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[1] All algebras considered here are supposed to be commutative, with unit, over the field of real numbers. A pre-norm of algebra on the algebra A is a real function π on A such that $\pi(x) \geq 0$, $\pi(x + y) \leq \pi(x) + \pi(y)$, $\pi(xy) \leq \pi(x)\pi(y)$, $\pi(\lambda x) = |\lambda| \pi(x)$, $\pi(I) = 1$ or 0. A topological algebra is assumed to have its topology defined by its continuous pre-norms of algebra.

[2] See N. Jacobson, *Structure of Rings* (Coll. Pub. *Am. Math. Soc.*, No. 37 [New York, 1956]).

[3] We assume that if U belongs to $\mathfrak{C}$ and U is isomorphic to V, then V belongs to $\mathfrak{C}$.

[4] See L. Nachbin, these PROCEEDINGS, **43**, 935–37, 1957. The algebras are considered as algebras of operators on themselves.

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*CYTOLOGICAL AND CYTOCHEMICAL CHANGES IN LIVERS OF WHITE MICE FOLLOWING INTRAPERITONEAL INJECTIONS OF DNA PREPARATIONS FROM BREAST CANCERS OF AGOUTI C3H MICE**

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Introduction.—In 1957 J. Benoit, P. Leroy, R. Vendrely, and C. Vendrely[1,2] reported changes in the characteristics of ducks of one breed following injections of deoxyribosenucleic acid (DNA) from another breed of ducks and stated that the modifications continued to be recognized in the progeny of the injected ducks. In view of the important implications which such observations may have for the role of the DNA as a genetic factor, we have started similar experiments, utilizing mice.

Although our own investigations are by no means far enough advanced to permit any conclusions in regard to the transforming activity of DNA in mice, a finding made incidentally during the course of these studies seems of sufficient significance to be reported in this communication.

It was noted that livers of white mice (BALB/C and CF_1) displayed abnormal cytological and cytochemical alterations after repeated intraperitoneal injections of DNA prepared from breast cancers of agouti C_3H mice. The alterations were not observed in controls or in white mice of the same strains after injections with DNA prepared from spleens and livers of the same C_3H mice.

Material and Methods.—Inbred strains of BALB/C mice of white color and low breast cancer incidence (less than 10 per cent) and of C_3H mice of agouti color with high breast cancer incidence (95 per cent) were obtained from the Jackson Memorial Laboratories. The white CF_1 hybrid mice were obtained from the Carworth Farms. The BALB/C and the CF_1 mice were bred further in our own laboratories, while the breast-cancer-bearing C_3H mice were sacrificed soon after their arrival and used for the DNA preparations. Livers, spleens, and the breast cancers were removed immediately after killing of the mice, and the DNA was prepared from each tissue according to the instructions of Vendrely and Vendrely.[3] Groups of newborn and

older mice from the BALB/C and CF_1 strains were injected intraperitoneally at repeated weekly intervals with the DNA preparations of the C_3H mice.

This report will be concerned only with groups of BALB/C and CF_1 mice which received their first DNA injection at the age of at least 3 months. Mice of the same age and sex were divided into four groups; the first group received DNA prepared from the breast cancer; the second, DNA from spleens; and the third, DNA from the livers. All three DNA preparations came from the same C_3H mice. Each group received a weekly intraperitoneal injection of approximately 0.3 cc. Density Units (D.U.)[4] of DNA for 3–6 weeks. The mice of the fourth group were not injected but were kept as controls.

At certain intervals after the injections, mice of each group were killed, and the organs were immediately removed and fixed in Lavdowsky's fluid under standardized conditions. Whenever possible, liver sections from control, spleen DNA-injected, liver DNA-injected, and breast cancer DNA-injected mice were mounted on the same slides so that simultaneous staining was possible. The PAS reaction for polysaccharides and Feulgen microspectrophotometry for the DNA determinations in individual liver nuclei were done as previously described.[5,6]

Results.—When BALB/C and CF_1 mice at 3–6 months of age received three to four weekly intraperitoneal injections of approximately 0.3 cc. D.U. of DNA[4] originating from the tumors of the C_3H strain, foci of unusually large atypical cells containing large nuclei and nucleoli, often multiple, were found scattered with varying frequency throughout the liver. Some of these bizarre cells were similar to the cell type observed in hepatomas, with chromatin which was frequently clumped and increased in quantity. Although extensive search of the livers revealed some mitotic figures, they were not too frequent; however, the microscopic appearance of many cells suggested an endomitotic or amitotic process.

