The Relation between Experimental Liver Carcinoma and Liver Cirrhosis Induced by Simultaneous Administration of p-Dimethylaminoazobenzene and Carbon Tetrachloride*  
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The effect of simultaneous administration of p-dimethylaminoazobenzene (D.A.B.) and carbon tetrachloride (CCl₄) was experimentally studied on rats.  
1) The morphological changes in the early stage of simultaneous administration of D.A.B. and CCl₄ were characterized by an earlier appearance of cholangiofibrosis or of liver cirrhosis than in those produced by D.A.B. or CCl₄ alone.  
2) Simultaneous administration of both agents invariably resulted in statistically significant higher incidences of liver carcinoma, liver cirrhosis, liver carcinoma with liver cirrhosis, and nodular hyperplasia than the administration of D.A.B. alone.  
3) There was no histological difference between the liver carcinomas caused by combined use of D.A.B. and CCl₄ and those by the administration of D.A.B. alone.  
4) CCl₄ caused a decrease in protein-bound azo-dye in the liver in the course of carcinogenesis by D.A.B.  
5) The mechanism of the activity of CCl₄ in the development of D.A.B.-induced liver carcinoma was discussed on the basis of the relation between the incidence of liver carcinoma and the amount of protein-bound azo-dye in the liver.  

In regard to the relation between liver carcinoma and liver cirrhosis, the following possibilities have hitherto been discussed,¹,² namely: 1) Liver cirrhosis develops first, and then induces liver carcinoma; 2) liver carcinoma grows first, and then causes liver cirrhosis; 3) liver carcinoma and liver cirrhosis can be produced simultaneously, or the one closely follows the other possibly as a result of one and the same cause; and 4) both liver carcinoma and liver cirrhosis appear independently but simultaneously in the same liver.  
The first and third possibilities are generally considered most plausible. In Japan where liver carcinoma without liver cirrhosis is observed more frequently than in Western countries, the third can be regarded as a fairly important one.³ However, it is not easy to evaluate these possibilities in the human subjects.  

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* Studies on the relation between experimental liver carcinoma and liver cirrhosis I.  
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The present experimental work was carried out to elucidate the relation between liver carcinoma and liver cirrhosis. Namely, p-dimethylaminoazobenzene (D.A.B.) and carbon tetrachloride (CCl₄) were administered to rats simultaneously or successively, and various effects of these compounds upon the rat's liver were examined.

It is widely known that some carcinogenic or non-carcinogenic substances, when used together with D.A.B., cause acceleration or suppression of carcinogenesis by D.A.B. As to the carcinogenic activity of CCl₄, the development of liver carcinoma by CCl₄ in mice and hamsters has been reported, but not in rats.

In Report 2 where CCl₄ was administered following D.A.B. feeding, it was possible for the author to demonstrate that CCl₄ works as a promoting factor in the process of carcinogenesis by D.A.B. In the present experiments, D.A.B. and CCl₄ were administered simultaneously, and histopathological features of their effect on the carcinogenesis in reference to the amount of protein-bound azo-dye in the liver were studied in order to make clear the relation between liver carcinoma and liver cirrhosis.

**MATERIALS AND METHODS**

One hundred and two hybrid rats, all 4 months old and weighing from 145 to 160 g, were divided into the following 3 groups.

(1) Group 1, consisting of 41 rats fed solely on a D.A.B. containing diet.
(2) Group 2, consisting of 20 rats injected with CCl₄ and fed on the basal diet.
(3) Group 3, consisting of 41 rats fed on a D.A.B. containing diet and injected with CCl₄ simultaneously.

The composition of the basal diet, vitamin mixture and salt are shown respectively in Tables 1, 2 and 3.

D.A.B. was mixed in 0.045 per cent with the basal diet and was permitted ad libitum.

