

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.

changes can be prevented to a large extent by the addition of casein to the diet, but this is accomplished more effectively by the combined oral administration of cystine and choline.

During the last 2 years similar investigations have been carried out with the purpose of elucidating the cause of cirrhosis and cancer of the liver in rats brought about by the addition of butter yellow (dimethylaminoazobenzene, dissolved in oil) to a diet consisting of rice and carrots, as first described by Kinoshita.<sup>2</sup>

The question arose whether or not the same mechanism that proved effective in the prevention of simple dietary liver injury<sup>1</sup> may also play a rôle in the prevention of changes in the liver which follow the administration of butter yellow.<sup>3</sup>

*Results.* 1. In a control group of rats fed basal diet A, consisting of rice, carrots, and dimethylaminoazobenzene in oil (0.6 g per kilo of diet), supplemented with 20  $\mu$ g daily each of thiamine, riboflavin and pyridoxine and with 100  $\mu$ g daily of pantothenic acid, cirrhosis, atypical, nodular proliferation of bile ducts and carcinoma<sup>§</sup> of the liver were a regular feature. In different groups the incidence of these changes fluctuated from 80 to 100%. However, only 40% of rats fed the same diet with the addition of 18% casein<sup>||</sup> showed similar changes. These observations are in close agreement with the results obtained by Kensler and his collaborators<sup>4</sup> just published.

2. Sixty rats were put on basal diet A (rice, carrots, dimethylaminoazobenzene) with the usual supplements of thiamine, riboflavin, pyridoxine and pantothenic acid. In addition sub-group 1 (16 rats) of this experiment received from 10 to 20 mg of choline

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<sup>2</sup> Kinoshita, R., *Trans. Soc. Path. Jap.*, 1937, **27**, 665.

<sup>3</sup> The special series of experiments dealing with the histologic character of these liver changes and with the effect of various vitamin-like substances including biotin, para-aminoazobenzoic acid and dried liver<sup>3</sup> will be reviewed in a separate paper. In this study, as well as in the continuation, as yet incomplete, of the experiments reported here, the staff of the research laboratories of the S.M.A. Corporation has taken and is continuing to take an extensive part.

<sup>3</sup> Nakahara, W., Fujiwara, T., and Mori, K., *Gann.*, 1939, **33**, 57.

<sup>§</sup> The lesion spoken of as carcinoma in these experiments is microscopically identical with that reported by other investigators who have used butter yellow but, so far, in our experience, it has not shown metastases and has not proved transplantable.

<sup>||</sup> Thiamine, riboflavin, pyridoxine and pantothenic acid have been kindly furnished by Merck & Co., Rahway, New Jersey, and casein by the S.M.A. Corporation, Chagrin Falls, Ohio.

<sup>4</sup> Kensler, C. J., Sugiura, K., Young, N. F., Halter, C. R., and Rhoads, C. P., *Science*, 1941, **93**, 308.

daily, sub-group 2 (12 rats) received from 25 to 50 mg of cystine daily and sub-group 3 (12 rats) received daily from 25 to 50 mg of cystine plus from 10 to 20 mg of choline. Sub-group 4 (20 rats) received no supplement. The results are summarized in Table I.

TABLE I.

| Basal diet A     |                                              | Supplement          | Liver changes produced |                                              |           |
|------------------|----------------------------------------------|---------------------|------------------------|----------------------------------------------|-----------|
|                  |                                              |                     | Cirrhosis              | Atypical nodular proliferation of bile ducts | Carcinoma |
| rice             |                                              | none                | +++                    | +++                                          | ++        |
| carrots          |                                              |                     |                        |                                              |           |
| thiamine         | dimethylamino-azobenzene (0.6 g per kg diet) | cystine             | +++                    | +++                                          | +++       |
| riboflavin and   |                                              | choline             | +++                    | ++                                           | ++        |
| pyridoxine       |                                              | cystine and choline | +                      | +                                            | 0         |
| pantothenic acid |                                              |                     |                        |                                              |           |

Incidence of lesions: + slight, ++ moderate, +++ great.

3. Twenty rats kept on diet B, composed of casein 18%, cane sugar 68, melted butter fat 8, salt mixture 4, and cod liver oil 2, and supplemented again with thiamine, riboflavin, pyridoxine (20 $\mu$ g of each daily), with pantothenic acid (100  $\mu$ g daily) and with the same amount of dimethylaminoazobenzene (0.6 g per kilo of diet) as in basal diet A, were conspicuously free of cancer or of atypical proliferation of bile ducts. Up to 175 days of the experimental period only 2 rats have shown mild patchy necrosis and slight cirrhosis<sup>5</sup> as the only sign of liver injury.

4. Eighty rats were put on diet C, composed of casein 6%, lard 23, cane sugar 15, cornstarch 50, salt mixture 4, cod liver oil 2, supplemented with dimethylaminoazobenzene in the same proportion as in diets A and B. The animals were subdivided into 4 groups. Sub-group 1 received no further supplement, sub-group 2 received 20 mg of choline daily, sub-group 3 received 50 mg of cystine daily and sub-group 4 received 50 mg of cystine plus 20 mg of choline. The 18 rats in sub-group 1 and the 30 rats in sub-group 2 all showed evidence of diffuse and severe hemorrhagic necrosis with or without cirrhosis. In sub-group 3, all 18 rats exhibited marked cirrhosis with or without additional necrosis, whereas in the 14 rats in sub-group 4 only 2 manifested severe cirrhosis, 3 slight cirrhotic or necrotic changes and the remaining 9 were free even of such changes.

In view of all these observations the conclusion appears to be

<sup>5</sup> György, P., and Goldblatt, H., *J. Exp. Med.*, 1939, **70**, 185.

warranted that the lipotropic activity of casein and in particular the combined oral administration of cystine plus choline afford a definite but not regular protection against pathological changes in the liver (necrosis, cirrhosis, atypical nodular proliferation of bile ducts, adenocarcinoma, malignant hepatoma) produced by a diet containing dimethylaminoazobenzene.

Further, it is noteworthy that no malignant changes were seen in rats fed rations containing dimethylaminoazobenzene but no rice (diets B and C). This may be due, apart from the effect of casein (present in diets B and C), either to a special carcinogenic property of rice or to other differences, such as content of butter fat, lard, sugar or corn starch in the rations used.

The experimental period lasted up to 175 days.

The experiments were performed on several hundred rats and are being continued.

### 13032 P

#### Centripetal Discharges in Dorsal and Ventral Roots Following Stimulation of Muscle by Ventral Root Volleys.

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When a ventral spinal root (L 7 or S 1) of the cat under light dial narcosis (0.5 ml/kilo Ciba) is stimulated, discharges arising in the periphery are conducted centripetally to both dorsal and ventral roots. Afferent activity consequent upon muscle contraction would be expected, but the discharges under consideration are such as to suggest that the mediation of muscle receptors is not involved. Centripetal discharges in ventral roots have been observed following the administration of eserine,<sup>1</sup> or prostigmine.<sup>2</sup> Apparently such discharges have not been observed previously in the non-eserinized preparation.

*Dorsal Root Discharges.* Record A of the accompanying figure shows the early centripetal activity (spike potentials 1 and 2) recorded from L 7, D. R. following a single shock to L 7, V. R. Similar discharges from the periphery may be recorded if S 1 roots

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<sup>1</sup> Dun, F. T., and Feng, T. P., *Chin. J. Physiol.*, 1940, **15**, 433.

<sup>2</sup> Masland, R. L., and Wigton, R. S., *J. Neurophysiol.*, 1940, **3**, 269.