

THE EFFECT OF LIVER REGENERATION ON TUMOR  
FORMATION IN RATS FED 4-DIMETHYL-  
AMINOAZOBENZENE\*

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PLATES 20 TO 22

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Many physical and chemical factors, without being carcinogenic themselves, are able to cause an augmentation of tumor production when properly combined with the action of a specific carcinogen. Physical factors shown to be capable of such an action are: mechanical irritation (1) and scalding (2). Chemical factors are: a basic fraction of creosote oil (3), turpentine and chloroform (4), croton oil and its active component, croton resin (5).

The most important fact, with respect to the mechanism of action of these irritants, is that none of them is effective when applied before rather than after the specific carcinogen (6). They act only when the carcinogenic process has been started by a specific carcinogen and carried on up to a certain point. This point may be such that the histological picture of the tissue under consideration cannot be distinguished from the normal one (4).

Valuable information concerning the effect of irritants may be gained by consideration of a series of investigations which deal with the tumor-promoting action of surgically induced cell regeneration. Wounds were produced by excision or cauterization of skin in areas previously treated with suboptimal doses of a carcinogen. The subsequent healing process was found greatly to augment tumor formation (4, 7). It seems probable, therefore, that the action of irritants is in some way similar to the action of traumatic factors in the sense that both induce cell regeneration.

These findings were considered to indicate the existence of a stage characterized by the formation of cells capable of forming tumors but not asserting

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themselves in this way unless stimulated to do so (4). Non-carcinogenic irritants or wound healing can act as stimuli during this stage (4, 6, 7). This work has been carried out for the most part upon the skin, with the use of carcinogenic hydrocarbons as tumor-inducing agents. Comparable results have been obtained on the thyroid with the use of a combination of 2-acetylaminofluorene and goitrogenic thiourea derivatives (8), and recent observations on the evolution of spontaneous mammary tumors in mice (9) can be considered as collateral evidence in the mammary tissue.

The present paper represents an attempt to investigate the relationship between regeneration and carcinogenesis in rat liver with the use of 4-dimethylaminoazobenzene (DAB) as the carcinogenic agent. Rat liver was chosen because of its great capacity to regenerate and its active response to the carcinogenic azo dyes.

Our procedure consisted in the induction of vigorous regeneration by partial hepatectomy in livers which were in the process of evolution towards neoplasia after a limited exposure to DAB. The incidence of tumors subsequent to regeneration would then reflect the impact of regeneration upon the carcinogenic process.

#### *Materials and Methods*

Five groups of male rats of the Harvard strain,<sup>1</sup> each containing approximately 30 to 60 animals, were used for this experiment. The semisynthetic diet of Kline *et al.* (10) containing 0.06 per cent of 4-dimethylaminoazobenzene was fed for varying lengths of time to the different groups. Thus group A was given the azo dye for 30 days, group B for 50 days, group C for 70 days, group D for 110 days, and group E for 135 days. At the end of each period the administration of the carcinogen was discontinued and the animals returned to the standard laboratory diet,<sup>2</sup> except for group E in which instance a large number of rats were kept on the semisynthetic diet without the carcinogen.

At the end of the feeding period, the peritoneal cavities were explored, and the animals which had already developed hepatic tumors were autopsied. Liver regeneration was induced by partial hepatectomy in half of the tumor-free animals in each group, and small hepatic biopsies were performed on the remaining half which were to serve as controls. The hepatectomy consisted of the removal of the two main lobes, namely the median and left lateral, constituting 68 per cent of the total liver (11). It was found that in each group a considerable variation in the degree of liver lesions existed in individual rats in spite of the fact that all the animals were exposed to the carcinogen for the same length of time. In some instances the lesions were of a moderate type, and consisted mainly of degenerative changes; proliferative activity was limited, and confined mainly to bile duct and connective tissue cells. In others a more advanced type of lesion was found, consisting primarily of extensive hyperplastic changes in all the mesenchymal and epithelial elements. It was considered that the type of the liver lesions at the time of the hepatectomy might be significant with respect to the possible effect of regeneration on the carcinogenic process. For this reason an attempt was made to

<sup>1</sup> Derived originally from Sprague-Dawley stock and maintained for the past 15 years at the Harvard Biological Animal Farm.

<sup>2</sup> Eshelman's red rose dog and puppy food.

distribute the animals presenting the same type of liver lesions equally between the control and hepatectomized series.

2 and 5 months after the operation exploratory laparotomies were performed. The livers were carefully palpated for tumor nodules and biopsies were taken. 12 months after the operation all remaining animals were sacrificed and autopsies performed.

All operative procedures were carried out under ether anesthesia.

#### RESULTS

**Group A:** This group contained 27 animals with an average body weight at the beginning of the experiment of  $174 \pm 24$  gm. DAB was fed for 30 days. The average weight change at the end of the feeding period was  $-8.0$  per cent.