<table>
<thead>
<tr>
<th>TABLE 1. Composition of basal diet</th>
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<tr>
<td>Alpha-starch</td>
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<td>Oil</td>
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<td>Salt mixture</td>
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<table>
<thead>
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<th>TABLE 2. Composition of vitamin mixture</th>
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<tr>
<td>Thiamine hydrochloride</td>
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<td>Nicotinic acid</td>
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<tr>
<td>Calcium pantothenate</td>
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<tr>
<td>Pyridoxine hydrochloride</td>
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<tr>
<td>Menadione</td>
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<tr>
<td>Folic acid</td>
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<td>Biotin</td>
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<td>Inositol</td>
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<td>Ascorbic acid</td>
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<td>Lactose</td>
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</table>
Ten per cent solution of CCl₄ in olive oil was injected subcutaneously twice a week in 0.1 ml/100 g body weight. Riboflavin was contained in 0.02% in the vitamin mixture, and choline chloride in 0.30%.

During the first 7 weeks, 3 rats from Groups 1 and 3 respectively were killed every week, some for histological investigation, but most of them for the quantitation of protein-bound azo-dye in the liver. For this purpose, the author employed Taki-Miyaji's method, a modification of Miller's method, making some further modifications in accordance with Ward's method.

The whole experiment required 24 weeks, and the animals were killed, and histopathological investigation was carried out. To fix the liver, 10% formalin and absolute alcohol were used, and hematoxylin-eosin stain, Bielschowsky's lattice fiber stain, periodic acid-Schiff's stain, and Sudan III stain were applied to histological sections.

**RESULTS**

1) **Average intake of D.A.B. in Groups 1 and 3**

Between Groups 1 and 3 there was no remarkable difference in D.A.B. intake during the early stages of the experiment (Table 5), but in the 24th week (Table 4), D.A.B. intake in Group 3 was much lower than that of Group 1.

2) **Increase of body weight**

Fig. 1 shows the increase in the average body weight in Groups 1, 2, and 3 from the
Fig. 1. Graph of average body weight in Groups 1, 2 and 3.

1st to the 24th weeks. The increase in weight was most remarkable in Group 2, a little less in Group 1 and the least in Group 3.

3) Histopathological observations

a) Histopathological changes in the early period (from the 1st to the 7th weeks) of experiment

The 1st week: In Group 1 multiplication of large mononuclear cells and of lymphocytes was observed in portal triads. Small fat globules were noticed here and there in the protoplasm of liver cells in the lobular periphery (Fig. 3). In Group 3, the above-mentioned changes were quite remarkable and in addition to them, fatty metamorphosis or necrosis of liver cells was observed rather in the central zones of liver lobules than in the peripheral zones (Fig. 4). Especially, cholangioles consisting of small oval cells were found in large numbers near the peripheral zones of the lobules (Fig. 5).

The 2nd week: In Group 1 cholangioles had begun to proliferate in peripheral zones of hepatic lobules and groups of lymphocytes were observed here and there in the middle zones (Fig. 6). In Group 3 these changes were much more conspicuous, and a large number of liver cells with mitotic figures were found (Fig. 7).

The 3rd week: In Group 1 fatty metamorphosis was observed in every part of hepatic lobules, and the protoplasm of liver cells in the peripheral zones of the lobules proved to be acidophilic (Fig. 8). Small regenerative liver cells with nuclei rich in chromatin were also found. In Group 3, besides the above-mentioned changes, cholangioles were found extending from the peripheral to the middle zones, trabeculas of the liver cells and lobular structures had begun to collapse (Fig. 9), and many mitotic figures of liver cells were observed.

The 4th week: In Group 1, the proliferation of cholangioles was much more remarkable than in the 3rd week, and they had extended from the peripheral to the middle zones of lobules, surrounding a small number of liver cells (Fig. 10). In Group 3 these changes were even more striking, and not only the mitoses of liver cells but also irregular-sized nuclei were prominently observed (Fig. 11).

The 5th week: In Group 1 fat globules were seen scattered from the central to the middle zones, and the cholangioles were extending from the peripheral zones. The changes were more remarkable here than in the 4th week. In Group 3, many cholangioles were found scattered diffusely all over the liver lobules (Fig. 12), and in other parts degenerative and
regenerative foci were co-existing mixedly. As compared with Group 1, the regeneration in the peripheral zones, mitoses and nuclear atypism of liver cells were more remarkable.