When the PAS reaction for polysaccharides[5] was carried out on slides on which sections of control liver, liver from mice injected with spleen DNA, liver from mice injected with liver DNA, and liver of the mice injected with the tumor DNA were mounted simultaneously, a striking difference in the glycogen content of the cytoplasm of the liver cells was noted. While the liver-cell cytoplasm of the controls and of the mice injected with liver DNA or spleen DNA showed an abundance of glycogen, the cytoplasm of the mice injected with the tumor DNA showed frequently a striking decrease and sometimes a complete absence of glycogen, particularly in the large, abnormal cell type. On the other hand, the nucleoli, which were PAS-negative in the liver cells of the controls and of the spleen and liver DNA-injected mice, were strongly PAS-positive in the livers of the tumor DNA-injected mice, especially, again, in the abnormal cell types. The PAS positivity of the large nucleoli could be used as a convenient marker to study the distribution of the abnormal cell types within the liver parenchyma. The PAS reaction, which showed varying degrees of intensity in the nucleoli, also permitted the recognition of different structures within the nucleoli, such as small globules and fiber-like elements which were either PAS-negative or PAS-positive.

When quantitative determinations of the DNA content in individual liver cells were carried out by Feulgen microspectrophotometry,[6] a marked increase and a larger scatter of the DNA values were found in the livers of mice injected with the DNA prepared from the breast cancers. The liver cells from mice injected with the

same or even larger doses of the DNA derived from the spleens or livers of the same C_3H mice had essentially the same DNA values as did the ones of the non-injected controls. The increase in the DNA of the liver cells of mice was noted following the injections with tumor DNA, irrespective of whether mice were used in which the liver cells were predominantly diploid, tetraploid, or where ploidy formation from the tetraploid state to higher classes seemed to take place. An example of the latter in a control liver of a 6-month-old BALB/C mouse is given in Figure 2.

In Figures 1 and 2, characteristic examples of the DNA data obtained in the livers by Feulgen microspectrophotometry are presented. It can be seen in Figure

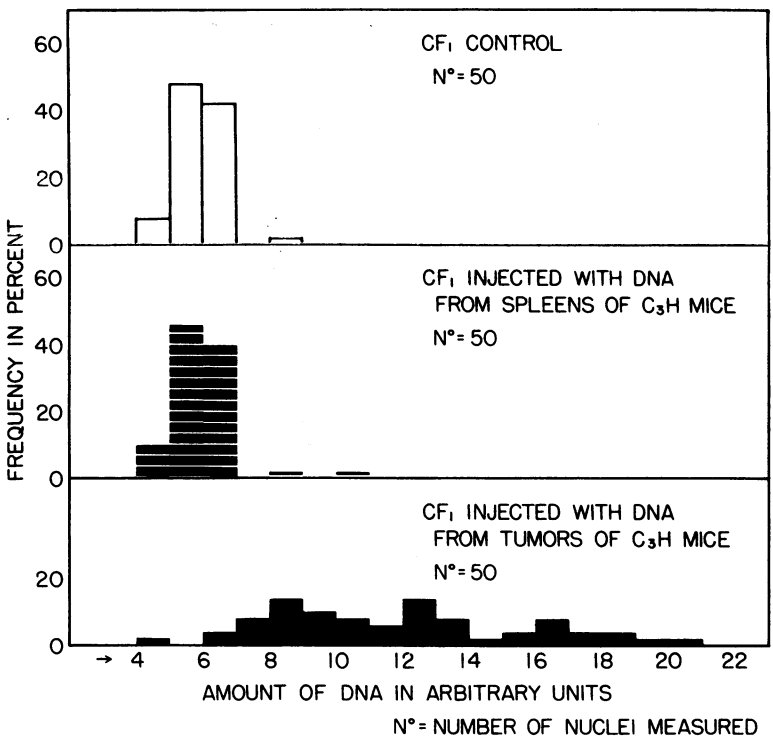

FIG. 1.—DNA content in individual liver nuclei of a control mouse, a mouse injected with spleen DNA, and a mouse injected with tumor DNA.