*At Time of Operation and Withdrawal of the Carcinogen.*—Macroscopic examination showed that most livers had a smooth yellowish surface. In a few of the animals the right lateral and median lobes were found to be slightly rough. Microscopic examination showed a variable amount of proliferation of bile duct cells and connective tissue elements in most of the livers. In some foci the proliferation was extensive (Fig. 2), whereas in other parts of the liver it was absent. Some livers showed only the moderate type of lesions consisting of fatty infiltration and slight proliferation of bile duct and connective tissue cells without disruption of the architectural pattern of the parenchyma (Fig. 1). The liver cells showed mostly degenerative changes consisting of pyknotic nuclei, fatty infiltration of the cytoplasm, and chromatolysis (12). Large liver cells with increased basophilia of the cytoplasm were found occasionally but typical foci of basophil hyperplasia (12) were not present. Hyperplasia of bile ducts, cholangiofibrosis (12), cysts, or tumors were entirely absent at this time in this series.

*2 Months after Operation.*—On microscopic examination most of the livers exhibited periportal infiltration, newly formed solitary small capillaries (Fig. 4), and enlarged blood vessels especially in the proximity of the liver capsule (Fig. 3). Cholangiofibrosis was found occasionally (Fig. 7). Some variability in the size of the liver cell nuclei was found. No foci of basophil hyperplasia were present.

*5 Months after Operation.*—Microscopic examination showed that the periportal infiltration still persisted. There was usually some fibrosis in the form of very narrow strands of connective tissue running through the liver parenchyma. Enlarged blood vessels and small, newly formed capillaries were present in almost all the livers. Cholangiofibrosis was again only an occasional finding and in some cases it was regressing (Fig. 8). Some hepatic cells with variable size nuclei were present, but this finding was less marked than before.

*1 Year after Operation.*—Microscopic examination showed that the lesions were still present although less pronounced. Only one animal, a control, had developed a tumor.

**Group B:** This group contained 30 animals with an average body weight at the beginning of the experiment of  $171 \pm 19$  gm. DAB was fed for 50 days. The average weight change at the end of the feeding period was  $-6.8$  per cent.

*At Time of Operation and Withdrawal of the Carcinogen.*—Macroscopic examination showed that most livers had a rough surface and that some of them were quite nodular. A moderate number of livers exhibited shrinkage of the right lateral and right median lobes where the changes were found always to be more pronounced than in the rest of the liver. The microscopic examination showed lesions of the moderate type in only 2 animals. All the remaining animals showed extensive lesions of the advanced type. The most characteristic change was very active proliferation of bile duct cells. These cells, together with connective tissue and hyperplastic blood vessels, were found to form wide bands running through the parenchyma and greatly disrupting its architecture. The liver cells showed degenerative changes, namely fatty infiltration, chromatolysis, and pyknotic nuclei, as well as extensive proliferation mainly in the form of focal hyperplasia. The liver cells in these foci were larger than normal, with increased basophilia of the cytoplasm and a tendency to form syncytia (Fig. 6). More than half of the animals showed the typical picture of cholangiofibrosis, and a few exhibited cysts of various sizes. No tumors were found at this time in this group.

*2 Months after Operation.*—The microscopic examination showed that almost all the livers had developed typical cholangiofibrosis. There were extensive periportal infiltration and hyperplasia of blood vessels. Bile duct hyperplasia (Fig. 5), fibrosis, and cyst formation were also present. No tumors were found at this time in this group.

*5 Months after Operation.*—The microscopic examination showed that in a great number of animals the extensive cholangiofibrotic lesions were regressing. 2 out of 13 surviving controls and 2 out of 13 hepatectomized animals had developed tumors.

*1 Year after Operation.*—10 out of 12 surviving control and 8 out of 12 hepatectomized animals had developed tumors.

**Group C:** This group contained 29 animals with an average body weight at the beginning of the experiment of  $200 \pm 24$  gm. DAB was fed for 70 days. The average weight change at the end of the feeding period was  $-8.1$  per cent.

*At Time of Operation and Withdrawal of the Carcinogen.*—Macroscopic examination showed almost all the livers to have a yellowish and moderately rough surface. 3 of the animals exhibited a few small cysts on the liver surface. Approximately one-half of the animals showed shrinkage of the right lateral and right median lobes. Microscopic examination of the liver showed lesions of the moderate type in only 3 animals. All the remaining animals showed lesions of the advanced type. The architecture of the hepatic parenchyma was greatly

disrupted by wide sheets and bands of rapidly proliferating bile duct and connective tissue cells. This finding was more pronounced here than in the previous group. The liver cells also showed a variety of changes similar to the ones described in the previous group and in more than half of the animals these changes were of an advanced hyperplastic nature. There was no bile duct hyperplasia except in the case of 2 animals which showed the typical picture of cholangiofibrosis. In addition to the 3 animals with macroscopically discernible cysts, 2 other cases showed cystic formations on sectioning. There was one tumor in the series at this time.