The 6th week: In Group 1, lobular structures had apparently collapsed in some lobules, and fat globules were noticed in the persisting lobules (Fig. 13). In Group 3 the histological expressions of degeneration were a little less remarkable than in the 5th week, and hepatic lobules were surrounded by proliferating cholangioles (Fig. 14), most of them forming pseudolobules and partly nodules (Fig. 15).

The 7th week: In Group 1, cholangioles were proliferating surrounding a small number of liver cells, and looked as if they were constituting pseudolobules at first sight (Fig. 16). No fibrosis was present (Fig. 17). The rest of the liver cells contained fat globules. In Group 3 cholangiofibrosis was observed (Fig. 18) and collagenous fibers accompanying cholangioles had greatly increased in number. Pseudolobules were already distinct. They were either multilobular or monolobular, and were partly forming nodular protrusions on the hepatic surface.

As mentioned above, Group 3 invariably showed severer histopathological changes than Group 1 every week, and these lesions were aggravated with the lapse of time. In other words, the degeneration of liver cells and the increase of cholangioles as well as that of stromas were quite notable in Group 3 as compared with Group 1. Consequently, liver cirrhosis or cholangiofibrosis were quite distinctly recognized in their initial stages.

These histopathological differences observed from the 1st to the 7th weeks between Group 1 and Group 3 are classified and arranged in Table 6.

b) Histopathological observations in the 24th week

The animals that were kept alive till the 24th week and then killed — 18 rats in Group 1, 20 in Group 2 and 18 in Group 3 — were compared with one other and studied with special attention to the following points.

Cyst (Fig. 25). Macroscopically many cysts were observed on the surface of the liver, and especially in a large number in the outer parts. Microscopically, these cysts were neither compressing nor invading the surrounding liver tissues. They were surrounded by connective tissues, frequently associated with cholangiofibrosis, and some of their lumens were filled with mucinous substance. The lining of the cysts were made of a single layer of epithelial cells, and some of them were cuboidal, while others were squamous in shape. This observation is in perfect accord with that presented by Stewart.14

Cholangiofibrosis (Figs. 26 and 27). Macroscopically, all the foci of cholangiofibrosis were found more or less sunken below the level of adjacent liver tissues. They were solid, white in color and of various sizes, some were large enough to occupy a whole liver lobe, but others are so small that they could only be microscopically recognized. Histologically, a single row of epithelial cells were lining glandular structures, which were circumscribed by connective tissues. The glandular structures were various in their forms; some were round, others crescent-like or spray-shaped. In many glandular epithelial cells the protoplasm was stained light-blue by hematoxylin-eosin. Their nuclei were generally clear, but some of them contained a large amount of chromatin. The stromas of the glandular structures were composed of immature connective tissues with infiltration of round cells. In the glandular cavity mucinous substance was found, and necrosis of glandular epithelial cells and round cell infiltration were also observed in some places. Mucinous substance was noticed in the protoplasm of glandular epithelial cells. This observation is in perfect agreement with that of Opie15 presented in his report of 'cholangiofibrosis'.

Nodular hyperplasia. Some nodular hyperplasias were already macroscopically nodular
<table>
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<tr>
<th>Group</th>
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<th>3</th>
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<th>5</th>
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<th>7</th>
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<tr>
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and tumor-like, but others were too small to the naked eye and were recognized only microscopically. The macroscopically visible ones were projecting in nodular shapes above the level of surrounding tissues on the surface of liver. They were forming larger nodules in the liver tissues than the surrounding liver lobules or pseudolobules. They were quite plainly distinguishable from the surrounding liver tissues on account of their different color. This kind of nodular hyperplasias was found in the lobules of non-cirrhotic livers all over, in a part of lobules of non-cirrhotic liver, in all or in a part of the pseudolobules of cirrhotic livers (Fig. 28). They were multicentric and their distribution was not confined to any particular places. Microscopically, the trabeculas of liver cells in pseudolobules were mostly composed of more than two cells and had greatly increased in number, evidently compressing the surrounding trabeculas of the liver cells. The liver cells that constituted these kinds of nodules were polygonal, large, stained in deep color, and contained a lot of chromatin in their nuclei, compared with the liver cells outside the nodules. The protoplasm of liver cells in the nodules contained glycogen. Ort16 said, referring to the differences between the nodules of benign regenerative liver cells and early trabecular hepatocellular carcinomas, that carcinoma cells contained no glycogen. Edwards and White7 also stated that no glycogen was detected in primary hepatocellular carcinoma or in its intraeutaclaneous transplants. But it is quite interesting that Stewart14 and Kinosita18 admitted that glycogen was detected in some kinds of carcinoma.

