On a Special Condition of the Interphase Nucleus in Normal and Cancerous Cells*

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In the course of histological and cytological studies of tissues of mice of tumor susceptible strains (Green (5) and unpublished), an atypical condition of some of the interphase nuclei was noted. No description of the exact condition has been found in the literature. Interest was aroused by the fact that the nuclei were seen in considerable numbers in the cells of tumors as well as in nontumorous tissues. The characteristics of the condition and its high frequency of occurrence in some individuals were suggestive of a similarity to certain types of virus inclusions. Since some animal tumors are known to be caused by viruses, a study was made of both normal and pathological tissues to further define the properties of the atypical nuclei, to determine how widespread the condition is and to look for possible relationships to the development of tumors or to other specific functional states.

CYTOLOGICAL CHARACTERISTICS OF THE ATYPICAL NUCLEI

General characteristics.—Three features distinguish the atypical nuclei from normal ones in fixed preparations (Fig. 1). These are (1) the presence of considerable amounts of granular material, in addition to the usual chromatin masses and nucleoli; (2) a refractile quality of the nuclear sap; and (3) translucent hexagonal crystals, which under some conditions are birefringent. Typically all three characteristics are found in a single nucleus, which then has a highly condensed and refractile appearance. However, there seems to be a progressive but not necessarily simultaneous accumulation of the substances involved. Nuclei may be recognized as of this type which contain relatively few granules or whose sap is only slightly or not at all refractile. Crystals are not always present, but when present they may be either in condensed nuclei or in more open ones, and in the cytoplasm as well. Since there is no independent correlation apparent of either the acidophilic or the refractile condition, with or without the presence of crystals, with other factors, nuclei which show either characteristic in marked degree are considered to belong to this type, and for convenience will be referred to as A-R (acidophilic-refractile) nuclei.

* Aided by a grant from the Elsa I. Pardee Foundation.
fixed material, the distribution of A-R nuclei is not suggestive of an artifact since it shows no relationship to the distance from the surface of the organ and therefore to the rate of penetration of the fixative or to the length of time of fixation. In all organs, cells with affected nuclei are found adjacent to those with normal ones, and in binucleate cells—for example, in the mouse liver—one nucleus may be normal, the other condensed and refractile.

The condition may be distinguished after a number of different fixatives, although some are more favorable than others for its demonstration.

Zenker’s with acetic, or sublimate-acetic, or alcohol-formalin-acetic (13, 10) brings out the refractive quality of the background and the birefringence of the crystals. Zenker-formol (Helly’s), Bouin’s and 10 per cent formalin leave the A-R nuclei dull and the crystals rarely birefringent; however, A-R nuclei may still be identified by the staining of the granular material.

**Microscopical properties of the components. Granules:** The granular material comprises two components. To the first of these (“coarse granules”) is due the characteristic over-all color of the refractile nuclei in stained sections. The granules take the form of irregularly shaped bodies of varying size. They are acidophilic, so that the nuclei appear pink after the Nocht-Maximow eosin-azure method or eosin-methylene blue and intensely red with acid fuchsin. With iron-alum-hematoxylin the A-R nuclei appear black almost throughout.

The second component of the granular material (“fine granules”) consists of the fine particles, visible both in living cells and in unstained sections, which appear alternately dark and refractile as the focus of the microscope is changed. These particles are sometimes found in otherwise normal nuclei, often in close association with the nucleolus. In refractile nuclei they are numerous and are distributed throughout the nucleus.

**Nuclear sap:** The substance responsible for the refractile appearance of the A-R type nuclei is located in the nuclear sap. In unfixed cells as well as in fixed ones, both with ordinary light and with phase contrast, it may be seen to occur in varying degrees in adjacent cells or in nuclei within the same cell, where conditions are presumably similar. It does not stain with either acid or basic dyes. It is usually uniformly distributed throughout the nucleus.

**Crystals:** In their broadest cross section, the crystals have a hexagonal form with all edges in focus at the same time (Fig. 4). Occasionally other faces may be seen indistinctly. They are almost completely transparent and usually unstained so that they are not readily distinguishable. In some instances, the crystals stain with fast green. The large crystal shown in Figure 4 was faintly stained with eosin. Crystals appear in both refractile and non-refractile nuclei. Furthermore, they are found also in the cytoplasm. There may be several crystals in a single nucleus or in the cytoplasm of a single cell.

