

Mice of strains C3H, C57 black, and A<sup>W</sup>, known to be among the most susceptible to subcutaneous growths after application of carcinogens, have not as yet (10 months) given any subcutaneous tumors following similar treatment with cholic acid and sodium desoxycholate.

A study of the effect of larger doses of desoxycholic acid on pulmonary tumor incidence in A strain mice is being continued.

*Summary.* A significant increase in the incidence of pulmonary tumors is recorded in A strain mice following subcutaneous injection of 10 mg sodium desoxycholate. No such response was indicated following similar treatment with cholic acid.

### 13030 P

#### Histophysiological Study on Activity of Stellate Cells of Frog Liver.\*

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Activity of stellate cells in the liver ordinarily is tested by their capacity to incorporate microscopic or submicroscopic particles, e. g., colloidal trypan blue. In isolated perfused frog liver this uptake is shown to be an active process by the fact that it is reversibly suppressed by addition to the perfusion fluid of an anesthetic or of iodoacetic acid.<sup>1</sup> Also, the color uptake is stopped by perfusing, for a short time, with Ringer's solution, but reappears upon the addition of serum or of various protein bodies.<sup>2, 3, 1</sup> While studying the influence of surface-active and nonpolar-polar organic compounds on erythrocytes, muscles, nerves, liver, kidney,<sup>4</sup> we were reminded of the statement of A. J. Clark<sup>5</sup> that the strikingly beneficial effect of serum on the heart of a frog, which has become

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<sup>1</sup> Ferrari, R., and Höber, R., *Pflüger's Arch.*, 1933, **232**, 299.

<sup>2</sup> von Jansc6, N., *Z. Exp. Med.*, 1928, **61**, 63.

<sup>3</sup> Höber, R., and Titajew, A., *Pflüger's Arch.*, 1929, **223**, 180.

<sup>4</sup> Höber, R., *Cold Spring Harbor Symposia*, 1940, **8**, 40.

<sup>5</sup> Clark, A. J., *J. Physiol.*, 1913, **47**, 66.

hypodynamic by a prolonged perfusion with Ringer, is due chiefly to presence of minute amounts in the serum of lipoids and soaps. We decided to study the influence of physico-chemically similar compounds upon the activity of the stellate cells.

*Methods.* The liver of female frogs was perfused through the abdominal vein with buffered Ringer solution (pH 7.5). In general, after 30 min. 2% ox serum was added, 10 min. later 0.02% trypan blue and a specified amount of a test substance were added. After another 45 min. the liver was placed in formalin and cut by a freezing microtom. In each experiment 2 to 4 frogs of approximately the same weight and belonging to the same group were compared. The same fresh stock of serum was used for all the perfusions in each experiment to avoid error resulting from varying amounts of activating substances in the sera.

*Results.* With livers identically treated the uptake of color varies very much, mainly because of seasonal variations. With an abundant uptake, the color appears in a fairly continuous blue network of the blood capillaries surrounding the unstained islets of liver cells. This network consists of irregularly shaped groups of stellate cells, which show a rather homogeneous coloration. With a small uptake one finds separate longer or shorter blue bars or rods. In general, the nuclei are unstained. A comparison of sections from the same set of livers shows that after Ringer perfusion almost no blue color can be detected. Addition of serum is followed by a definite coloration, which however differs according to the amount of activating matter being present in the serum. Further addition in rising concentrations of surface-active nonpolar-polar compounds results in increased color uptake which is indicated either by a stronger coloration of the stellate cells or by the fact that with higher concentrations the cells begin to swell and that the dye spreads over the enlarged cell body.

*Bile Salts.* Due to their marked nonpolar-polar molecular configuration as well as to their powerful choleric activity the bile salts were expected also to influence the behavior of the stellate cells. As a matter of fact, they increase the amount of trypan blue deposited more consistently than any of the tested substances. Therefore, the aforementioned blue network is particularly conspicuous. An increase of the cell volume can contribute to the distinctness of the picture. This is most evident with about  $3 \times 10^{-5}$  molar taurocholate or glycocholate. Beyond this concentration, the areas showing the diffuse blue coloration are enlarged to a greater extent and appear to be formed by aggregates of more greatly swollen cells and by irreg-

ularly formed pale-blue cell rests, parts of which extend into the lumina of the broader capillaries. At this stage of structural alteration one meets more or less marked amounts of nuclear staining, even in the liver cells proper.

Similar results are obtained with other organic nonpolar-polar substances, by means of which a dispersing effect in the colloidal systems can be produced and, therefore, the normal properties of the surface film of cells can be altered. Among such compounds investigated, detergents and wetting agents (aerosols, sulphated fatty acids, hexylresorcinol) have proved effective.

*Discussion.* According to the histological effects described, the substances tested in this study can be compared to cytolytics, although such a classification would appear to deprive the results of their physiological meaning. However, cytolysis is only the end effect of a range of structural and ultrastructural changes, which, at their very beginning, are reversible and are indicative of changes falling into the range of physiological manifestations. This has been found already in earlier experiments with the Ringer perfused frog liver insofar as cytolytics have been shown to increase the secretory transport of dyestuffs in low concentrations and to abolish it in higher concentrations.<sup>6</sup>

### 13031 P

#### Necrosis, Cirrhosis and Cancer of Liver in Rats Fed a Diet Containing Dimethylaminoazobenzene.\*

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It was recently reported<sup>1</sup> that dietary liver injury (necrosis, cirrhosis) seen in rats fed a synthetic ration is determined in great part by the absence of the lipotropic activity of casein. These

<sup>6</sup> Höber, R., and Moore, E., *J. Gen. Physiol.*, 1939, **23**, 191.

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<sup>1</sup> György, P., and Goldblatt, H., *Proc. Soc. Exp. Biol. and Med.*, 1941, **46**, 492.