*2 Months after Operation.*—The macroscopic examination showed that lesions were more pronounced than before. Half of the animals had developed cholangiofibrosis. Periportal infiltration and new hyperplastic blood vessels were found in approximately one-third of the animals. Fibrosis, bile duct hyperplasia, and cyst formation were also present in some of the livers. 3 out of 13 surviving control and 3 out of 12 hepatectomized animals had developed tumors.

*5 Months after Operation.*—The microscopic examination showed that there was only one case of regressing cholangiofibrosis. In all other cases the lesions were persisting or progressing toward definite tumor formation. 7 out of 13 surviving control and 8 out of 11 surviving hepatectomized animals had tumors.

*1 Year after Operation.*—10 out of 13 surviving control and 9 out of 11 hepatectomized animals had developed tumors.

**Group D:** This group contained 41 animals with an average body weight at the beginning of the experiment of  $146 \pm 21$  gm. DAB was fed for 110 days. The average weight change at the end of the feeding period was +1.9 per cent.

*At Time of Operation and Withdrawal of the Carcinogen.*—Macroscopic examination showed that 6 animals had livers with a yellowish smooth surface. The livers of the remaining animals exhibited a rough surface and many had gross tumor nodules or cysts. Microscopic examination showed lesions of the moderate type in 3 animals. The remaining animals showed lesions of the advanced type. As a whole, the histological picture gave the impression that proliferation of connective tissue and bile duct cells was less extensive than in group C. On the other hand, it was found that cholangiofibrosis and new formation of hyperplastic bile ducts were more pronounced in this series than in group C. Thus one-fourth of the animals showed the typical picture of cholangiofibrosis and one-fifth showed areas of new formation of bile ducts. The liver cells, for the most part, showed advanced lesions predominantly hyperplastic in nature. Foci of basophil hyperplasia with definite syncytium formation were found in six livers. These lesions indicated a much more advanced reaction to the carcinogen than in the previous series. 19 animals of this group had developed tumors.

*2 Months after Operation.*—The microscopic examination showed that the

lesions persisted and that there was no perceptible increase in cholangiofibrosis. Periportal infiltration and formation of hyperplastic blood vessels were insignificant. These findings are in contrast with the sequence of events in group C. 2 out of the 8 control and 3 out of the 8 surviving hepatectomized animals had developed tumors at this time.

*5 Months after Operation.*—3 out of 8 control and 5 out of 8 surviving hepatectomized animals had developed tumors.

*1 Year after Operation.*—8 out of 8 control and 7 out of 8 hepatectomized animals had developed tumors.

**Group E:** This group contained 60 animals with an average body weight at the beginning of the experiment of  $302 \pm 31$  gm. DAB was fed for 135 days, at the end of which only 8 rats were returned to the stock diet, the rest continuing on the semisynthetic diet but without the azo dye. The average weight change at the end of the feeding period was  $-8.9$  per cent.

*At Time of Operation and Withdrawal of the Carcinogen.*—Macroscopic examination showed that 23 animals had livers with a yellowish, smooth or slightly rough surface. The remaining livers exhibited a rough and nodular surface and a few of them had obvious tumor masses. Microscopic examination showed that about one-third (19 animals) had lesions of the moderate type. The liver changes in these animals consisted of fatty infiltration, especially in the central area of the lobules, and a moderate amount of bile duct and connective tissue cell proliferation with only partial or no disruption of the pattern of the hepatic parenchyma. There was also slight periportal infiltration. The liver cells exhibited mostly degenerative changes consisting of pyknotic nuclei, fatty infiltration, and chromatolysis. Large liver cells with increased basophilia of the cytoplasm were found occasionally but no focal hyperplasia was present. The remaining livers showed extensive lesions of the advanced type. In these, the most characteristic change was fibrosis with formation of long, narrow bands completely disrupting the architecture of the hepatic parenchyma. In addition, there was proliferation of bile duct cells, but in contrast to the findings in the previous series, their proliferative activity lagged behind that of the connective tissue and they were never found in large sheets, but only in narrow bands. Many of the livers showed newly formed hyperplastic bile ducts in clusters. Small hyperplastic capillaries were also present mainly in the central area of the lobules. Cholangiofibrosis was found in only six of the livers in this group. Focal basophil hyperplasia of liver cells with formation of typical syncytia was present in many of these livers. There were 16 animals with tumors at this time in this group.

*2 Months after Operation.*—It was found that the lesions of 11 of the control animals, which at the time of the previous observation were found to belong to the moderate type, were progressing at a very slow rate. The macroscopic appearance of the livers was the same as before, and microscopically fibrosis

and periportal infiltration were found to be slightly increased. None of these animals had developed tumors. On the other hand, the lesions of the hepatectomized rats, which at the previous observation were found to belong to the moderate type, now had progressed rapidly toward definite tumor formation. Out of 8 surviving animals in this category, 6 were found to have tumors. In the animals with lesions of the advanced type at the time of the previous observation, 3 out of 6 controls and 5 out of 9 hepatectomized rats had developed tumors.