Examination of suitably fixed tissues with the polarizing microscope shows crystals that are birefringent. These occur only in refractile nuclei. Here the position in which the crystal lies is of importance. Although with ordinary light hexagonal forms can be distinguished, with polarized light it is only when the crystals lie at an angle to the surface of the section that birefringence appears.

**CYTOCHEMICAL CHARACTERISTICS OF THE A-R NUCLEI**

A summary of the cytochemical properties of the four unusual nuclear components is given in Table 1.

**Coarse granules—**

- Protein—They do not stain with acid fuchsin after digestion of sections with pepsin (HCl pH 2.0, 4 hrs., 37° C.) or with trypsin (phosphate buffer pH 7.0 or citrate buffer pH 6.8, 4 hrs., 37° C.) but do after similar treatment with buffer alone. They continue to stain with acid fuchsin after acid hydrolysis (1 N HCl, 1 hr., 60° C.) but not after hydrolysis with alkali (1 N HCl, 45 min., room temp.).
- Lipid—The material does not stain with Sudan III before or after hydrolysis.
- Nucleic acid—There is no more staining than in adjacent normal nuclei with the Feulgen reaction or with methyl green-pyronin.
- Carbohydrate—There was no color in the refractile nuclei after the iodine test, the Molisch reaction, the Schiff reaction without hydrolysis, or the reaction of McManus as used by Hotchkiss (7).  

**Fine granules—**

- Protein—No fine granules were visible in nuclei digested with pepsin or trypsin or extracted with 6 M urea (2).
- Lipid, Nucleic acid and Carbohydrate—It is not possible to say whether or not they contain lipid, nucleic acid or carbohydrate because they are so small and so
dark or so refractile that one cannot tell whether or not they are colored by any of the reactions applied above.  

**Refractile substance of the nuclear sap—**  
Protein—The refractile material is removed by digestion of sections with pepsin or trypsin but not by buffer alone. It is also removed by extraction of tissue before fixation with 6 M urea (24 hrs., 5° C.) while it is present in material from the same organs fixed without extraction. Under certain conditions the substance undergoes a form of precipitation that results in the formation of a shrunken distorted mass that is brownish in color and highly refractile. This effect is seen occasionally in living cells and in fixed but unstained sections, but is enhanced by certain procedures which would be expected to affect proteins—namely, after staining by the Pappenheim-Urna method in which the dye solution contains a shrunken distorted mass that is brownish in color and highly refractile. This effect is seen occasionally in living cells and in fixed but unstained sections, but is enhanced by certain procedures which would be expected to affect proteins—namely, after staining by the Pappenheim-Urna method in which the dye solution contains glycerine, phenol and methanol and staining is carried out at 37° C., or in sections from which water has been evaporated by boiling to dryness (Fig. 5).

Lipid—The refractile quality is still present after treatment of sections with hot organic solvents—ethyl alcohol, ether-alcohol (1:3), chloroform-methanol (3:1), acetone, or pyridine. It is also removed by extraction of tissue before fixation with 6 M urea (24 hrs., 5° C.) while it is present in material from the same organs fixed without extraction. Under certain conditions the substance undergoes a form of precipitation that results in the formation of a shrunken distorted mass that is brownish in color and highly refractile. This effect is seen occasionally in living cells and in fixed but unstained sections, but is enhanced by certain procedures which would be expected to affect proteins—namely, after staining by the Pappenheim-Urna method in which the dye solution contains glycerine, phenol and methanol and staining is carried out at 37° C., or in sections from which water has been evaporated by boiling to dryness (Fig. 5).

Nucleic acid—The crystals are not stained by toluidine blue or by methyl green-pyronin and they are Feulgen negative. The reaction of St. Hilaire for purines (8) was negative, and the crystals are not blackened by AgNO3.

**Carbohydrate—** All of the tests used above were negative.

**DISTRIBUTION AND INCIDENCE OF A-R TYPE NUCLEI**

In order to evaluate the significance of the fact that nuclei of the A-R type are found in tumors, it seemed advisable first to investigate their distribution generally with respect to species of animals and to organs and tissues within the individual. The incidence in a single organ was examined more thoroughly in order to determine the variability among individuals and to look for possibilities of relationship to normal factors such as age, nutritional condition or growth and cell division. The condition was also considered in relation to certain general pathological features with which it seemed to be associated, and finally with respect to its occurrence in tumors and tumor susceptible animals.

