20-METHYLCHOLANTHRENE AS AN INHIBITOR OF HEPATIC CANCER INDUCED BY 3-METHYL-4-DIMETHYLAMINOAZOBENZENE IN THE DIET OF THE RAT: A DETERMINATION OF THE TIME RELATIONSHIPS

by

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A THESIS

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(Chairman of the Graduate Council)

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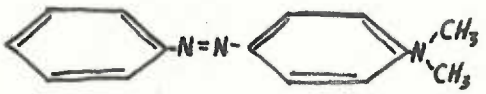
I N T R O D U C T I O N

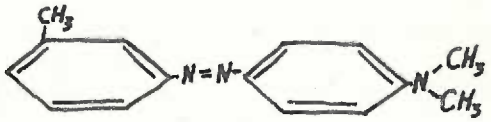
Cancer research has been directed along many lines, but the goals are to find a cure for cancer or a means of preventing it. It is through the determination of the etiology and the pathogenesis that these goals may be achieved. Animal experimentation affords the most fundamental observations in the etiology of cancer. To understand the processes involved in the pathogenesis of cancer, one must begin with the experimental production of this disease, and then modify the factors concerned in such a manner that as few variables as possible appear. If one were able to initiate the growth of a cancer, and then, by altering some part of the process, arrest the growth, a large step towards the realisation of the second goal would be accomplished. For this reason, much of cancer research has been directed towards studies in the inhibition of experimentally induced cancer.

REVIEW OF THE LITERATURE

It has been 20 years since hepatic cancers were first produced experimentally. An azo dye used in enhancing the yellow color of butter had been suspected of inducing hepatomas in man. This led Sasaki and Yoshida [1], [2] in 1935 to attempt to produce hepatomas experimentally by adding this and related dyes to the diet of rats. Various types of hepatic cancer resulted. The most effective dye used by them was o-aminoazotoluene. A few years later Kinosita [3] tested additional compounds of related chemical composition. He found that 3-methyl-4-dimethylaminoazobenzene induced the greatest incidence of hepatomas. This compound, herein referred to as m'MeDAB, is the same as butter yellow with the addition of a methyl group in the position shown in the following structural formula.



Butter yellow
p-dimethylaminoazobenzene



m'MeDAB
3-methyl-4-dimethylaminoazobenzene

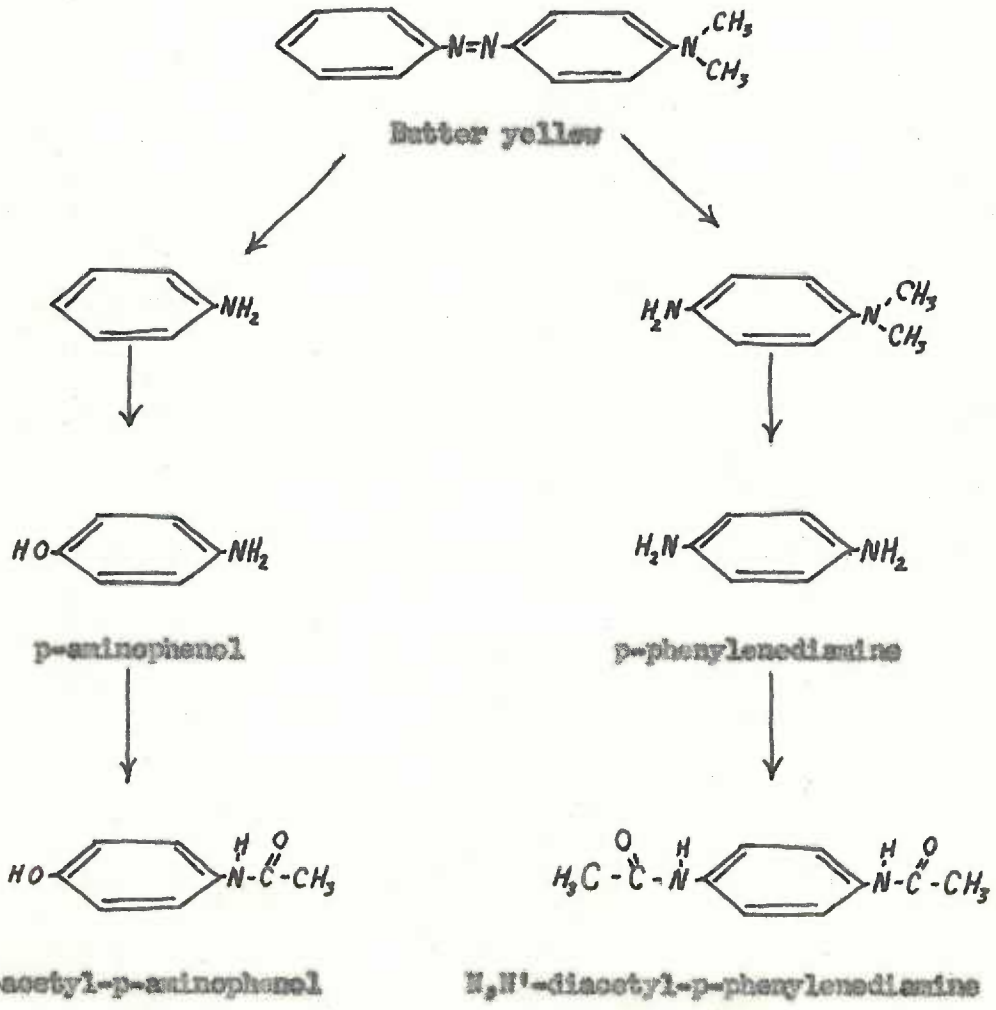
There is a direct relationship between the daily amount of m'MeDAB consumed and the incidence of tumors. The quantities used in the diet varied, but the maximum effectiveness was found to be 0.06 percent concentration in the diet. [4]