*5 Months after Operation.*—In the animals with lesions of the moderate type at the time of the first observation, 3 out of 10 controls and 7 out of 8 hepatectomized animals had now developed tumors. The lesions of the tumor-free animals in this category ranged from an almost imperceptible periportal infiltration and normal liver parenchyma to marked fibrosis with disruption of the hepatic architecture and new formation of hyperplastic bile ducts. In the animals with lesions of the advanced type at the time of the first observation, 4 out of 4 controls and 8 out of 9 hepatectomized rats had developed tumors.

*1 Year after Operation.*—In the animals with lesions of the moderate type at the time of the first observation 5 out of 7 controls had developed tumors. There were no other survivors at this time in this group.

The incidence of tumors at the various time intervals in all five groups has been summarized in Table I. The incidence at time of operation increased with time of exposure to the carcinogen, except that group E, which was exposed to the azo dye for 135 days, had a smaller percentage of tumors than group D which was exposed for only 110 days. On the other hand, the incidence of tumors at 12 months after the operation was of the same order of magnitude ( $82 \pm 12$  per cent) in all groups except group A. The appearance of only one tumor at the end of the 12 month period in group A seems to imply that in this group the 30 day exposure time to the carcinogen was almost totally inadequate for tumor formation. The findings in the four other groups indicate that beyond the threshold of 50 days the time of exposure to the azo dye was effective only as far as the rate of the appearance of the tumors was concerned, but was without effect on the final tumor incidence. The distribution of the tumors at the final time interval of 12 months was equal between control and hepatectomized animals in all series.

In groups B, C, D, and E the findings in the 2 and 5 month intervals after the operation show that in the majority of cases the incidence of tumors in the hepatectomized animals was higher than in the controls. In order to obtain a large enough number of animals for a valid statistical analysis, and since final tumor formation was shown to be independent of the time of exposure to the carcinogen, the individual findings in these groups were combined. The bottom line of the table shows the cumulative results of groups B, C, D, and E considered as a whole. The findings at the 2 and 5 month intervals were analyzed with respect to the significance of the difference between proportions

TABLE I  
Comparison of Tumor Incidence in Control and Hepatectomized Rats

Group	Initial weight	Length of time on DAB	At operation	Controls			Hepatectomized			Total controls and hepatectomized at 12 mos.	
				2 mos.	5 mos.	12 mos.	2 mos.	5 mos.	12 mos.		
A	174 ± 24	30	0/27*	0/12	0/12	1/11	0/11	0/11	0/11	1/22	
B	171 ± 19	50	0/30	0/14	2/13	10/12	0/13	2/13	8/12	18/24	
C	200 ± 24	70	1/29	3/13	7/13	10/13	3/12	8/11	9/11	19/24	
D	146 ± 21	110	19/41	2/8	3/8	8/8	3/8	5/8	7/8	15/16	
E	302 ± 31	135	16/60	3/17	7/14	9/11	11/17	15/17	15/17	24/28	
Total, B + C + D + E.....				8/52†	19/48§	37/44	17/50†	30/49§	39/48	76/92	82 ± 12 per cent

\* Number of rats with tumors/number of rats alive.

Standard error of the difference between proportions  $\sigma_p$ , per cent =  $\sqrt{pq \left( \frac{1}{N_1} + \frac{1}{N_2} \right)}$

$p$  = total per cent of occurrence,

$q$  = 1 -  $p$ ,

$N_1$  = number in first sample,

$N_2$  = number in second sample,

$X$  = difference between proportions,

$P$  = probability of difference as great or greater than that obtained being due to chance.

†  $\sigma_p$  = 0.083,  $X$  = 0.172,  $\frac{X}{\sigma_p}$  = 2.03,  $P$  = 0.046.

§  $\sigma_p$  = 0.102,  $X$  = 0.216,  $\frac{X}{\sigma_p}$  = 2.1,  $P$  = 0.035.

TABLE II  
Comparison of the Kinds of Tumors Found in All Groups before and after Operation

Kinds of tumors	Trabecular hepatomas	Adenohepatomas	Cholangiomas	Cystadenomas	Tumors without diagnosis*	Total
At the time of operation.....	13	15	2		6	36
Hepatectomized rats—1 yr. after operation.....	12	12	4	6	5	39
Control rats—1 yr. after operation.....	7	10	1	10	10	38

\* No histological examination was performed because of postmortem changes.

of tumor formation in controls and hepatectomized animals (13).  $P$  was found to equal 0.046 and 0.035 respectively.<sup>3</sup>

<sup>3</sup> See footnotes to Table I.