Liver cirrhosis and liver fibrosis. Histologically, the necessary conditions of liver cirrhosis are firstly that remodeled liver lobules are present, and secondly that fibrous connective tissues are increased. On the findings obtained by Mallory’s azan stain, the author apply the term of liver cirrhosis (Figs. 34 and 35) only when increased fibers are arranged in perfectly annular shape around pseudolobules, and used the name of liver fibrosis (Figs. 32 and 33) when the fibers are extending around the Glisson’s sheath or central vein, and perfect lobules or pseudolobules are not circumscribed by fibers.

Liver carcinoma. Many histological classifications of D.A.B.-induced liver carcinomas have been attempted to date. However, these traditional classifications have to be reexamined fundamentally,15,16,19-21 now that many investigators admit of H. Miyaji’s24 opinion that hepatocellular carcinoma and cholangiocellular carcinoma can be transformed into each other, as ascertained with transplantable liver carcinoma under various conditions. Therefore, the author will only tentatively classify the liver carcinoma into two groups, hepatocellular carcinoma (Fig. 37) and cholangiocellular carcinoma (Fig. 41), and then call liver cell cancer with the evidence of transition ‘mixed carcinoma’.

(A) Hepatocellular carcinoma: Hepatocellular carcinoma is made of tumor cells which are arranged in trabecular (Fig. 39), solid (Fig. 38) or glandular (Fig. 40) shapes. In trabecular and solid forms, sinusoids are present among the trabeculas as well as among the solid masses of tumor cells. Little or no bleeding is observed. As regards glandular ones, connective tissue stromas are recognized in some of them, but other trabecular or solid hepatocellular carcinomas may partly form glandular cavities (Fig. 40). The tumor cells are of various kinds, ranging from those in which atypism, mitoses, increased nuclear chromatin and infiltrative growth are clearly demonstrated as in ordinary carcinoma cells, to those in which only slight histological signs of malignancy was demonstrable as compared with the cells constituting benign nodular hyperplasia. The malignant features, such as atypism, mitoses, increased nuclear chromatin and infiltrative growth are more remarkable in solid hepatocellular carcinomas than in trabecular ones. The differentiation between glandular hepatocellular carcinoma and cholangiocellular carcinoma is to be dealt with later on the basis of the following three points.

1) Compared with cholangiocellular carcinoma, glandular hepatocellular carcinoma has less stroma, and less pronounced infiltration of round cells. Sinusoid-like structures are recognized on exact histological examination.

2) No papillary proliferation is noticed, and tumor cells themselves have much greater resemblance to liver cells.
3) When a digestion test with diastase is made prior to periodic acid Schiff's stain, mucus productivity as Firminger called it cannot be demonstrated.

(B) Cholangiocellular carcinoma: Cholangiocellular carcinoma shows glandular structures of various sizes, among which the stroma of connective tissue nature is recognized with infiltration of round cells.

The tumor cells forming glandular cavity are generally arranged in a single sheet, but they are sometimes piled to two or three cell layers and proliferate in papillary shapes. Tumor cells themselves are various in form: some are squamous, some cuboidal and others columnar. Atypism, mitoses and increased nuclear chromatin are distinctly observed and signs of infiltrative growth are also seen in many of them. The protoplasm of tumor cells is found basophilic. A histological classification is attempted based on the above-mentioned standards, as is shown in Table 7.

