical properties are progressively diminished. The depolymerase for yeast nucleic acid, ribonucleodepolymerase, is heat-stable, while the depolymerase for thymonucleic acid, desoxribonucleodepolymerase, is heat-labile.

Greenstein and his co-workers (61, 62), in their extensive studies on enzymes in tumors, tested these 2 depolymerases on milk, and on several tumors and the corresponding normal adult and embryonic tissues. Since their results showed variations from one type of tumor to another, and from similar tumors in different species of animals, it is difficult to correlate their findings with other observations. The 2 depolymerases do show a striking and parallel behavior concerning their content in various tissues.

**Histochemical and cytochemical evidence of disturbance of thymonucleic acid in tumors.**—Histochemistry is the study of the chemical constitution of tissues, whereas cytochemistry deals with the chemical constitution of the elements of a cell. Numerous workers have made visual observations on cancer cells stained by the Feulgen technic. Cowdry (38) compared tissue cultures of rat sarcomas and of normal fibroblasts, and reported an increased amount of Feulgen-stained material in the nuclei of sarcoma cells. On the other hand, Ludford (83) found no difference in the amounts of thymonucleic acid in the nuclei of a tar tumor as compared with the surrounding skin. He was unable to demonstrate any relation between the amount of chromatin in the nucleus of a tumor cell and the rate of growth of the tumor. Eckert and Cooper (56), studying epidermoid carcinoma of the cervix, did not observe any significant differences in the amount of Feulgen-stained material in malignant and normal cells.

As a part of the research project on experimental carcinogenesis of the skin under the direction of Dr. E. V. Cowdry (39, 40), Stowell determined the relative thymonucleic acid content of normal, hyperplastic, and neoplastic epidermis of mice (114). He employed a photometric histochemical method in which the relative absorption of monochromatic light in sections of tissues stained by the Feulgen reaction
for thymonucleic acid was determined by a special microphotometer.

With the same methods similar measurements were made on biopsy material from patients with epidermoid carcinoma of the skin (117), and results similar to those for carcinoma of the skin in mice were obtained. The order of decreasing mean amounts of thymonucleic acid per unit volume of tissue and per cell was carcinoma > normal > hyperplastic epidermis. Half of the carcinomas contained increased amounts of thymonucleic acid per cell that were statistically significant and in no instance was it significantly decreased. Additional investigations will be necessary to determine the reason for the variations in the increase in the relative amount of thymonucleic acid in specific tumors.

Preliminary investigations by Stowell (115) have shown that the amount of thymonucleic acid is increased in the leukemic cells of patients with lymphoid leukemia. There is some evidence that irradiation produces a disturbance in the balance of nucleic acid of malignant cells, accompanied by a decrease in the amount of desoxyribonucleic acid (116) in the nucleus and an increase in the amount of ribonucleic acid (91) in the cytoplasm.

Caspersson, using cytochemical methods, concluded that the heterochromatic section of the nucleus plays a specific role in carcinogenesis (Caspersson and Santesson, 28; and Koller, 74, p. 246). In a study of human carcinoma Caspersson (27, 28) found that the cytoplasm of tumor cells contained larger amounts of ribonucleic acid than corresponding normal cells. The amounts varied in different parts of the same tumor, and were larger in areas of more rapid growth. In view of the large amounts of ribonucleotides in the cytoplasm of rapidly growing normal cells Caspersson and Schultz (30) believe that the vigorous growth character of the malignant cell is evidenced by the large nucleoli, which play an important role in protein synthesis. It is a recognized fact that large nucleoli are characteristic of malignant cells (41, 85).

Mitchell (91), using ultraviolet absorption for nucleic acids, did not observe any significant difference between normal and hyperplastic tissues and malignant tumors, but relatively few cells were measured in each specimen.