1 that the livers of the control and spleen DNA-injected CF_1 mice have not only the same mean tetraploid value of approximately 6 arbitrary units but that there are also a very similar distribution and a narrow range of the DNA values within the individual liver nuclei. This picture is very different from the DNA values in the liver of the mouse which has been injected with the tumor DNA; here no definite mean value is present, but, instead, a larger spread of DNA values above the tetraploid DNA can be noted. These DNA values are similar to those found in actively dividing and proliferating tissues, such as tumors.[7] Essentially the same findings are demonstrated in Figure 2, although, as mentioned above, the DNA val-

ues in the liver of the BALB/C control have a somewhat wider range, with a tendency toward a DNA peak near the octaploid value.

Discussion.—While it is not possible at the present time to interpret fully the various findings in the livers of BALB/C and CF_1 mice after injections with the DNA prepared from tumors of C_3H mice, nevertheless it is justifiable to state that the changes described point to a disturbance in the liver-cell metabolism. The occurrence of large, atypical cells with large nuclei and nucleoli and increased amounts of DNA strongly suggests a change in the nucleic acid metabolism, indicating nucleic acid synthesis and proliferation of the cells, although the latter appears somewhat atypical because of the scarcity of mitosis. The decrease in glycogen and the presence of PAS-positive nucleoli suggest an additional derangement in the carbohydrate metabolism. Whether there is an interrelationship between the nucleic acid and carbohydrate disturbances in these cells remains

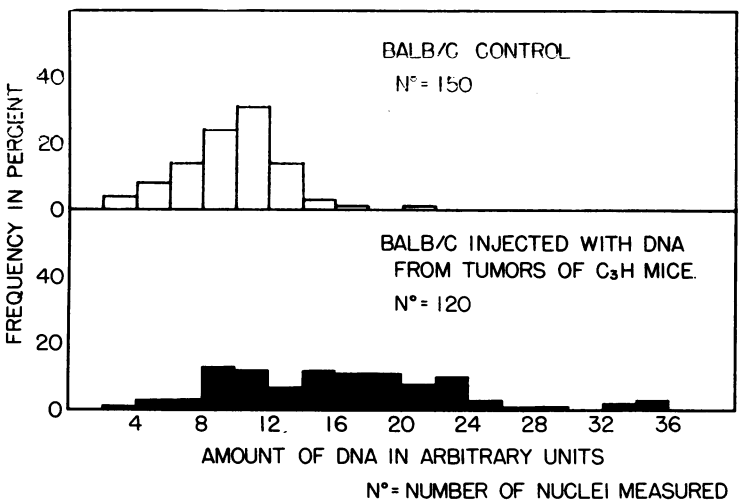

FIG. 2.—DNA content in individual liver nuclei of a control mouse and a mouse injected with tumor DNA.

to be seen, although there may be an inverse relationship similar to what has been observed in the salivary gland cells of *Helix pomatia* by Leuchtenberger and Schrader.[8]

The finding that the changes in the livers were noted, so far, only in mice which had been injected with tumor DNA, but not in those injected with DNA from spleen or liver derived from the same tumor-bearing mice, assumes special significance, since neither biochemical nor microspectrophotometric studies of the tumor, spleen, and liver DNA preparations revealed any significant differences between them. On the other hand, it is not too surprising that there exists a difference between the biological activity of the DNA derived from a tumor and of the DNA derived from a non-tumorous tissue. In recent years, evidence has been accumulated which indicates that the presence of a tumor in animals stimulates the nucleic acid and, in particular, the DNA metabolism of the normal organs of the host.[9]

C. Leuchtenberger, in 1954,[10] using Feulgen microspectrophotometry, also re-

ported an increase and wide scatter of DNA values in liver cells of mice bearing the rapidly growing subcutaneously transplanted Sarcoma 180. The similarity between these DNA data in the livers of tumor-bearing mice and the DNA data in liver cells of mice after injection with DNA prepared from tumors is indeed so striking that it may be suggested that the DNA contained in the tumor cells is perhaps responsible for the change of the nucleic acid metabolism in the normal tissues of tumor-bearing animals.