**Table 7.** Histological changes in the last week (24th week)

<table>
<thead>
<tr>
<th>Histological changes</th>
<th>Group 1</th>
<th>Group 2</th>
<th>Group 3</th>
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<tbody>
<tr>
<td>Number of rats</td>
<td>18</td>
<td>20</td>
<td>18</td>
</tr>
<tr>
<td>Cyst</td>
<td>13</td>
<td>0</td>
<td>18</td>
</tr>
<tr>
<td>Cholangiofibrosis</td>
<td>15</td>
<td>0</td>
<td>16</td>
</tr>
<tr>
<td>Nodular hyperplasia</td>
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<td>0</td>
<td>11</td>
</tr>
<tr>
<td>Liver fibrosis</td>
<td>10</td>
<td>9</td>
<td>10</td>
</tr>
<tr>
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<td>11</td>
<td>8</td>
</tr>
<tr>
<td>Liver carcinoma</td>
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<td>13</td>
</tr>
<tr>
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<td>7</td>
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<tr>
<td>Cholangiocellular type</td>
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<td>8</td>
</tr>
<tr>
<td>Mixed type</td>
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<tr>
<td>Liver carcinoma with liver cirrhosis</td>
<td>0</td>
<td>0</td>
<td>13</td>
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**Table 8.** The differences in the incidence of some pathological findings between Groups 1 and 3 examined by the chi-square test

<table>
<thead>
<tr>
<th>Pathological finding</th>
<th>Group 1</th>
<th>Group 3</th>
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<tbody>
<tr>
<td>Cyst</td>
<td>Significant (below 1%o)</td>
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<tr>
<td>Cholangiofibrosis</td>
<td>Not significant (above 5%o)</td>
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</tr>
<tr>
<td>Nodular hyperplasia</td>
<td>Significant (below 1%o)</td>
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<tr>
<td>Liver cirrhosis</td>
<td>Significant (below 1%o)</td>
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<tr>
<td>Liver carcinoma</td>
<td>Significant (below 5%o)</td>
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<tr>
<td>Liver carcinoma with liver cirrhosis</td>
<td>Significant (below 1%o)</td>
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</table>

Comparing Group 1 and Group 3, the incidence of cyst, cholangiofibrosis, nodular hyperplasia, liver cirrhosis, liver carcinoma, and liver carcinoma with liver cirrhosis were examined by the chi-square test, respectively, and the results are given in Table 8.

Compared with Group 1, Group 3 shows a significantly higher incidence in such items as liver carcinoma, liver cirrhosis, and liver carcinoma with liver cirrhosis. but as for the histological pattern of liver carcinoma developing in such livers, no significant correlation is confirmed.

*Amount of protein-bound azo-dyes in the liver.* In regard to this, Group 3 constantly showed a downward trend compared with Group 1, as is seen in Fig. 2.
Both Groups 1 and 3 have a trait of showing two peaks in the shift of azo-dye contents with time. The former group had the lower peak in the 2nd week and the higher in the 6th week, while the latter had the lower one in the 2nd, and the higher one in the 4th.

**DISCUSSION**

The present experiment shows that simultaneous administration of D.A.B. and CCl$_4$, as compared with that of D.A.B. alone, invariably brings about higher incidences of liver carcinoma, liver cirrhosis, liver carcinoma with liver cirrhosis, nodular hyperplasia and cholangiofibrosis, from both histopathological and statistic points of view, in spite of rather small average intake of D.A.B. Moreover, the histopathological changes in the early period of combined use of D.A.B. and CCl$_4$ clearly indicated a positive correlation between the aggravation of liver cirrhosis and the rise in the incidence of liver carcinoma. In the first week of the experiment, there was little or no histological change in the group given D.A.B. alone. In the group of simultaneous D.A.B. and CCl$_4$ administration, however, not only localized centrilobular necrosis of liver cells but also proliferation of cholangioles was observed in the peripheral zones of the lobules. The former change could be induced by D.A.B. alone. The latter was caused neither by D.A.B. nor CCl$_4$, when these agents were given separately. From the 2nd to 7th weeks, the proliferation of cholangioles was more remarkable in the group given D.A.B. and CCl$_4$ simultaneously than in the group of D.A.B. alone. Similar phenomena, however, are known to occur when D.A.B. is administered simultaneously with tannic acid, thioacetamide, or ethionine, and it is well known that in each case a higher incidence of liver carcinoma is observed. Furthermore, concerning the histopathological observations of liver carcinomas, neither in the hepatocellular type nor in cholangiocellular type nor in mixed type was there any significant difference between the group of simultaneous D.A.B. and CCl$_4$ and the group of
D.A.B. alone.