Morton investigated the carcinogenic activity of various hydrocarbons and tried to find a common chemical denominator in the structural formula. The most effective carcinogens had at least two benzene rings. [5], [6], [7] Various methods of administration were tried, and the oral route was found to be most effective. [8]

While alteration in the chemical structure of the carcinogen occupied some investigators, others studied the chemical changes in the carcinogenic agents within the experimental animal. According to Mueller [9] there are two methods by which o'MeDAB is altered by the liver. The methylated parent compound combines with liver protein, or the parent compound is metabolised by the following reactions:

1. The reductive cleavage of the azo linkage.
2. Demethylation of the N-methyl groups.
3. Hydroxylation of the 4' position.

These reactions are represented by the following diagrams:



These degradation products have been examined for carcinogenic activity without any general agreement among various investigators. At best, all of these are far less effective than the parent compound, p-dimethylaminoazobenzene.[10], [11] This suggests that the carcinogenic action must occur before metabolic degradation, or the carcinogenic action occurs after the dye has combined with protein.[12] The action of agents which inhibit carcinogenesis may be explained partially by an alteration in the rate of metabolic degradation.

The reductive-cleavage is catalyzed by riboflavin which may account for the partial protection of the liver by this substance.[13] [14] Other agents have been reported to retard tumor development. Substances such as yeast and rice bran may act through their riboflavin content. The effectiveness of ample dietary casein may be attributed to the retention of riboflavin within the liver.[15], [16] Liver, liver extracts, lanolin, nitrogen mustard, and egg albumin have been reported to inhibit carcinogenesis, but these results have been poor and many are equivocal. Their mode of action in cytochemical terms remains unexplained.[17], [18], [19], [20], [21], [22], [23], [24], [25], [26], [27], [28], [29]

In an attempt to explain this carcinogenesis on a cyto-chemical basis, the carcinogenic dye has been traced electrophoretically.[30], [31], [32], [33], [34], [35], [36], [37] It is bound in the liver to a protein molecule. This dye-bound protein complex reaches a maximum concentration between the third and sixth week of continuous feeding. When the dye is removed from the diet, the concentration falls to zero within two weeks. When the dye is continued in the diet, it begins to disappear from the liver after the sixth week, paralleling the individual

tissue changes within the liver more closely than the length of feeding. The dye continues to disappear and by the time carcinoma appears, from the sixth week on, little dye is found in the altered, but non-neoplastic tissue. No protein-bound dye has ever been found in the carcinomas.

Referring again to riboflavin, it was observed that the addition of this vitamin in a concentration of 10 mg/ Kg of food led to a lower hepatic content of the dye. (38), (39) Thus, one may theorize that riboflavin, by increasing the rate of reductive-cleavage reduces the quantity of parent dye to be combined with the protein. While this might explain the protective action of riboflavin, it does not account for the diminution of protein bound dye with the appearance of hepatic tumors. Another theory is that the liver protein, bound by the dye, is removed by excretion in bile and urine, thereby depleting the liver cells of protein, and thereby leading to liver cells which have lost their normal growth. Such cells are cancerous. (33) The implication of this is that protein depletion is the cause of hepatic carcinomas.