The results of the histological examination of the tumors are summarized in Table II. Opie's terminology (12) was followed in describing the four main types of tumors found: trabecular hepatomas, adenohepatomas, cholangiomas, and cystadenomas (Figs. 9 to 12). There were no significant differences between the types of tumors in the various groups, nor was there any difference between control and hepatectomized animals. With respect to the time of appearance of the different types of tumors, it was noted that cystadenomas tended to appear later than the others. As shown in the table, they were never found at the time of operation, and most of them did not occur until 1 year afterwards.

#### DISCUSSION

In comparing the findings of the five experimental groups, the most pronounced differences in reaction to the azo dye appear in groups A and E. It should be noted that the observations were made at fixed intervals beginning with the point of withdrawal of the carcinogen. Therefore the actual length of time from the start of administration of DAB to the time of observation was progressively longer for each successive group. However, if the start of administration of the carcinogen is considered as the point of reference, the over all relationships between the various groups are not altered.

In group A, the incidence of tumors at the end of the 12 month period was minimal, in contrast to the high incidence which all other groups exhibited. This finding, as well as the limited histological changes shown by this group, may be considered indicative of a sub-threshold exposure to DAB.

Groups B, C, and D present a rather uniform picture if the variation in exposure time is taken into consideration.

The reaction of group E, however, differs from that of the other groups in three points: first, although this group was exposed to the azo dye for 135 days, *i.e.* a longer time than any other group, the percentage of tumors at the time of operation was lower than in group D which had an exposure of only 110 days; second, at the time of operation, the moderate type of histological changes occurred in a greater number of animals than in groups B, C, and D; third, the difference in the incidence of tumors between control and hepatectomized animals at 2 and 5 months after the operation was greater in this group than in any other (Table I). All three points seem to be interrelated. Thus the relatively lower incidence of tumors at operation and the presence of a considerable number of animals with the moderate type of liver lesions seem to indicate that in group E the reaction to DAB was slower and of a lower intensity than in the other groups. The greater difference in the incidence of tumors between control and hepatectomized animals after the operation may also be attributed to the slow and less intense response to the carcinogen, since it was in the animals with lesions of the moderate type that this difference was most strikingly

manifested. All the other experimental conditions being the same, the slow and weak reaction to the carcinogen in group E may be attributable to at least two possible causes. One possibility is that in the particular lot of casein which was used in the preparation of the diet for the animals in group E, the riboflavin content may have been elevated, although we do not know this. The well known protective effect of riboflavin could then be held responsible for the relatively low intensity of the response to DAB in this group in spite of the longer feeding period.

A second possibility is that the weak response to the carcinogen may have been related to the age of the animals in group E. The exact age of the rats in the different groups was not known, but if body weight can be taken as a criterion of age, the animals in group E, with an average weight of 302 gm., were older than the rats in all other groups in which the average weight was 172 gm. There is evidence in the literature that aging might delay and inhibit, to a certain extent, the effect of some carcinogenic agents (14).

The main conclusion which may be drawn from the results of this work seems to be that carcinogenesis in the liver of rats fed DAB proceeds in at least two stages. The first stage appears to consist in the development of a particular state of the cells exposed to the azo dye. The main characteristic of this state is that although the cells are capable of neoplastic growth they may remain in latency for many months if the exposure to the carcinogen is discontinued. The development of this state seems to take place rather rapidly after the beginning of the administration of the carcinogen to the animal. In the present experiments the contrasting findings in final tumor production in group A, on the one hand, and the rest of the groups on the other, indicated that the necessary exposure time for the development of this state lay between 30 and 50 days. The variability of exposure to the azo dye in groups B, C, D, and E was without sizable effect on the final total tumor production. Thus it appears that the particular cellular state had developed in the same time interval in all four groups.

The findings at operation, especially in group E, indicate that during the development of this particular cellular state, the histological picture may not be characterized by specific preneoplastic lesions, but may show only non-specific degenerative or slight hyperplastic changes.

The second stage of chemical carcinogenesis in the liver of rats fed DAB consists in the evolution of cells from the particular state to definite neoplastic growth.

The increase in incidence of tumors found at the time of operation from zero in group B to 46 per cent and 27 per cent in groups D and E respectively, shows that this process can be greatly influenced by the time of exposure to the carcinogen.

The difference, especially in group E, in the incidence of tumors between

control and hepatectomized animals at 2 and 5 months after the operation seems to imply that regeneration can also be effective in enhancing the carcinogenic process at the second stage.

Since liver regeneration in the rat is practically completed in about 2 weeks after partial hepatectomy (11, 15) the enhancing effect on tumor formation would be expected to appear at the time of the early observations, 2 and 5 months after the operation rather than after later intervals.

Thus at the end of the 12 month period there was no difference in the final total incidence of tumors between control and hepatectomized animals in any of the groups, nor was there any difference between the four groups themselves. Therefore it seems that the rate by which the transition proceeds from the particular cellular state to tumor formation may be accelerated by a prolonged exposure to the carcinogen, or by the induction of regeneration. On the other hand, the similarity in the final tumor incidence in the four groups may be considered as evidence in favor of the irreversible nature of the first stage (4, 16).