On the other hand, liver cirrhosis was not induced in the group of D.A.B., and only a remarkable proliferation of cholangioles was noticed with a slight increase of collagenous fibers. In the group dosed with D.A.B. and CCl\textsubscript{4} simultaneously, liver cirrhosis was noticed in the 6th week, and some nodular hyperplasias in the form of pseudolobules were found in the 7th week. In this group the proliferation of bile ducts was much more remarkable, and cholangiofibrosis was found in earlier stage of experiments than in the group dosed with D.A.B. alone. This result was in accord with the report of Sutton\textsuperscript{25} However, the incidence of the development of liver carcinoma from liver cirrhosis was strikingly higher in the group of simultaneous administration of D.A.B. and CCl\textsubscript{4} than in the group of D.A. B. alone, and in this connection the results obtained by Sutton\textsuperscript{25} and Maltoni\textsuperscript{26} are not contributory, as will be explained later.

The activity of CCl\textsubscript{4} as a factor accelerating carcinogenesis with D.A.B. was indicated by a higher incidence of liver carcinoma in the group of simultaneous D.A. B. and CCl\textsubscript{4} administration than in the group of D.A.B. alone, in spite of the remarkable decrease of protein-bound azo-dye in the liver under the former experimental condition. This experimental result obviously disagrees with 'protein-depletion' asserted by Miller\textsuperscript{27} Similar results were obtained in experiments of simultaneous administration of D.A.B. and tannic acid\textsuperscript{22} or of D.A.B. and ethionine.\textsuperscript{25} Carcinogenic action of tannic acid\textsuperscript{28} or ethionine\textsuperscript{29,30} being well known, possible carcinogenic effect of CCl\textsubscript{4} ought to be carefully pursued also, though such an activity of CCl\textsubscript{4} on rats has never been mentioned in the literature. The author will demonstrate the effect of CCl\textsubscript{4} as a promoting factor of carcinogenesis in Report 2,\textsuperscript{4} where CCl\textsubscript{4} is given to rats following D.A.B. administration. Now, no combination of CCl\textsubscript{4} and 'slow-h\textsubscript{2}' protein having been proved, a different approach will be necessary to find out the reason for the decrease of protein-bound azo-dyes when D.A.B. and CCl\textsubscript{4} are given simultaneously. According to Miller's theory,\textsuperscript{27} the decrease in the amount of protein-bound azo-dyes means a depletion of 'slow-h\textsubscript{2}', a special protein that combines with D.A.B., and the deficiency of this protein is considered to play some role in carcinogenesis. The amount of protein-bound azo-dyes during the first several weeks is supposed to allow prediction of the incidence of liver carcinoma.\textsuperscript{12,27} In the present experiments, however, the incidence of liver carcinoma was found very high, although the protein-bound azo-dye was rather small in quantity, and this result is clearly opposed to the above-mentioned Miller's theory.\textsuperscript{27} The author would like to advance the following hypothesis to explain this result.

1. CCl\textsubscript{4} hinders or delays the protein synthesis in the liver.
2. CCl\textsubscript{4}, as well as D.A.B., combines with 'slow-h\textsubscript{2}', which is a specific protein fraction.

As regards (1), it is well known that D.A.B. combines with protein in ribosome\textsuperscript{31} in the course of its synthesis, but not with pre-existing protein. In the present experiment, CCl\textsubscript{4} caused morphologically centrilobular degenerative changes or
necrosis, which suggested hampered or delayed protein synthesis in the liver, and consequently a decrease in the quantity of protein-bound azo-dyes. As to (2), when both CC14 and D.A.B. combine with the specific protein fraction 'slow-h2', decrease in the amount of protein-bound azo-dyes is to take place even though protein synthesis proceeds normally. It goes without saying that the present hypothesis implies the possibility of carcinogenesis by CC14.

