EFFECT OF TRYPAN RED AND METHYL ORANGE ON THE EXPERIMENTAL PRODUCTION OF LIVER CANCER

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Recently the authors[1)2)3)] and Sayama *et al.*[4)] have found trypan blue to have an inhibitory effect on the development of tumor of the liver induced in rats by the administration of *p*-dimethylaminoazobenzene (DAB). It may be significant that both are somewhat related in chemical structure to each other. Trypan red is a sulphonated dis-azodye and resembles trypan blue very much in chemical structure. Methyl orange is a sulphonated mono-azo dye and is mostly related to DAB in chemical structure. From these aspects, it may be suspected that these mono- and dis-azo dye have some effect on the experimental production of liver cancer by DAB.

The present study was undertaken to determine whether trypan red and methyl orange have an inhibitory effect on the tumor incidence by DAB under approximately comparable conditions as when trypan blue is administrated.

METHODS

Albino rats weighing from about 80 to 100 g were given DAB orally and either trypan red or methyl orange subcutaneously. DAB dissolved in olive oil was mixed with the basic diet in the proportion of 0.06 per cent and fed to the rats for the 120 experimental days and thereafter DAB was removed from the basic diet. The basic diet consisted of unpolished rice which was supplemented by green vegetables. The aqueous solution of trypan red or methyl orange was injected subcutaneously into the back of the experimental animals, each in an amount of 1 ml of a 1 per cent aqueous solution of the dye; the injections were made every two weeks from the first week of experiment and continued until a total dose of 60 mg of dye had been given. Besides the experiment mentioned above, the control animals were given DAB alone without receiving the injections of trypan red or methyl orange. The experimental animals were killed between the 120th and 150th experimental days and the incidence of liver cancer of the group injected with trypan red or methyl orange was compared with the controls on the basis of histological examinations.

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RESULTS

A. Control group: This group consisted of 26 rats, 11 males and 15 females, which were fed DAB without any other treatment. Through the course of experiment, they lost weights during the first 3 or 4 weeks, thereafter regained and increased by about 50 to 70 g over their starting weights towards the termination of experiment. Within 2 months of experiment, two rats died of hepatic insufficiency plus pneumonia and they were discarded from the data of present study. All the other rats survived over the 4 months of experiment. On the 120th day after the beginning of experiment, 8 rats, 4 males and 4 females were killed and histological examinations of the liver were performed. Liver cancer was found in one case macroscopically and in four cases microscopically. Therefore, the tumor incidence at this stage was 62.5 per cent (five out of eight cases examained). After 4 months 2 rats died and the remaining 12 rats were sacrificed on the 150th day. Among these 14 cases liver cancer was noticed in 4 cases macroscopically and 9 cases were detected by histological examination. Therefore tumor formation occurred in 13 out of 14 cases (tumor incidence 92.9%).

Detailed data on the macroscopical and histological findings of the liver at 4 and 5 months will be presented in a separate publication.[5]

B. Trypan red group: This experimental group started with 15 rats, 9 males and 6 females. Their general condition was worse than in the control group and 7 cases died mainly due to respiratory infections by the 140th day when the remaining 8 rats were killed.

The body weight did not generally increase as in the control and even at the termination of experiment only about 20 g in average increase than their starting weight was noted. This may suggest the toxicity of trypan red. Macroscopically the liver was diffusely stained red in colour occasionally intermingled with yellowish flat lesions of irregular shape. Generally pathological changes of the liver were slight although some of them showed yellowish nodules devoid of red tinge or early signs of cirrhosis (Fig. 1). Macroscopically liver cancer was not noticed in all cases and detected in only one case by histological examination.

Thus the tumor incidence amounted to 12.5 per cent on the 140th experimental day (on out of 8 cases). As mentioned above, in the control group, the tumor incidence on the 120th and 150th days amounted to 62.5 and 92.9 per cent respectively. This fact indicates that trypan red shows an inhibitory effect on the experimental production of liver cancer by DAB. The data on the number of cases, survival, average body weight, liver ratio and tumor formation are summarized in Table 1.

Histological liver changes were examined for the types of tumors (hepatoma, cholangioma, mixed) as well as the precancerous benign changes (cirrhosis, nodular hyperplasia, cholangiofibrosis). The details of the interpretation of these classifications will be described and illustrated in a separate publication.[5] As Table 2 shows, generally the histological changes were slight. It may be note-

worthy that trypan red did not produce such a characteristic proliferation of histiocytic cells as is seen in the trypan blue treatment in spite of the close resemblance of both dyes in chemical structure.

