Mechanism of Growth

II. The Influence of *p*-Dimethylaminoazobenzene on Rat Liver Regeneration after Partial Hepatectomy*

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SUMMARY

The immediate and prolonged effects of *p*-dimethylaminoazobenzene on the increase in weight, net amount of pentose- and deoxypentosenucleic acids and acid deoxyribonuclease activity in the rat liver after partial hepatectomy have been studied.

A slight depression of the rate of dry weight increase was noted after feeding DAB to the rats for 60 days. Discontinuation of the carcinogen brought about normal conditions. No immediate or prolonged effects of DAB on the rate of PNA formation were recorded. A considerable reduction of the rate of DNA formation was found after only 25 days' administration of DAB. The same reduction was found at 60 days, but a return to normal conditions was noted after discontinuation of the carcinogen. An initial stimulation of the postoperative DNase increase was demonstrated. This stimulation after 25 days' administration of DAB was followed by a depression at 60 days. The DAB-induced reduction of the capacity of the liver cells to respond to a growth stimulus with an increase in the average cellular DNase activity was sustained even after discontinuation of the carcinogen.

Chemical carcinogenesis is considered to be a multi-stage process. The first phase is sometimes referred to as the initiation period (12, 21). Malignant transformation usually occurs after completion of this period, even if administration of the carcinogen is discontinued.

These observations indicate that certain irreversible changes take place rather early during carcinogenesis. These changes affect one or several sectors of the cellular metabolic machinery. They seem to appear before any definite histological or cytological signs of carcinomatous transformation are visible (12, 20).

The acid deoxyribonuclease (DNase) activity in nonmalignant and malignant growth of the rat liver has been studied in earlier investigations (7, 9). It was here shown that nonmalignant growth induced by partial hepatectomy is associated with an increase in the average cellular DNase activity, whereas malignant growth induced by feeding *p*-dimethylaminoazobenzene (DAB) is accompanied by a decrease in this activity.

It has been clearly demonstrated that nonmalignant growth is invariably associated with increased cellular DNase activity levels (5, 7, 9, 10, 11). In studies on the time-relationship between the increase in the average cellular DNase activity and the net amount of deoxypentosenucleic acid (DNA) during normal growth it has been shown that the rise in enzymatic level preceded the net synthesis of DNA (10, 11). Furthermore, the highest levels of DNase activity were associated with the period of most intense DNA net synthesis.

The parallelism between DNase activity and DNA net synthesis demonstrated in normal tissues is apparently lacking in DAB-induced malignant

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growth of the rat liver (7). It would appear that during carcinogenesis irreversible damage has been inflicted on cellular systems concerned with DNase-synthesis.[1]

On the assumption that the cancerous state is characterized by, *inter alia*, an impairment of the DNase-synthesizing capacity of the cell, one would expect that during carcinogenesis this capacity would become gradually impaired. If this be so, it would imply that during precancerous stages certain cellular changes are brought about which are manifested by a gradual decrease and the eventual disappearance of the capacity of the cell to respond to a growth stimulus in terms of an increase in DNase activity.

Experimental proof of this hypothesis requires an organ in which cancer can be induced and which at different precancerous stages can be exposed to a strong growth stimulus. Rat liver is a tissue that lends itself to such studies. This paper presents some results of the effect of partial hepatectomy on rats fed on DAB (16) for various periods. The hepatic response has been measured in terms of increase in weight, nucleic acids, and DNase activity. The experiments were designed with the ntention of studying the early effects of the carcinogen and of ascertaining any long-time effects produced by the short-time administration of the carcinogen.

MATERIALS AND METHODS

Male Sprague-Dawley rats, weighing 280–300 gm., were used. The following diets were given:

Group 1: Purina chow diet (36 rats).

Groups 2 and 3: rice, carrots, and water for 25 and 60 days, respectively (24 and twelve rats, respectively).

Group 4: rice, carrots, and water for 60 days, Purina chow for a further 110 days (twelve rats).

Groups 5 and 6: rice, carrots, and water with the addition of 60 mg. DAB dissolved in olive oil per 100 gm. rice. The groups were fed this diet for 25 and 60 days, respectively (fourteen and sixteen rats, respectively).

Group 7: the same diet as that given to Groups 5 and 6 for 60 days. Purina chow was then fed for a further 110 days (sixteen rats).