In this experiment, however, no liver carcinoma was found in the group given CC14 alone. Nor is there any literature to date in which the development of liver carcinoma in the rats given CC14 alone is described. In view of the inhibitory action of CC14 on the formation of protein-bound azo-dye and the increased incidence of D.A.B.-induced liver carcinoma by CC14 as mentioned above, a consideration about the mechanism of serious influence of CC14 on carcinogenesis with D.A.B. is necessary.

On the assumption that the process of the development of D.A.B.-induced liver carcinoma is constituted by a series of different steps and the incidence of liver carcinoma depends on the degree of the change at the final metabolic step, CC14 has presumably a mechanism in common with D.A.B. at some steps excepting the final step. On account of this common mechanism CC14 can indirectly bring about an increase in the incidence of liver carcinoma at the final stage of carcinogenic process. In the present experiment the administration of CC14 alone could not induce liver carcinoma, because CC14 had apparently no mechanism in common with D.A.B. at the final step of the development of D.A.B.-induced liver carcinoma. However, the above-mentioned explanation is possible only under limited circumstances where the metabolism of these two substances administered are not subjected to mutual interference. It must be admitted that the changes in some steps of metabolism can be augmented or even the metabolic pathway itself may be influenced by the simultaneous administration of these two agents.

It was proved by means of a quantitative determination of protein-bound azo-dye that the simultaneous administration of CC14 induced a remarkable decrease in the dye. According to Miller's principle, a decrease in protein-bound azo-dye should bring about a reduction in the rate of development of D.A.B.-induced liver carcinoma. The increase in the incidence of liver carcinoma observed in the present experiment by the simultaneous administration of CC14 could not be due to the intensification of the mechanism in the development of D.A.B.-induced liver carcinoma by CC14, because an exaggeration of carcinogenic mechanism of D.A.B. would be possible only with an increase of protein-bound azo-dye.

In this connection, Sutton25 once made a report of his experiment with D.A.B. and CC14 to the effect that the simultaneous dosage of D.A.B. and CC14 caused more remarkably proliferation of bile ducts as compared with that of D.A.B. alone. In spite of early appearance of cholangiofibrosis, there was no difference in the changes of liver cells nor in the incidence of liver carcinoma. Neither did Miller32 and Sutton25 notice any difference in the incidence of liver carcinoma in their
experiment with D.A.B. and CCl4. They both used D.A.B. in a concentration of 0.06%, while the author used it at a rate of 0.045%, and this difference in D.A.B. concentration caused perhaps the difference in the results. In the author’s opinion, however, in order to examine the accelerating effect of a certain factor in the carcinogenesis by D.A.B., D.A.B. itself ought to be employed in the minimum effective amount, since the influence of the agent is elicited most clearly under this condition. In the experiments conducted by Sutton,26 for instance, D.A.B. was used in a rather high concentration, and therefore its strong potency of carcinogenesis might have obscured rather weak effect of CCl4 in carcinogenesis. On the other hand, Maltoni and Prodie27 admit of the rise in the incidence of carcinoma as a result of simultaneous use of CCl4 with D.A.B., but unfortunately their reports do not include a histopathological study and they make no reference to possible carcinogenesis by CCl4 or to its accelerating activity to carcinogenesis.

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References


Legends

Figs. 3-18. Microscopic observations in the early period (from the 1st to the 7th weeks).

Fig. 3. Group 1. 1st experimental week. Multiplication of large mononuclear cells and of lymphocytes in Glisson’s sheath is observed. Small fat-globules are noticed in the protoplasm of liver cells in the perportal area. H-E. × 250.

Fig. 4. Group 3. 1st experimental week. Fatty metamorphosis or necrosis of liver cells is observed in the central zones of liver lobules. H-E. × 250.

Fig. 5. Group 3. 1st experimental week. Cholangioles are found in a large number in the peripheral zones of the lobules. H-E. × 250.