It seems then, that chemical carcinogenesis in the skin or the liver with either carcinogenic hydrocarbons or azo dyes proceeds in essentially the same way. The results of the present experiments, in which partial hepatectomy was used as a non-specific growth stimulus, are in agreement with those of other investigators, in which wound healing, turpentine, croton oil, etc., were used as non-specific stimuli. Rous *et al.* described an initiating and a promoting process (4); Mottram a sensitizing factor with specific cellular reaction, and a developing factor (17); Berenblum a precarcinogenic and an epicarcinogenic action (6). The characteristics of these stages are similar to those of the first and second stages which, in these experiments, were found to occur in liver carcinogenesis.

#### SUMMARY

Partial hepatectomies were performed on five groups of rats which had been maintained on a diet containing 4-dimethylaminoazobenzene for various lengths of time. The effect of regeneration on the incidence of hepatic tumors was compared with that in similarly treated non-hepatectomized controls.

Regeneration, and the time of exposure to the carcinogen as well, were relatively effective in accelerating the rate of appearance of tumors, although not markedly so. These factors were without effect on final total tumor formation.

These findings support the concept that during chemical carcinogenesis tumor formation proceeds in stages.

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#### EXPLANATION OF PLATES

Tissues were fixed in Zenker's fluid and stained with eosin and methylene blue. The magnification of the sections is 177.

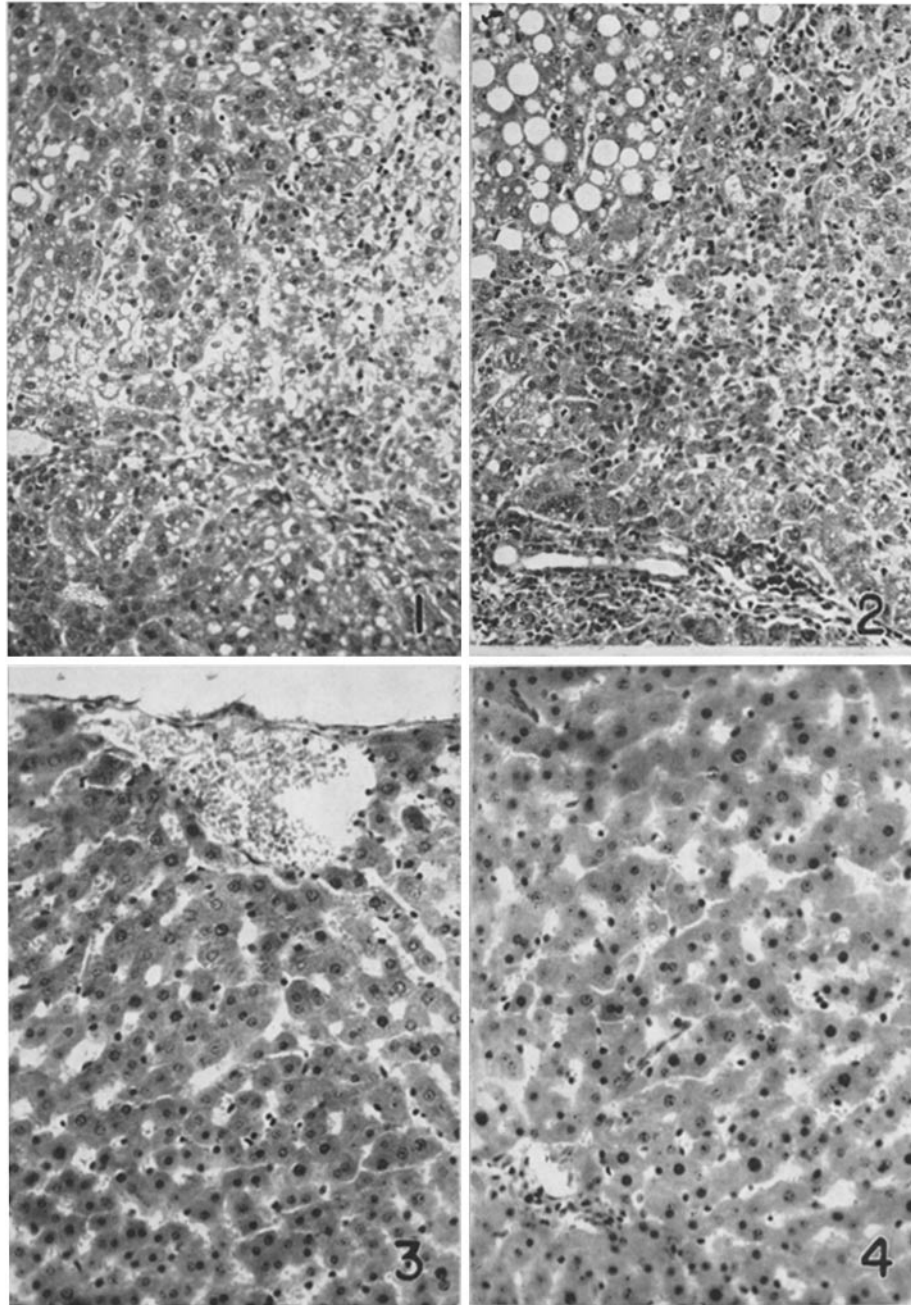
#### PLATE 20

FIG. 1. Moderate type of lesion found at the time of operation, showing fatty infiltration of liver parenchyma, and slight proliferation of bile duct cells and connective tissue elements without disruption of the architectural pattern of the lobules.