**General distribution of A-R nuclei.**—The nuclear condition has been found to occur in several vertebrate species.

Slides of human organs fixed in Zenker-formol, examined through the courtesy of Dr. Clark E. Brown, showed refractile nuclei in considerable numbers in one individual, in smaller numbers in several others. Material obtained from a 3 months human foetus and fixed in Zenker's fluid with acetic showed refractile nuclei in every organ examined—liver, kidney, adrenal, intestine, lung and spleen. They were present also in the uterus and placenta of the mother. A Syrian hamster which died showing congestion of the lungs, and 2 rabbits which were freshly killed, showed considerable numbers of refractile nuclei in several organs. Properly fixed tissues from rats and guinea pigs have not been examined. The condition was also found in both tadpoles, and adult...
frogs of the species *Rana pipiens* and in a bullfrog, *Rana catesbiana*.

Nuclei of the A-R type have been seen in mice of the Swiss, C3H, A, Bagg albino, C, dba, C57 black, “62” (Lynch), and “5” (Lynch) strains. No marked strain differences in incidence have been noted. Neither is there any sex difference.

The most extensive study of the incidence has been made in the C3H and Swiss strains. Here nuclei of the A-R type have been found at some time in every organ examined. Frequently they are prevalent in both the parenchymal cells and the connective tissue but if they are absent from the former they are still found in the latter. Here they occur in all types of cells—fibroblasts, macrophages, endothelial and reticular cells and in lymphocytes. One gains the impression that the connective tissues may act as a reservoir from which the effect spreads at times into the functional cells of the organs.

Because of its homogeneity and the relatively large size of its nuclei, the liver was chosen as a representative organ in which to observe the incidence of the A-R nuclei.

Counts were made on a single section (6μ) from a piece of liver taken in each case from the left lateral lobe. The average number of refractile and/or completely acidophilic nuclei per microscopic field (obj. 44×, oc. 15×) was obtained by counting adjacent fields in rows spaced 1 mm. apart. Counts on more than one section from a single animal were in agreement, on the average, within less than 10 per cent.

The total number of nuclei, normal and abnormal, included in a single count was found to range from 1000 to 7000. Sample counts on sections of liver from mice of different ages showed that there was an increase in cell size and hence a decrease in the total number of nuclei per field with age. The decrease, exponential in form, runs from about 90 nuclei per field at 1 month to 35 at 20 months. The curve thus obtained was used in estimating the percentage of the total number of nuclei represented by actual counts of the number of A-R nuclei.

**Incidence of A-R type nuclei with respect to age.**

The mice from which liver counts were made range in age from foetal stages to 22 months. A-R nuclei are found at all ages. Individual counts from 135 stock mice of several strains are shown in Figure 6. It can be seen that there is a wide range of variability in young mice than in older ones. The proportion of counts higher than 5 A-R nuclei per microscopic field is significantly greater in mice 5 months of age and younger than in mice that are over 5 months (χ² test, P < 0.001). There is also a decline in the successive median values at each age.

At each age, the counts range from 0 upward and there are more low values than higher ones. Therefore, the median is a better representation in each instance than the mean. It is necessary also to take into account the decline in total number of nuclei per field with age mentioned above. The logarithmic lines of the medians of both A-R nuclei (B) and total nuclei (A) are shown in Figure 6. It can be seen that the rate of decline in A-R nuclei per field is faster than can be accounted for by the decrease in total nuclei per field until a level is reached after which no further falling off occurs.

The decrease in variability and the decline in median values with age shown in the composite group of stock mice can be seen in smaller groups of mice of a single strain.

![Count of A-R type nuclei per microscopic field in the livers of freshly killed stock mice. Lines A and B represent the logarithms of the median values of total nuclei and A-R nuclei per field respectively at each age.](image)

The relatively high incidence of A-R type nuclei in young mice and their presence in foetal stages might indicate that there is a correlation with the actively growing phase of organs and tissues. One piece of evidence suggests that this is not the case. In the mouse intestine, the frequency of A-R nuclei is lower in the deeper portions of the glands, where mitoses are common, than in the more differentiated superficial epithelium.