Fig. 6. Group 1. 2nd experimental week. Cholangioles have begun to appear in peripheral zones of lobules and groups of large lymphocytes are observed here and there in their middle zones. H-E. × 250.

Fig. 7. Group 3. 2nd experimental week. A great number of liver cells with mitotic figures are found. H-E. × 500.

Fig. 8. Group 1. 3rd experimental week. Fatty metamorphosis is observed in every part of the lobules. The protoplasm of liver cells in the peripheral zones of the lobules proved to be acidophil. H-E. × 100.

Fig. 9. Group 3. 3rd experimental week. Trabeculas of liver cells and lobular structure have begun to collapse. H-E. × 100.

Fig. 10. Group 1. 4th experimental week. Proliferation of cholangioles surrounding one or few liver cells. H-E. × 1,000.

Fig. 11. Group 3. 4th experimental week. Not only mitosis of liver cells but also anisokaryosis is prominently observed. H-E. × 500.

Fig. 12. Group 3. 5th experimental week. All over the liver lobules are found many sections where cholangioles are diffusely distributed. H-E. × 100.

Fig. 13. Group 1. 6th experimental week. The lobular structure has apparently collapsed in some lobules and in the remaining lobules fatty globules are seen. H-E. × 100.

Fig. 14. Group 3. 6th experimental week. Liver lobules are surrounded by cholangioles. H-E. × 100.

Fig. 15. Group 3. 6th experimental week. Most of the liver lobules are transformed to pseudolobules and partly make nodules. H-E. × 100.

Fig. 16. Group 1. 7th experimental week. Liver lobules simulate pseudolobules at first sight. H-E. × 100.

Fig. 17. Group 1. 7th experimental week. Collagenous fibers are not increased. M-A. × 100.

Fig. 18. Group 3. 7th experimental week. Cholangiofibrosis. H-E. × 250.

Figs. 19-24. Macroscopic observations in the last week (24th week).

Fig. 19. Group 1. Liver surface is rough.

Fig. 20. Group 1. Liver carcinoma without liver cirrhosis.

Fig. 21. Group 2. Liver fibrosis.

Fig. 22. Group 2. Liver cirrhosis.

Fig. 23. Group 3. Liver cirrhosis.

Fig. 24. Group 3. Liver carcinoma with liver cirrhosis.
Figs. 25–41. Microscopic observations in the last week (24th week).

Fig. 25. Cyst with cholangiofibrosis. H-E. ×250.

Fig. 26. Cholangiofibrosis. H-E. ×250.

Fig. 27. Cholangiofibrosis. H-E. ×500.

Fig. 28. Group 3. Nodular hyperplasia is found in a part of pseudolobules. H-E. ×100.

Fig. 29. Enlargement of a part of Fig. 26. These liver cells that constitute these kinds of nodules are polygonal, large, stained deep in color, and contained a lot of chromatin in their nuclei, when compared with adjacent liver cells. H-E. ×100.

Fig. 30. Group 3. Nodular hyperplasia was found in a pseudolobule all over cirrhotic liver. H-E. ×100.

Fig. 31. Enlargement of a part of Fig. 28. Trabeculas of liver cells are mostly composed of cell cords of more than two cells and had greatly increased in number evidently oppressing the surrounding connective tissues. H-E. ×600.

Fig. 32. Group 1. Liver fibrosis. H-E. ×30.

Fig. 33. Group 2. Liver fibrosis. H-E. ×30.

Fig. 34. Group 2. Liver cirrhosis. H-E. ×30.

Fig. 35. Group 3. Liver cirrhosis with liver carcinoma. H-E. ×30.

Fig. 36. Group 1. Liver carcinoma without liver cirrhosis. H-E. ×30.

Fig. 37. Hepatocellular carcinoma. H-E. ×100.

Fig. 38. Solid hepatocellular carcinoma. H-E. ×100.

Fig. 39. Trabecular hepatocellular carcinoma. H-E. ×500.

Fig. 40. Glandular hepatocellular carcinoma. H-E. ×500.

Fig. 41. Cholangiocellular carcinoma. H-E. ×250.
