Increased Viscosity of Cells of Induced Tumors*

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In an earlier study (1) the authors found that tumor cells (carcinoma, sarcoma, adenofibroma, adenocarcinoma) of rats remained unstratified or stratified but feebly when centrifuged at extremely high speeds. In that series of experiments the tumor tissue was first transplanted to such organs as adrenal gland, kidney, pancreas, liver, spleen, stomach, and intestine. After growth was well established, bits of the transplant together with pieces of the host tissue were rotated in a Beams air-driven ultracentrifuge at a speed which produced a displacement pull of about 400,000 times that of gravity. Invariably the cells of the normal tissues became markedly stratified; those of the tumor tissue remained largely unchanged. It was inferred therefore that the protoplasm (both cytoplasm and karyoplasm) of the latter had become so viscous that the cellular contents were unable to move freely and take positions according to their respective densities.

In the present study advantage has been taken of the well-known tendency of butter yellow, p-dimethylaminooazobenzene, to induce cancer of the liver after prolonged feeding. The liver tissues used were from rats which had lived on a ration containing butter yellow for from 1 to 689 months.

We are indebted to Van Rensselaer Potter of the McArdle Cancer Research Institute for liver tissue from the 24 rats used in this study. After treatment was begun the animals were killed, 3 at a time, at the end of 4, 6, 8, 10, 13, 18, 21, and 26 weeks respectively. The photomicrographs (Figs. 1 to 7), illustrating the cytological effects observed, were made from paraffin sections of tissue fixed in Bouin's fluid and stained with Harris' hematoxylin and eosin or acid fuchsin.

The purpose of our investigation was to find out if the cells of such induced tumors showed increased viscosity in comparison with adjoining normal cells centrifuged at the same time, and if so, what could be determined about the onset of the condition.

For purposes of general histological guidance and comparison we depended mainly on Orr's (2) recent careful study on the histology of the rat's liver during the course of carcinogenesis by butter yellow. Of 136 rats fed on a diet containing butter yellow and killed at intervals through a period of from 1 to 11 months, Orr found that 56 displayed tumors, of which 43 were definitely malignant. The tumors described by him were of three types: (a) bile duct carcinomas (cholangiomas); (b) bile duct cystadenomas; and (c) liver cell carcinomas (hepatomas). Any particular liver might contain all three kinds or any one or two.

Occurrence of excessive proliferation of ducts and the presence of cystic bile ducts were usually the first indication of a definitely affected liver. Almost invariably, in the vicinity of such proliferative centers, certain areas of the liver cells lose their characteristic corded arrangement and become more or less nodular in appearance, although nonneoplastic liver cells may remain scattered throughout the developing tumor. Within such disorganized masses of cells, tubules or cysts begin to appear—first with small lumina and thick walls of columnar cells. As the tubules become fully developed the cells of the walls become cuboidal and eventually flattened. Such cells in all stages of

Fig. 7.—Photomicrograph of a section from a carcinomatous nodule in the liver of a rat after six months' treatment with butter yellow. Mag. X 430, approx.

DESCRIPTION OF FIGURES 1 TO 7

Fig. 1.—Photomicrograph of section of normal liver of the rat showing stratification of the component cells after centrifuging for 1 hour. The vacuoles below the nuclei represent regions originally occupied by glycogen which has been dissolved out by treatment with water. Mag. X 430, approx.

Fig. 2.—Photomicrograph of section of centrifuged normal liver stained for glycogen only. The glycogen has been dissolved out to the centrifugal side of the cell. Mag. X 430, approx.

Fig. 3.—Photomicrograph of section of a nodule of the liver of the rat showing effects of administration of butter yellow. Mag. X 430, approx.

Fig. 4.—Photomicrograph of section of tissue from a nodule in the same liver as that from which the section shown in Fig. 3 was taken. After an hour of severe centrifuging the cells remain unstratified. Mag. X 430, approx.

Fig. 5.—Photomicrograph of a representative area of centrifuged tissue from the liver of a rat after 6 months' treatment with butter yellow, showing proliferation of bile ducts, granulation, and pyknosis. Mag. X 100, approx.

Fig. 6.—Photomicrograph of section of the same liver as that from which the tissue shown in Fig. 5 was obtained. After an hour of severe centrifuging the cells of the ducts and cysts remain unstratified. Mag. X 430, approx.

Fig. 7.—Photomicrograph of a section from a carcinomatous nodule in the liver of a rat after six months' treatment with butter yellow. Mag. X 430, approx.
Figs. 1 to 7
their development remain unstratified after centrifuging at high speed for one hour (Figs. 4 and 6). In addition to the cystic systems just described, vacuolation of greater or less degree is often also present in the adjacent intercellular substance.