The variability of counts in a group of animals of uniform age, strain and environmental experience proves to be great. Since, as already indicated, there are usually more low values than high, the frequency distribution of the counts is not normal, and may be so skewed as to be exponential in form (Fig. 7A). When there are a larger proportion of high counts, it may take the form shown in Figure 7B. Therefore, in comparing the incidence in
different groups, those statistical tests which depend upon a normal distribution are not applicable.

Incidence of A-R nuclei with respect to nutritional condition.—If the nuclear condition is related to some phase of the normal metabolism of the cell it must be to a general and not a specific process, since it is found in the parenchymal cells of many organs with different specific functions and in cells of the connective tissue as well.

In order to see whether the incidence of the atypical nuclei is directly related to the state of nutrition of the cell, a group of C3H mice were left without food for 24 hours. These animals lost 2 to 4 grams in weight and at autopsy showed fatty liv-

ers. Nevertheless, the counts of A-R type nuclei in the liver did not differ in range or distribution from those of litter mate controls which had a continuous supply of food.

In the livers of mice generally there is no appar-
tence of large numbers of A-R nuclei is associated.

In organs of apparently healthy animals, in which there are considerable numbers of A-R nuclei, either general or localized areas are found which show these features. Here the cytoplasm of the parenchymal cells is more acidophilic than in neighboring regions; there is congestion of capillaries and small blood vessels, or even diffuse hemorrhage; frequently there is diffuse infiltration with lymphocytes, many of whose nuclei are in the refractile or acidophilic condition; polymorphonuclear leukocytes are absent; the cytoplasm of macrophages in the affected organ often contains a pigment-like substance that is greenish gray in eosin-azure preparations.

In the brains of apparently healthy animals, refractile nuclei are seen occasionally in the glia and ependymal cells. Groups of nerve cells in the cerebral cortex or of Purkinje cells in the cerebellum frequently show shrunk-
en basophilic cytoplasm and nuclei that are deeply acidophilic throughout, although not refractile. Trans-
lucent hexagonal crystals are present in both the nuclei and the cytoplasm of these cells. Neighboring nerve cells are normal in appearance or may contain diffusely scattered acidophilic granules. While congestion has been noted only rarely in these brains, there is sometimes diffuse infiltration with lymphocytes, particularly in the granular layer of the cerebellum.

The foregoing features are especially prominent in certain animals that have died showing relatively high counts of A-R nuclei in the liver. Gross symptoms have been diarrhea and/or a nodular type of pneumonia. No bacteria are found, either histologically or in cultures except in the intestine. In the organs of these animals, the A-R nuclei seem to have undergone pyknosis. They stain more heavily with basic dyes—they contain much Feulgen positive material—yet still show the refractile quality of the background, the acidophilic and refractile granules and birefringent crystals. A check was made to see that the high numbers of A-R type nuclei in the mice that died was not due to postmortem changes. A mouse was killed and allowed to remain at room tempera-
ture. Sections from pieces of liver fixed after 2, 4, 6 and 24 hours showed no increase in the number of A-R nuclei.

Another type of degenerative transformation is seen in certain nuclei that contain hexagonal crystals but are not refractile (Fig. 8). While a few masses of chromatin are applied against the nuclear membrane, the nucleoli and remaining chromatin are clumped in the center of

RELATIONSHIP OF A-R TYPE NUCLEI TO

PATHOLOGICAL CONDITIONS

In the foregoing sections, the incidence of A-R type nuclei has been considered with respect to various factors that are normal or physiological in nature. The possibility also exists that they may be related to pathological conditions.

Association with general pathological features.—While the widespread occurrence of the A-R type nuclei in young and apparently healthy animals and in cells whose cytoplasm seems unaffected indicate that the nuclear condition, in certain of its phases, is not detrimental or degenerative in nature, nevertheless, one gains the impression from histological observations that there is a consistent set of pathological features with which the presence of large numbers of A-R nuclei is associated.
the nucleus about one or more hexagonal crystals. The relatively clear zone between the central mass and the nuclear membrane contains some small eosinophilic particles and a number of refractile granules.