FIG. 2. Advanced type of lesion found at the time of operation, showing extensive proliferation of bile duct cells and connective tissue elements, with extreme fatty infiltration of liver parenchyma and disruption of architectural pattern of the lobules.

FIG. 3. Large blood vessel lying just beneath the liver capsule. Biopsy 2 months after operation and return to normal diet. Note relatively normal appearance of the hepatic cells.

FIG. 4. Material from same biopsy as Fig. 3. Newly formed, solitary small capillaries running through the liver parenchyma.



(Glinos *et al.*: Liver regeneration and tumor formation)

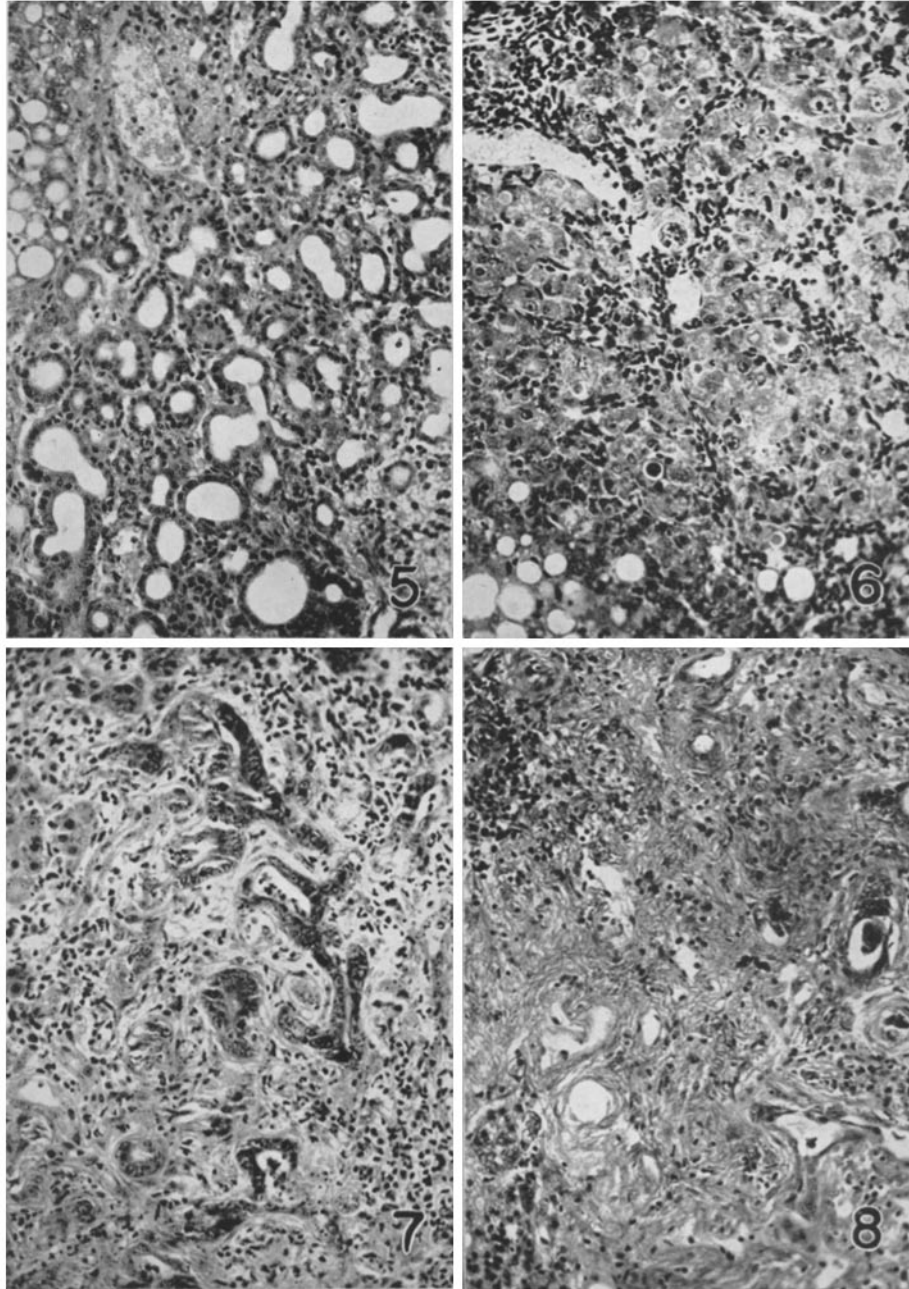
PLATE 21

FIG. 5. Focus of benign hyperplasia of bile ducts.