Partial hepatectomy was performed according to the method of Higgins and Anderson (15). The operations were carried out between 8:00 and 9:00 A.M. The rats were hepatectomized in groups of two to five animals. Analyses were performed on pooled material from such groups. If several batches of animals treated in the same way were analyzed, the results are given as weighted means.

At various intervals after the operation the rats were anesthetized by ether and killed by bleeding. The livers were immediately removed and frozen on dry ice. After being thawed, the material was homogenized in distilled water for 5 minutes with a Virtis homogenizer at 40,000 r.p.m. The tissue concentration was approximately 10 per cent. Precautions were taken to keep the temperature of the homogenate near to 0° C.

The DNase activities were determined as described earlier (4), in 0.2 M acetate buffer at pH 5.25. Calf thymus DNA prepared according to Hammarsten (14) was used as substrate. The reaction mixtures were incubated with continuous shaking at 37° C. for 30 and 60 minutes. The reaction was stopped by adding trichloroacetic acid-lanthanum acetate (7). After centrifugation, the increase in deoxypentose concentration in the supernatant was determined colorimetrically (3), and from this the amount of degraded DNA (expressed as DNA-phosphorus-DNAP) was calculated. The DNase activities are expressed as μg DNAP liberated/hour/μg tissue DNAP, per mg. dry weight and per total organ.

Pentosenucleic acid-phosphorus (PNAP) and DNAP were determined as described earlier (2, 3, 6).

The water content of the tissues was determined by drying small liver samples to a constant weight at 105° C.

RESULTS

Liver weight.—The increase in the wet and dry weights of rat liver following partial hepatectomy was rather rapid (Tables 1 and 2). In the Purina chow-fed group the dry weight was 136 per cent above that noted for the fraction left after the operation. The influence of the rice-carrot diet was inconsiderable (Groups 2 and 3), and DAB seemed to cause only a slight additional depression in this respect. Discontinuation of the rice-carrot as well as the rice, carrot, and carcinogen diet brought about a return to normal conditions.

Pentosenucleic acid.—The PNA concentration (expressed as mg PNAP/gm dry weight) showed a slight temporary decrease immediately after partial hepatectomy in the Purina chow-fed group (Table 1). After this an increase above initial values, particularly noticeable 48 hours after the operation, was observed. The postoperative decrease was not found in any other group. On the other hand, a rise in the PNA concentration above

[1] The increase in DNase activity following partial hepatectomy is here referred to as due to synthesis of the enzyme. The precise mechanism underlying the recorded rise in enzymatic activity is not known. It may also be brought about by an increase in an activator or a decrease in an inhibitor.

zero-hour values was demonstrated in every group.

Neither the rice-carrot diet nor DAB seemed to have any effect on the rate of PNA net synthesis. After 72 hours the PNA content of the pre-operative liver was completely restored in all groups (Tables 1 and 2).

Deoxypentosenucleic acid.—In all groups partial hepatectomy initially caused a more or less pronounced decrease in DNA concentration (expressed as mg DNAP/gm dry weight). In Group 7, however, the decrease seemed to be insignificant. In some groups this decrease was followed by an increase in DNA concentration (Table 1).

The rice-carrot diet had a considerable influence on the rate of DNA net synthesis. In the Purina chow-fed group the DNAP content per liver at 72 hours was 154 per cent above the zero-hour value (Tables 1 and 3). The protein-poor diet caused a reduction to 93 and 102 per cent in Groups 2 and 3, respectively. When the rats were again put on Purina chow diet, normal conditions in this respect were restored (Group 4). DAB caused an additional decrease in the rate of new formation of DNA. In Groups 5 and 6 the DNAP content per liver at 72 hours was 61 and 57 per cent, respectively, above zero-hour values. Discontinuation of the carcinogen (and the rice-carrot diet) brought about an increased postoperative rate of DNA net synthesis. In Group 7 the 72-hour value was 125 per cent above the zero-hour value, i.e., almost the range observed in Group 1.