Two other formations have been noted which may or may not be associated with the A-R condition of the nucleus. (1) In the livers of stock mice, there may be seen in as many as 2 per cent of the nuclei discrete inclusion-like bodies. These vary in appearance from small brilliantly eosinophilic globules to large pale-staining spherical bodies or even vacuoles containing diffuse acidophilic granular material is suggestive of a virus. Although crystals have not often been reported in virus infected animal cells, they are characteristic in one instance.

The condition described here resembles that found in the polyhedral disease of silkworms (4) in that the nuclei contain crystalline bodies accompanied by fine granules, but differs in that here neither the crystals nor the granules give a positive test for nucleic acid. In its distribution with respect to species, organs and types of cells affected, as also in the presence of acidophilic granular material and of basophilic “minute bodies,” the appearance of the A-R nuclei is suggestive of herpes simplex, but intranuclear and cytoplasmic crystals and a refractile substance in the nuclear sap have not been reported for herpes.

If the features described above constitute a consistent pathological syndrome, the A-R nuclear condition may represent a general physiological response attributable to a number of different agencies, or it may indicate the presence in the cell of a specific infectious agent. The presence of eosinophilic material (Fig. 9). Similar bodies have been described by Shibatsuji (14, 15) in C3H mice. Sometimes a vacuole or homogeneous inclusion lies outside of but adjacent to the nucleus. (2) In dividing cells, basophilic granules have been noted in the cytoplasm around and within the spindle area. The granules appear after fixatives that do not preserve the mitochondria. They are not Feulgen positive. They are conspicuous in tumor cells, but have been seen also in dividing cells in normal tissues.

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The occurrence of A-R type nuclei in the frog as well as in mammals suggests that if the condition is due to a virus it must be one of low specificity, but it need not be less specific than the virus of psittacosis, which, although native to birds, is infective for man.

Incidence of A-R type nuclei with respect to tumors.—Nuclei of the A-R type are found in all types of tumors that have been examined. These include spontaneous, induced and transplanted tumors and carcinomas as well as sarcomas. They are found in fibroblasts and other cells of the stroma and also in varying percentages of the tumor cells themselves. Here, as in other locations, the nuclei may be very refractile and granular, or they may be non-refractile but acidophilic in varying degree. Hexagonal crystals are found, not only in the refractile and/or acidophilic nuclei, but also in nuclei that are vesicular in appearance and contain only small amounts of acidophilic material. The crystals may often be seen in dividing cells, lying either among the chromosomes at metaphase or anaphase or in the cytoplasm. They occur with considerable frequency also in the cytoplasm of non-dividing cells.

In cases where it was possible to make counts of A-R type nuclei in the tumor and in adjacent normal tissue, as shown in Table 2, the incidence in the two locations proved to be closely similar. The correlation between tumor and normal tissue in a single animal is close, whereas there is considerable variation in different animals with the same type of tumor. Spontaneous tumors of the mammary gland in C3H mice show greater variation among fields of the same tumor than the lung or liver tumors.

We have seen that A-R type nuclei are found in both mammary tumors and normal mammary glands of mice with the mammary tumor milk agent. In view of the fact that the agent is known to be at times widely distributed throughout the animal (1), tissues were examined from mice with lungs of Swiss, Bagg and strain A mice—yet their presence in a given organ does not necessarily result in the appearance of a tumor, since they also occur in the lungs of C3H, dba and C57 black mice, the livers of Swiss mice and the salivary glands of Swiss and C3H mice, where tumors are rare. The abnormal nuclei are widespread throughout the individual, yet tumors tend to appear in only one or at most a few organs. In spite of the fact that all of the characteristics associated with the A-R nuclei are found with considerable frequency in tumor cells, the evidence from their incidence does not give a decisive answer concerning a possible relationship of the nuclear condition to the origin of tumors.

**DISCUSSION**

In the literature there are descriptions of the independent occurrence of certain intranuclear structures similar in some respects to those described here.

Acidophilic material has been reported in several connections. Heidenhain (6) described acidophilic granules in nuclei of amphibian and mam-

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<th>Animals</th>
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<th>Normal tissue</th>
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**TABLE 2**

INCIDENCE OF A-R TYPE NUCLEI IN TUMORS AND ADJACENT NORMAL TISSUE
malian cells which he called "oxychromatin." Pfuhr (11) illustrates a difference in staining of the nuclei in binucleate cells in the livers of rabbits, where one nucleus is sometimes more acidophilic than the other. Acidophilic bodies are described by Scharrer and Scharrer (12) in nerve cells of certain locations in the brain of fish, reptiles and man where one nucleus is sometimes more acidophilic than the other. It is also characteristic—either in diffuse form or as formed inclusions—of the presence of intranuclear viruses.