FIG. 6. Focal basophilic hyperplasia of liver cells. Note great enlargement of cells and nuclei. Mitotic figure at right, near center.

FIG. 7. Cholangiofibrosis. Mitotic figure near center.

FIG. 8. Cholangiofibrosis regressing. 5 months after operation and return to normal diet.



(Glinos *et al.*: Liver regeneration and tumor formation)

PLATE 22

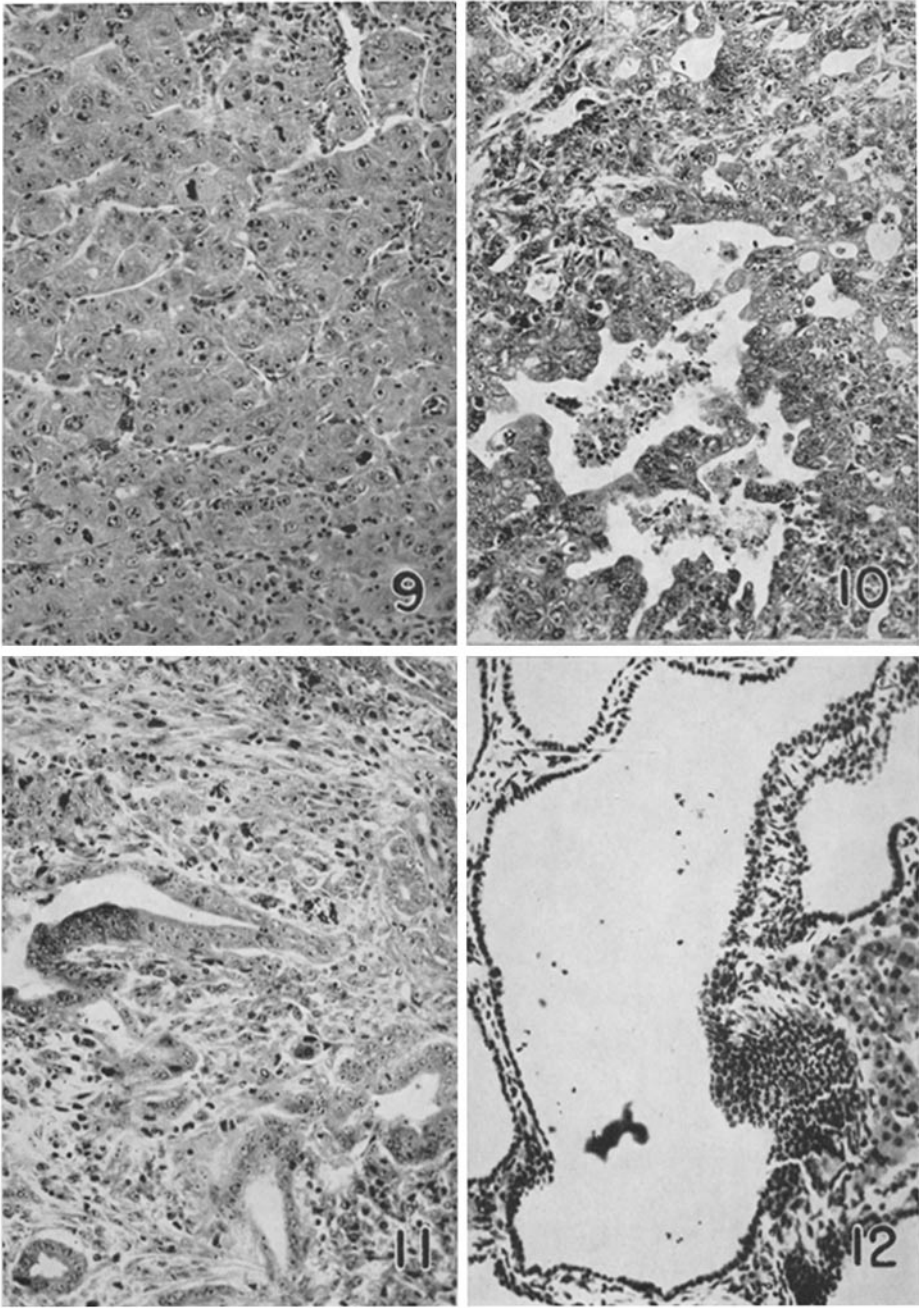
FIG. 9. Trabecular hepatoma. Numerous mitotic figures.

FIG. 10. Adenohepatoma.

FIG. 11. Cholangioma. Note large abnormal mitotic figure near center.

FIG. 12. Cystadenoma.





(Glinos *et al.*: Liver regeneration and tumor formation)