Cytological evidence of abnormalities of nucleic acids in chromosomes and nucleoli in tumors.—Koller (74), who carried out a cytological analysis on 565 neoplasms of the human skin, esophagus, colon, rectum, larynx, lung, cervix, uterus, and breast, presents interesting ideas regarding the nature of the disturbed nucleic acid metabolism in tumors. It was found that nucleoli in cells of the same tumor, as well as in separate tumors, differed in their chemical content and in their size. The small, deep-staining nucleoli represented heterochromatic regions in the chromosomes that retained the desoxyribonucleic acid during the resting stage. The larger nucleoli, which varied greatly in size, contained large amounts of ribonucleic acid and histone. The proportions of these two types of nucleoli varied in the same and in different tumors. With the development and growth of the neoplasm the proportion of Feulgen-negative nucleoli containing ribonucleic acid increased in proportion to other cells.

Abnormalities of mitosis were expressed in chromosome structure and behavior, and in lack of coordination between spindle mechanism and chromosome movements. A close relationship was present between the frequency of abnormalities and the type of nucleolus. The tumors with few Feulgen-negative nucleoli had few abnormalities of mitoses, while those with a high proportion of these nucleoli showed polyploid and giant cells with various chromosome and spindle abnormalities. The abnormalities observed were: stickiness of chromosomes, nondisjunction of chromosomes, displacement of chromosomes, clumping, binucleate cells, polyploid cells, multinucleate cells, giant cells, and spindle abnormalities.

The aberrant chromosome and spindle behavior was attributed to stickiness and to increased rate of division, alterations that are caused by a quantitative change in the nucleic acid synthesis. The polymerization of thymonucleic acid is responsible for the coiling, the reduplication, and the visibility of chromosomes. The desoxyribose for the synthesis of thymonucleic acid may be obtained by reduction of the ribonucleic acid, which is produced under the influence of histone. An increase in the nucleic acid of tumors, as described by Caspersson and his co-workers, may be a fundamental cause of an increased rate of cell division and of malignancy. This quantitative change in nucleic acid metabolism, as indicated by abnormal chromosome behavior, may be an important differential criterion of a tumor cell.

In his discussion Koller suggests that in the first stages of neoplasia a disturbance in the heterochromatin-euchromatin balance or a slight increase in nucleic acid may cause a shortening of the resting stage between divisions that is not evident morphologically. An irregular segregation of the chromosomes at anaphase would produce further imbalance and increase in nucleic acid, giving more pronounced abnormal chromosome behavior and change in size and contents of nucleolus. An excess of nucleic acid makes the chromosomes sticky because they are coated with fluid nonpolymerized nucleic acid. In the next stage of the development of cancer there is nuclear division without cell division. In the final stage the nucleic acid content of the cells is further increased, abnormal
chromosome behavior and irregular division are frequent, and there are many giant polynucleate cells. These 3 stages, which were observed in the same and in different tumors, may be used as criteria of the degree of malignancy. It is known from genetic and cytologic evidence that the chromatic regions of the chromosomes, which are primarily concerned with nucleic acid synthesis, are more readily susceptible to mutation and structural change (43, 44, 92).

A chain of reactions exists in which the chromosome thread controls the polymerization of its thymonucleotide charge. This charge in turn controls the spiralization and the reproduction of the thread itself with its genes. The whole course of events can be altered by temperature and other cell conditions, as well as by the balance of heterochromatin and euchromatin and by the organization of the nucleolus. Koller suggests that the initial change in nucleic acid metabolism is brought about by a gene mutation occurring in the heterochromatic region responsible for the nucleic acid supply.