Deoxyribonuclease activity.—The DNase activities found in the various groups are summarized in Table 1. The enzymatic activities have been calculated per μg. tissue DNAP, per mg. dry weight, and per total organ. Except in Groups 2 and 6, where a postoperative decrease was found, the

TABLE 1

CHANGES IN WEIGHT, PENTOSE- AND DEOXYPENTOSENUCLEIC ACID CONTENTS, AND DEOXYRIBONUCLEASE ACTIVITY IN REGENERATING LIVER OF RATS FED VARIOUS DIETS FOR VARIOUS PERIODS

Diet	Hours after partial hepatectomy	Dry weight of livers	mg PNAP/gm dry weight	mg DNAP/gm dry weight	DNase/μg tissue DNAP	DNase/mg dry weight
Purina chow	0	1.10 (6)	4.19	0.71	9.4	5.6
	24	1.38 (15)	3.90	0.55	16.4	9.1
	41	2.00 (4)	4.02	0.67	18.0	10.6
	48	2.28 (4)	6.17	0.74	17.1	12.7
	72	2.60 (7)	5.27	0.76	16.1	12.1
Rice, carrots, and water for 25 days	0	0.86 (8)	3.94	0.88	9.9	8.6
	24	1.28 (4)	4.45	0.52	12.6	6.6
	41	1.76 (4)	5.03	0.68	13.0	8.9
	48	1.91 (4)	4.51	0.61	12.1	7.4
	72	1.96 (4)	5.28	0.74	14.7	10.9
Rice, carrots, and water for 60 days	0	1.34 (4)	4.67	0.79	9.7	7.7
	41	2.12 (4)	4.53	0.70	14.7	10.3
	72	2.75 (4)	6.84	0.78	14.4	11.2
Rice, carrots, and water for 60 days, Purina chow for a further 110 days	0	1.31 (4)	4.02	0.73	9.1	6.6
	41	2.34 (4)	4.24	0.67	18.5	12.4
	72	2.99 (4)	5.42	0.77	16.0	12.4
Rice, carrots, water and DAB for 25 days	0	1.30 (4)	4.25	0.86	10.1	8.6
	41	2.18 (5)	4.84	0.65	18.1	11.8
	72	2.69 (5)	6.30	0.67	18.1	12.0
Rice, carrots, water and DAB for 60 days	0	1.26 (4)	4.25	1.07	15.3	16.4
	41	1.90 (6)	5.36	0.88	18.0	15.8
	72	2.46 (6)	5.35	0.86	18.2	15.7
Rice, carrots, water and DAB for 60 days, Purina chow for a further 110 days	0	1.49 (4)	4.39	0.88	12.1	11.5
	41	2.61 (6)	5.45	0.87	14.1	12.2
	72	3.61 (6)	5.70	0.88	13.9	12.2

Figures within parentheses are number of animals investigated.

The zero-hour values are for the liver not removed in hepatectomy. They can be converted to the value for total liver by multiplying by 3.

DNase activity per mg. dry weight showed a more or less pronounced increase throughout the whole period studied.

When the activities were expressed with reference to the tissue-DNAP, a maximum was observed at 41 hours. The increase in enzymatic level, however, varied considerably in the different groups (Tables 1 and 2). In the Purina chow-fed rats the DNase activity/μg DNAP following partial hepatectomy rose to a maximum of 92 per cent above the level found in pre-operative livers. The influence of the rice-carrot diet was considerable. In Groups 2 and 3 the 41-hour enzymatic level was only 31 and 52 per cent, respectively, above the zero-hour values. Return to the Purina chow diet brought about an increase to the normal range in the postoperative response (Group 4, Tables 1 and 2). Addition of carcinogen to the rice-carrot diet seemed initially to stimulate the postoperative increase in DNase activity. The 41-hour value in Group 5 was 79 per cent above the zero-hour value. This increase should be compared with that exhibited in Group 2, which amounted to 31 per cent. Further exposure of the rats to the carcinogen caused spectacular changes in the DNase system. In Group 6 the 41-hour level was only 18 per cent above the zero-hour level. In Group 7, finally, where the rats had been returned to the Purina chow diet, a considerable reduction in the postoperative DNase increase was still noted (compare Groups 4 and 7, Tables 1 and 2).