It is hoped that enzymatic studies which are under way will help to answer the question whether the biological activity is due to the tumor DNA itself, or whether the traces of proteins found in the DNA preparations (tumor, 0.8 μg. per D.U.; spleen, 0.3 μg. per D.U.; liver, 1.7 μg. per D.U.) may also be contributing factors. Regardless of the outcome of these studies, the fact remains that intraperitoneal injections of purified DNA preparations from tumor cells have a similar biological activity on normal tissues in vivo as has the presence of the tumor itself. While it is significant to find that the DNA from malignant tumor cells (breast cancer) displays a different biological activity from that of the DNA derived from non-tumorous tissues of the same animals, one is, of course, not justified to ascribe this activity to the malignancy per se. It may very well be possible that the activity of the tumor DNA is due to the fact that it is prepared from a rapidly dividing tissue. This is, however, not too likely a possibility, since the DNA from the spleen, which is also a dividing tissue, did not show such an effect on the liver. In order to explore this possibility further, we have prepared DNA from the embryonic tissue of the C_3H mice.

Another question, which also can be answered only in the future, concerns the fate of the atypical cells found in the liver after the tumor DNA injections. Will they progress to tumor formation (hepatomas), especially when the DNA injections are continued? Will perhaps partial resection leading to regeneration of liver tissue hasten the development of hepatomas? If the tumor DNA injections should ultimately produce tumors in normal tissues, then the concept that an aberrant DNA which may occur in cells due to virus infection, radiation, or other injuries may itself become a tumor-initiating factor will gain further support. Perhaps this approach may ultimately bring the long-sought answer to the question whether DNA itself can act directly as a tumor-promoting agent or whether it acts only in an indirect way as a mediator or cocarcinogen.

Summary.—Livers of white BALB/C and CF_1 mice show cytological and cytochemical alterations after repeated intraperitoneal injections of DNA prepared from breast cancers of agouti C_3H mice. The livers show foci of abnormally large cells containing large nuclei and large PAS-positive nucleoli. There is a decrease of the glycogen in the liver cytoplasm and an increase in the amounts of DNA in the liver nuclei.

These alterations were not present in livers of control mice or mice similarly injected with DNA derived from spleen or livers of the same C_3H mice.

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*A MECHANISM OF CELLULAR THERMOGENESIS IN COLD-ADAPTATION**

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Introduction.—The adaptation of the non-hibernating, homeothermic mammal to lowered ambient temperatures could be achieved in theory either by reduction of heat losses or by an increase in energy exchange and heat production. It is by now well established[1–5] that, in general, the cold-adapted mammal, including man,[6,7] achieves this solution mainly by increasing its total metabolic energy production through higher caloric intake and increased oxygen utilization. Evidence from in vitro studies on slice[8–10] and liver brei[11] is also adequate to show that this response reflects adaptive changes at the tissue and cell levels, indicating use of preferred metabolic pathways of substrate utilization and energy exchange [12–14] by the cold-adapted system.

For optimal adaptation from the energetic standpoint, the required increase in heat evolution should be obtained by metabolic processes yielding the maximal heat compatible with the minimal requirements for basal maintenance work; whence the optimal system would appear to be one having, in terms of work, a relatively low efficiency, with a greater balance of its energy yield from substrate oxidation being diverted into a heat pool rather than into its usual form of potential energy in the chemical bond. With this, it would follow that, in the formation of ATP,[15] we should observe in the tissues of the cold-adapted rat a net decrease in phosphate fixation relative either to oxygen utilization or, implicitly, to total heat production. Under suitable experimental conditions this would appear as a decrease in the P/O ratio of the corresponding in vitro system.

The studies reported in this paper were undertaken primarily to test the foregoing proposition and, contingent upon the outcome, to examine either the metabolic pathways by which the expected result was achieved or, alternatively, the mechanism by which the cells of the cold-adapted system might achieve the necessary heat