suggestions concerning biochemical methods used in this work.

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Inhibiting Effect of Trypan Blue on the Experimental Production of Liver Cancer

Recently, Gillman *et al.*[1] and Simpson[2] reported that trypan blue injected subcutaneously into rats at weekly or bi-weekly intervals induced tumour (reticulum cell sarcoma) of the liver. Trypan blue is a sulphonated dis-azo dye and somewhat resembles in chemical structure dimethylaminoazobenzene, which is a potent carcinogen for the liver of rats.

The present communication deals with the results of the subcutaneous injection of trypan blue on the incidence and course of tumours induced by *p*-dimethylaminoazobenzene. In these experiments 1–2 ml. of a 1 per cent aqueous solution of trypan blue was injected subcutaneously at intervals of one to two weeks into rats of mixed breed weighing 80–100 gm. which were also given 0·06 per cent dimethylaminoazobenzene diet. The injections were continued until a total dose of 60–80 mgm. of trypan blue had been given. The control animals were given 0·06 per cent dimethylaminoazobenzene diet, but received no trypan blue. The basal diet consisted of unpolished rice and contained 1·33 mgm. of riboflavin per kgm., which encourages tumour induction and survival, and this was supplemented by green vegetables every two days. The dimethylaminoazobenzene given to each rat amounted to about 600–700 mgm. over a period of six to twelve months. The experiments were terminated within one year and the incidence of liver cancer of the group injected with trypan blue was compared with the controls at periods of 6, 7–8 and 9–12 months after the commencement of the experiments.

These results indicate that trypan blue delays the production of liver cancer by *p*-dimethylaminoazobenzene. In the controls, the induced liver cancer reached a great size after six months and often metastases appeared in the abdominal lymph nodes and lungs. In the groups of animals which received trypan blue for seven to eight months, the tumour incidence amounted to 33 and 100 per cent respectively; but these tumours were much smaller and more localized compared with the controls, in which the tumour incidence was practically 100 per cent. During early trypan blue treatment, the rats showed less gain in weight and higher mortality due to respiratory infections than the controls, suggesting toxicity of the drug, while after five to six months they showed a considerable gain in weight whereas the controls showed a sustained loss.

Histologically, a marked hepatic response could be observed in the liver of treated groups. During the first one to two months, the stellate cells in the liver sinusoids were large and plump, and filled with trypan blue particles, whereas in the later months of the experiment a characteristic proliferation of the histiocytic cells developed in the portal tracts with the stellate cells growing smaller and narrower. The hyperplasia and hypertrophy of the parenchyma cells of the liver, however, were more inhibited than in the controls.

Recently, Miller *et al.*[3] have shown that the levels of protein-bound carcinogenic azo-dyes in the liver are lower when the carcinogen is fed in diets containing high levels of riboflavin. We measured the total protein-bound dimethylaminoazobenzene in the livers of the animals receiving trypan blue and in the control animals, using the method of Miller *et al.*[4]. It was found that the difference in the two groups of animals was too small to explain the inhibiting effect of trypan blue. *p*-Dimethylaminoazobenzene, however, was found in a slightly higher concentration in the trypan blue group, which suggests that trypan blue does not reduce the absorption of dimethylaminoazobenzene from the intestine or increase the rate of metabolism of it in the liver.

We wish to thank Profs. Fukuzo Oshima and Kazuo Hotta for their encouragement and advice.

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Oct. 10.

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Determination of the Life of Human Blood Platelets using Labelled Di*iso*propylfluorophosphonate

Hitherto no satisfactory method for the determination of the life of blood platelets has been described. Recently a method has been reported for determining the life of erythrocytes using di*iso*propylfluorophosphonate labelled with phosphorus-32[1]. This method was based on the irreversible combination of this compound with esterases present on the red cell membrane. Since thrombocytes have also been shown to contain esterase, the same method suggested itself as a way of measuring the life of these cells. It seemed reasonable to assume that conditions similar to those described for red cells would be met. This implies that the decay of radioactivity associated with platelets would be entirely due to the destruction of these cells and therefore an index of their normal breakdown. This would only be true if no re-incorporation of labelled material in newly formed platelets were to take place. This assumption seems justified, since it has been shown that the only demonstrable metabolite of di*iso*propylfluorophosphonate in the human body is di*iso*propylphosphate. This compound is not further metabolized but quickly excreted in the urine. The labelled di*iso*propylfluorophosphonate used was synthesized by Mr. Oosterbaan (M.B.L., Rijswijk) by a method to be reported in the near future.