The DNase activities per total organ are given in Table 2. In all groups there was a progressive increase during the whole experimental period. The postoperative response calculated as the percentual increase at 72 hours above the zero-hour values (Table 2) is 335 per cent in Group 1. The rice-carrot diet caused a considerable reduction, but return to the Purina chow diet restored normal conditions. When DAB was given with the rice-carrot diet, an additional effect, particularly noticeable in Group 6, was caused. Here an increase of only 87 per cent above the zero-hour level was found. Discontinuation of the carcinogen (and rice-carrot diet) did not restore normal conditions as regards the postoperative DNase increase. In Group 7 the rise in DNase activity per total organ was considerably below that noted in the control group (compare Groups 4 and 7, Tables 1 and 2).

DISCUSSION

In a variety of experimentally induced cancers a multi-stage development is discernible (1, 12, 13, 19–21). During the first stage certain cellular changes ultimately leading to cancer are apparently initiated.

TABLE 2

PERCENTUAL INCREASE OVER ZERO-HOUR VALUES

Diet	Dry weight*	PNAP/total organ*	DNAP/total organ*	DNase/μg tissue DNAP†	DNase/total organ*
Purina chow	136	197	154	92	335
Rice, carrots, and water for 25 days	128	206	93	31	187
Rice, carrots, and water for 60 days	105	201	102	52	200
Rice, carrots, and water for 60 days, Purina chow for a further 110 days	128	209	143	103	328
Rice, carrots, water, and DAB for 25 days	107	208	61	79	189
Rice, carrots, water, and DAB for 60 days	95	198	57	18	87
Rice, carrots, water, and DAB for 60 days, Purina chow for a further 110 days	142	193	125	17	158

* 72-hour values.

† 41-hour values.

The completion of this first stage is somewhat rapid. In DAB-fed rats it was found to lie between 30 and 50 days (13). The main characteristics of cells in this stage seemed to be lack of histological or cytological signs indicating malignancy (12, 21) and irreversibility of the cellular changes produced by the carcinogen (12, 13, 21). Discontinuation of the carcinogen may prolong the period of latency before tumor formation but cannot prevent it.

The present investigation has shown that, among the different parameters studied, the only irreversible change induced by the short-time feeding of DAB seems to reside in the DNase-synthesizing system. An early effect of the carcinogen (25 days' feeding) is the stimulation of this system. Further administration of the carcinogen (60 days) causes an impressive decrease in the postoperative DNase response. Rats fed on DAB for 60 days may be considered to have completed the first stage of carcinogenesis (13). A group of such rats was returned to Purina chow diet for 110 days

and thereafter partially hepatectomized. These liver cells are characterized by a considerably reduced capacity to react to the growth stimulus in terms of increased cellular levels of DNase activity. Irreversible damage seems to have been inflicted on the DNase-synthesizing system.

Of particular interest in this connection is the relationship between the postoperative extent and rate of DNA net synthesis and the increase in the average cellular DNase activity (Table 1). The rice-carrot diet brings about an associated decrease in both these metabolic activities. Return to Purina chow diet normalizes the postoperative response. The carcinogen reverses the picture initially. There is initially a considerable reduction in the rate of DNA net synthesis accompanied by a stimulation of the DNase system. The further course is characterized by a permanent depression of the DNase system, accompanied by a return of the rate of DNA net synthesis to the normal range. It may be said that malignant growth is characterized by, *inter alia*, progressive changes along these lines. Considerable net synthesis of DNA is here associated with damage to the DNase-synthesizing system, manifested as very low levels of DNase activity (7).

It would seem that the results of earlier work on the relationship between normal growth and DNase activity (5, 7–11) together with the present data point to a functional relationship between the DNase and DNA metabolism. It appears that the enzyme might be engaged in the well balanced and rigidly organized process of normal growth and cellular reduplication. Its involvement in mechanisms controlling and regulating the DNA metabolism during normal growth is conceivable. According to this line of thought a possible function of the DNase might be that of counterbalancing the DNA-synthesizing enzyme of a tissue. In the light of this interpretation of the cellular function of DNase the demonstrated subnormal activities in malignant tissues become meaningful.

The mechanism by which the DNase might exert a restrictive and regulating function on the DNA metabolism of normal cells may be illustrated by the findings of Mantsavinos and Canellakis (18). These authors studied the properties of an enzyme system derived from regenerating rat liver and catalyzing DNA synthesis. They found that the addition of minute amounts of pancreatic DNase inhibited the synthesizing enzyme and caused a decrease in the incorporation of deoxyribonucleotides into DNA. These findings have also been arrived at by Krakow and Kammen (17).

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