Biesele, Poyner, and Painter (13), using aceto-carmine squash preparations, measured nuclear volumes and did chromosome counts on 10 mouse tumors, as well as on some normal adult and embryonic tissues of the mouse. They found that cancer nuclei fell into volume classes that were less distinct than those of nuclei of normal tissues. The Class I nuclei were small, diploid nuclei with chromosomes similar to those of normal diploid embryonic cells. Class II, the most frequent class in tumors, contained nuclei with chromosomes twice as large as normal and with approximately the diploid number. With these larger chromosomes, twice as many nucleolar organizers and twice as many plasmosomes were present. Class III nuclei, which were ideally twice as large as the Class II nuclei, contained tetraploid and diploid nuclei. The nuclei of Class IV contained octoploids with chromosomes twice the normal size, tetraploids with chromosomes 4 times normal size, and diploids with chromosomes 8 times normal size and strand number. Thirty-two nuclear organizers were present in the chromosomal complement. The other succeeding classes of nuclei similarly were presumably derived from the nuclei of the next lower class by endomitosis with or without division of the centromeres. The formation of diplochromosomes in carcinogenesis seemed irreversible. All cancer chromosomes except those in nuclei of Class I were larger than normal and had more strands. These observations agree with the general findings of other workers, which have been reviewed by Biesele (13), demonstrating that polytene chromosomes are widespread in the cancers of man and the lower animals, which are often characterized by greater nuclear volume, an increase in the number or size of nucleoli, and enlarged or doubled size of chromosomes. The increased basophilia of the cytoplasm of malignant cells is a result of higher concentration of ribonucleic acid associated with increased protein synthesis. Malignancy is paralleled in degree by frequency of endomitosis and concentration of nucleic acids.

Biesele's observations (7, 10, 12) on the chromosomes of an ovarian adenoma or adenocarcinoma of the goldfish, on 2 human mammary carcinomas, and on 2 rat neoplasms (hepatoma 31 and Walker carcinosarcoma 256) confirmed his work on mouse tumors. He concluded that the volume of the chromosomes in normal tissues does not vary in accordance with the cytoplasmic concentration of ribonucleic acid, nor with the relative development of heterochromatin and plasmosomes, but rather with the development of the euchromatin (9).

Biesele's suggestion (9) that there is a relationship between the volume of chromosomes, the euchromatin of the chromosomes, and the vitamin content of tissues is very interesting. However, since this hypothesis is based largely on numerous assumptions, for which direct evidence has not yet been obtained, we must await the accumulation of this information. Biesele's own measurements of chromosome volume do not correlate with his observations on the amount of ribonucleic acid in the cytoplasm of normal cells, so he assumes that the large size of the chromosomes in both normal and neoplastic cells is due to a disproportionate increase of euchromatin and not of heterochromatin. The euchromatin is thought to form more complex proteins than heterochromatin. He makes additional assumptions regarding the relationship between euchromatin and vitamin synthesis, on the basis of a correlation of his measurements on chromosome volume with the measurements of other workers who analyzed the vitamin B content of tissues and nuclei. Some of the measurements of these investigators were related to the weight of fresh tissue and others to the weight of dried tissue. One might question the significance of the correlation of measurements on chromosomes with other measurements that are not directly related to such criteria as amount per nucleus, per cell, or per unit volume of fresh tissue, since it is recognized that there are significant differences in the actual and relative size and water content of cells and nuclei in various normal and neoplastic tissues.

Biesele and Cowdry (11) found diplochromosomes, and even more greatly enlarged chromosomes, from the second day on to carcinoma production in the epidermal cells of mice painted with methylcholanthrene. Only normal chromosomes were present in the epidermis of control mice that were untreated or
concluded that the appearance of polytene chromosomes of normal and regenerating rat liver, Biesele (8) containing dibenzanthracene. Additional evidence is needed to establish that the abnormalities of nucleic acid and of mitosis represent the fundamental process initiating neoplastic growth, and that they are not just another result of the aberrant functional physiology of neoplastic cells.

The one property common to all malignant cells is their capacity for unlimited, uncontrolled proliferation, which is evidenced cytologically by the abnormalities of cell division that have been mentioned. Whatever be the cause of neoplasia, the fact is clear that malignant cells are specifically altered cells and not merely normal cells excited to increased proliferation (Ludford, 84). It seems probable that this neoplastic alteration is transmitted through the nucleoproteins of the nucleus or cytoplasm.