While they were obviously multifocal in origin, there was no way of determining whether the nodules just mentioned start as single cells or as groups of cells. The cells of the nodules are characterized by obvious variability in size with here and there much larger ones (Fig. 3). The nuclei of the latter are also enlarged and may contain several nucleoli instead of the usual single one. Moreover a greater number of cells than is usual in the liver cells of the rat display two nuclei. There is considerable pyknosis and the cells in general stain more deeply with the conventional hematoxylin and acid fuchsin dyes. In some preparations relatively few mitotic figures are observable; in others, they are plentiful (Fig. 7).

In liver cells of such foci (Fig. 3), centrifuging for an hour failed to produce appreciable stratification in either cytoplasm or nucleus (Fig. 4), although the nonneoplastic cells of the same liver tissue were markedly stratified (Fig. 1), with glycogen thrown to the extreme centrifugal side of the cell (Fig. 2) and nucleus surrounded by mitochondria next on top of it. The lighter cytoplasmic inclusions graded off toward the opposite side of the cell where a vacuole usually occurred. This vacuole evidently represented a region from which some lipoidal substance, probably the Golgi apparatus (1), had been dissolved by fat solvents in the later treatment of the sections. Within the nucleus of such normal cells the chromatin and the nucleolus were thrown into a crescentic mass against the nuclear wall with strands of achromatic substance stretching across to the opposite side.

In the livers of 3 rats fed on butter yellow for 2 months, where evidence of neoplasms was just becoming visible, (Fig. 3) the affected regions showed intensified staining indicating chemical change, and, when centrifuged, stratification did not occur (Fig. 4). Whether or not this indicates the onset of malignancy is impossible to decide surely. That it may be such is perhaps indicated by the occasional occurrence of areas containing scattered large cells. Longer feeding of butter yellow led to the occurrence of definite tumors the cells of which, like those of typical carcinomas, remain unstratified after being centrifuged. This was also true of the cells constituting the walls of the superabundant cysts and ducts (Figs. 5 and 6).

Normal liver cells centrifuged for 1 hour showed stratification (Fig. 1). Some of the centrifuged cells appear to show two vacuoles, one on the centrifugal (heavy) and one on the centripetal (light) side of the cell. This was puzzling for a time until similar cells were fixed in alcohol and stained specifically for glycogen. The centrifugal side of the cell was thus shown to be occupied by glycogen and the apparent vacuoles were obviously areas from which glycogen had been dissolved in the aqueous treatment of such cells. The vacuole on the lighter side of the cell was that left by dissolution of lipoidal substance, probably the Golgi apparatus, by fat solvents used in treatment of the sections. Glycogen is evidently the heaviest substance in the centrifuged normal liver cells stained for glycogen only (Fig. 2). According to Orr (2), glycogen does not occur in the cells of fully malignant liver cell carcinomas. The contents of cells comprising nodules in the liver of a rat which had been on a diet of butter yellow for 2 months (Fig. 3) were not stratified by centrifuging for one hour (Fig. 4). Similarly, the cells of proliferated bile ducts and cysts in the liver of a rat after 6 months of feeding with butter yellow (Fig. 5) were not stratified by severe centrifuging (Fig. 6).

Since it is now well established that the respiratory metabolism of cancer cells is so altered that the carbon dioxide output is largely replaced by formation of lactic acid, and with the well-known clotting effect of this acid on milk proteins in mind, one is prompted to inquire if the generation of excess lactic acid may not be the cause of the increased viscosity observable in cancer cells. At least the two phenomena are concurrent and it would seem worth while to determine, if possible, whether or not they are interrelated. Further investigations in this field are in progress.

**Summary**

High speed centrifuging of tumor cells induced in the livers of rats by the feeding of butter yellow (p-dimethylaminoazobenzene) leaves such cells unstratified. Normal liver cells stratify readily, with glycogen appearing heaviest, nucleus and mitochondria next, and lipoidal substances, mainly Golgi apparatus, lightest. Inside the normal nucleus of such cells the chromatin and nucleolus are forced to one side, with achromatic strands of material left stretching across to the opposite wall. The failure of either cytosome or nucleus of induced tumor cells to stratify when centrifuged along with adjacent normal liver cells is evidently due to the increased viscosity of their cellular contents. Such viscosity apparently comes on fairly early since it is evident in incipient tumor cells seen after some 2 months of feeding butter yellow. It is suggested that the increasing lactic acid output of the abnormal cell may be the cause of its enhanced viscosity.

**References**


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