TABLE 1. Response of Rats Fed DAB Treated with Trypan Red and Methyl Orange (I)

	Trypan red group	Methyl orange group
Number of caes (sex)	8: 5♂, 3♀	9: 7♂, 2♀
Survival	8/5 (53.3%)	9/10 (90.0%)
Average body weight	108	154
Average liver ratio	6.3	6.4
Tumor formation		
Massive tumor	0	3
Small tumor	1	1
Total	1 (12.5%)	4 (44.4%)

TABLE 2. Response of Rats Fed DAB Treated with Trypan Red and Methyl Orange (II)
Histological Liver Changes

	Trypan red group	Methyl orange group
Types of tumors		
Hepatoma	0	1
Cholangioma	0	0
Mixed	1	3
Total	1	4
Benign changes		
Cirrhosis		
Mild	6	4
Moderate	2	4
Nodular	0	1
Islet-like	0	0
Total	8	9
Nodular hyperplasia		
Mild	6	2
Extensive	0	4
Total	6	6
Cholangiofibrosis		
Mild	0	3
Extensive	0	0
Total	0	3

C. Methyl orange group: This consisted of 10 rats, 7 males and 3 females. The general condition of the experimental animal was rather better than in the controls: The rats of this group showed considerable gain in weight (80–90 g) up to the 120th experimental day when the animals were killed for the comparison of liver cancer development with the controls. Three out of 9 cases had

grossly massive tumor of the liver and 1 case, microscopical small tumor (tumor incidence, 44.4 per cent).

The data on the number of cases, survival, average body weight, liver ratio, tumor formation and those on the histological changes of the liver are shown in Tables 1 and 2 respectively. From these data, it was found that the difference between this group and the control was not so significant in many respects and it may be concluded that methyl orange exerts no definite effect on the production of liver cancer by DAB.

DISCUSSION AND SUMMARY

Recently, the author has suggested that the proliferation of the reticuloendothelial cells of the liver following injections of trypan blue may have some bearing on the inhibitory mechanism for the production of liver cancer[3]. However, it may not seem essential from the fact that trypan red having a closely related chemical structure to trypan blue shows a similar inhibitory effect without producing the histiocytic response of the liver. At any rate, the inhibitory mechanism of both trypan blue and trypan red may be based on the common factors of their chemical structures.

The interaction of methyl orange and DAB seemed to be somewhat interesting in the respect that the former is a sulphonated azo dye of the latter but noticeable results were not obtainable.

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EXPLANTATION OF FIGURES

FIG. 1. Livers of trypan red group at 140 experimental days.
FIG. 2. Livers of methyl orange group at 120 experimental days.

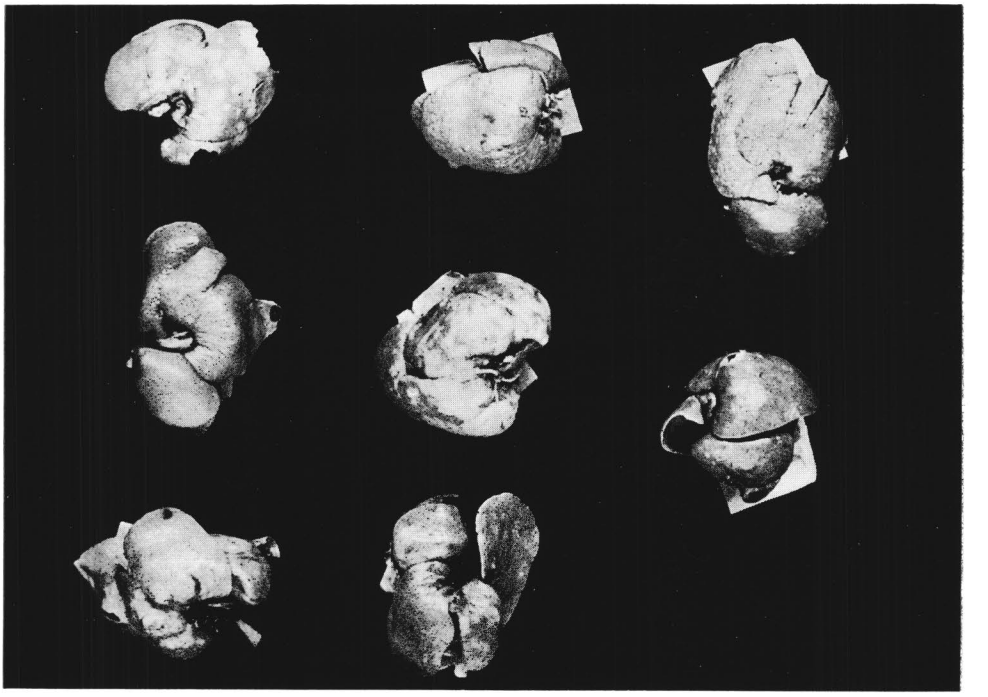

FIG. 1

FIG. 2