Nucleoproteins in tumor inciting agents.—Certain agents that elicit the formation of neoplastic growths contain nucleic acids as one of their chief constituents. That the Shope papilloma virus contains desoxyribonucleic acid has already been mentioned (118). Kahler, Bryan, and Sipe (72) found that the substance in the milk of mice that incites mammary carcinomas contains a ribonucleic acid complex. Although the proof is not available, it is interesting to speculate on the possibility that these nucleic acids may exert their carcinogenic action by producing a disturbance of the normal nucleoproteins of cells.

SUMMARY AND CONCLUSIONS

Desoxyribonucleic acid, a most important constituent of the chromatin of cells, has a significant part in the transmission of hereditary characteristics by the genes, in mitosis, in nucleic acid synthesis and balance, and in the protein synthesis of the cell. The various studies on tumors by means of macrochemical analysis or visual inspection of Feulgen-stained material are not in agreement regarding disturbances of thymonucleic acid. Photometric histochemical observations, using the Feulgen reaction, have shown that some epidermoid carcinomas of mice and men, and leukemic blood cells from the human subject, contain increased amounts of thymonucleic acid. Cytochemical studies show that the cytoplasm of malignant cells contains increased amounts of ribonucleic acids, and suggest that the heterochromatic region of the chromatin plays a specific role in carcinogenesis. The observations in tumor tissues of stickiness, nondisjunction, displacement and clumping of chromosomes, of polyploid cells with increased number and volume of chromosomes, of more frequent mitoses, of enlarged nucleoli, and of multinucleate and giant cells represent cytologic evidence of abnormalities of nucleic acids in neoplastic cells. These findings are especially frequent in more anaplastic tumors.

Extracts of cells containing nucleic acids and their breakdown products have a growth-promoting effect on other cells. That desoxyribonucleic acid may induce a specific change in cells that is predictable and transmissible to subsequent generations has been shown in work with pneumococci. The Shope papilloma virus and the mammary tumor-inciting milk factor of mice, two agents that elicit tumors, contain desoxyribonucleic acid and ribonucleic acid, respectively. It is possible that nucleic acids may also induce transmissible changes in cells that become neoplastic.

Desoxyribonucleic acid is located in the chromatic regions of the chromosomes, which are susceptible to physical changes leading to mutation. It has been shown that similar wave lengths of ultraviolet light produce a breaking down of the polymerized form of sodium thymonucleate, mutations in chromosomes, and carcinoma of the skin. It is suggested that somatic mutations leading to neoplasia may be produced by alterations in the extremely complex macromolecule of thymonucleic acid. An initial slight modification in the complex polymerized thymonucleoprotein could, during a variable latent period, lead to a progressive and ultimately irreversible imbalance of nucleic acid—an imbalance characterized by the excessive amounts of ribonucleic acid found in rapidly proliferating cells. Quantitative or qualitative alterations in the nuclear and cytoplasmic nucleic acids and nucleoproteins—substances which seemingly directly or indirectly regulate many reproductive, synthetic and enzymatic activities of cells—could explain many of the properties of malignant cells. The presented evidence of a disturbance of nucleoproteins forms the basis for a relatively new concept of an intracellular cause of neoplasia, which will be established or disproved by subsequent investigation.

REFERENCES


91. Neave, J. Response of Bone Marrow to AKH and RFI
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ADDENDUM

Among the articles on nucleic acids published since this manuscript was prepared, three reviews are of especial interest. Greenstein has published a general review on nucleoproteins. Hadlow discussed the possible relation of cytoplasmic structures containing nucleic acids to viruses and the malignant properties of cells, and Davidson and Waymouth reviewed the literature on nucleic acids and tissue growth.
Thymonucleic Acid in Tumors

Robert E. Stowell


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