Crystalline bodies have been seen occasionally in animal cell nuclei. Weatherford (16) reviews the literature. The most extensively investigated crystals are those found in the livers of dogs and other Canidae. Weatherford regards the canine crystals as probably bound up with purine metabolism, although he discusses the possibility of association with a virus (17). He also finds homogeneous inclusion bodies in the liver nuclei. The crystals in the Canidae differ from those described here in that they are elongated prisms, stain regularly with acid and basic dyes, and were not birefringent under the conditions under which they were observed. They were found only in the liver and the kidney, and never in more than 2 per cent of the nuclei. Like the crystals described here, the crystals studied by Monné (9) in the nucleus of the sea-urchin egg give evidence of being composed of lipid and protein. They differ in that they are rod-shaped rather than hexagonal; are birefringent before but not after fixation; are not preserved by fixatives containing acetic acid; and after suitable fixation, stain with iron-alum hematoxylin.

No report has been found of a refractile condition of the nuclear sap or of the formation of a refractile precipitate.

The association of the characteristics described here as constituting a composite nuclear condition seems not to have been recognized previously. The question at once arises as to its nature and what may cause it. Three possibilities present themselves: (1) either the condition may be involved in a particular pathological process, such as the development of tumors; (2) it may be the visible expression of a more general type of pathological response, or be due to a specific infective agent; or (3) it may represent an accumulation of normally occurring substances.

The occurrence of the A-R nuclei in several types of spontaneous tumors and in induced and transplantable tumors as well suggested a possible relationship between the nuclear condition and the development of tumors. Since the strains of mice primarily examined were known to carry the mammary tumor milk factor, it seemed possible that the nuclei might represent the location of this agent. However, nuclei of this type were found also in mice known not to carry the milk factor. Subsequently, the nuclei were found to occur with equal frequency in normal tissues adjacent to the tumors and in organs of animals which are not ordinarily susceptible to the production of tumors in that organ. On the one hand, it cannot be said that their presence will be followed by the appearance of a tumor. On the other hand, they are of such general occurrence that it cannot be stated at present whether or not a tumor can arise without their presence. Thus, the possibility is not ruled out that, given the proper combination of secondary factors, such as hormonal balance, genetic susceptibility, and nutritional requirements, the condition may be related to the genesis of tumors.
of the nucleus and hence a special phase of nuclear activity with respect either to cell division or to cell metabolism, a predictable distribution might be expected with respect to some factor or factors such as organs, position within the organ or definite stages in a cell cycle.

On the basis of the data now available it is not possible to say into which of the three categories mentioned above the functional role of the A-R type nuclei will fall. The point of significance at present is the establishment of the characteristics of the A-R nuclei as a definite nuclear condition or syndrome.

SUMMARY

1. An unusual condition of the interphase nucleus has been observed in tissues of man, rabbit, hamster, mouse and frog.
2. In addition to the usual constituents, the nuclei contain acidophilic granules, fine refractile granules, a refractile element in the ground substance, and birefringent crystals.
3. Cytochemical tests indicate that all four of these components contain protein. The crystals probably contain lipid as well.
4. Nuclei of this type are found in all organs and in derivatives of all cell layers.
5. The incidence of affected nuclei in the livers of mice shows a decrease in variability and in median values with age. It is not influenced by 24 hour fasting.
6. The condition is considered with respect to certain pathological features that seem to be associated with higher numbers of affected nuclei and to certain virus diseases.
7. The incidence in tumors is closely similar to that in adjacent normal tissues, and the nuclei appear in organs of animals that are not ordinarily susceptible to the production of tumors in that organ as well as in those that are.
8. It is not possible to say what causes the nuclear condition, but its wide occurrence suggests a process of some functional significance.

ACKNOWLEDGMENTS
The author is indebted to Miss Jeanne Miller and Miss Evelyn Schuler for technical assistance in making the histological preparations.

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On a Special Condition of the Interphase Nucleus in Normal and Cancerous Cells

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