Besides some cytochemical changes, there are a number of different morphologic changes in the liver of significance. Prior to the development of carcinomas the liver shows such changes as fatty infiltration, cirrhosis, bile duct proliferation, benign hepatomas, and cholangiofibromas. Cirrhosis begins at three weeks and is present in all animals at six weeks. Bile duct proliferation begins at nine weeks and progressively becomes more severe. The majority of animals have benign hepatomas at twelve weeks. The types of liver cancers have been variously classified. (40), (41), (42), (43), (44) Price (45) feels that all tumors of the liver regardless of their appearance, arise from areas of benign bile duct adenoma.

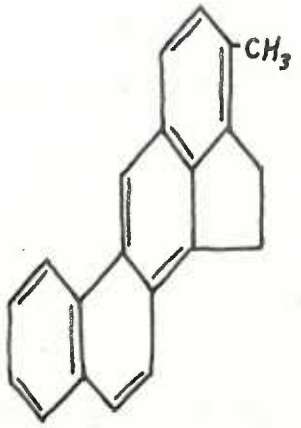
One of the major questions in the pathogenesis of the hepatic carcinomas is whether the effects, once begun with cirrhosis, are progressive to carcinoma, or whether successive phases of injury and tissue change are necessary. Fritz-Niggle [46] was of the opinion that carcinogenic agents produce irreversible lesions, the effects of which are additive. However, Cortell [47] found that rats fed m'MeDAB 39 to 46 days, then followed 150 days developed no tumors. This would indicate that the cirrhotic stage alone did not lead to cancer. If the dye was fed 69 days, 93 percent on fox chow developed tumors, and 80 percent on Basic diet developed tumors.

On a shorter experiment Clayton [48] found very little significance in the differences of tumor incidence when interrupted feedings of the dye were practiced.

Various factors influence the appearance of tumors even though the same dye is used. Rumsfeld [49] feels that males develop the tumors more readily. Harris [50] reports no evidence of strain susceptibility. However, Engel [51] reports a difference in strains, although he used only 5 animals in his study. White [4] finds that the incidence of tumors decreases as the incidence of pneumonia increases.

In 1951 Richardson [52] reported the inhibition of the liver tumors by the use of 20-Methylcholanthrene. The oral route was found to be the most effective. This substance was synthesized in 1936. [53], [54] It was found to be a powerful carcinogen, producing both carcinomas and sarcomas. [55], [56], [57] It will be referred to as MCA. When this strong carcinogen is given subcutaneously, sarcomas of the skin are produced. Since these two carcinogens, m'MeDAB and MCA, independently would cause cancer in the majority of the animals, it was felt that the

two substances added together should increase the incidence and shorten the time of cancer production. The results showed that when m'MeDAB in the concentration of 0.06 percent and MCA in the concentration of 0.067 percent were combined in the diet there was a 98 percent decrease in the incidence of liver carcinoma.



MCA

20-Methylcholanthrene

Dauben [58] found that MCA is excreted in the bile and in the urine.

Although it is very carcinogenic some inhibitory activity has been observed. MCA in concentrations of 60mg/100 Grams of food retards the growth of the rat. Cystine and methionine overcome this inhibition. [59]

The comb-growth response to androgens in the female chick was found to be retarded by MCA. (60) O'Flynn (61) noted that the preliminary administration of a minute dose of MCA appeared to produce a refractory state in the tissues to subsequent carcinogenic doses of MCA. Various workers have found no inhibitory action of MCA upon tumors of other organs. They did not study hepatic cancers. Since m'MeDAB did not inhibit the sarcomas induced by MCA, it was postulated that MCA and m'MeDAB acted independently. (62), (63) There is some evidence to suggest that weak carcinogenic agents inhibit the strong carcinogens. (64), (65), (66) However, this would not apply to the two strong carcinogens, MCA and m'MeDAB.

Some workers have attempted to produce liver tumors with MCA, but they were unable to do so. It was noted that MCA depletes the Vitamin A stores of the liver in 3 to 6 weeks. (67), (68), (69), (70) Bile salts did not augment the carcinogenic action of MCA upon the gastric mucosa of the mouse. (71)

Since there is a similarity of structure between MCA and the adrenal-cortical hormones, (72), (73) Richardson (74) noted changes in the adrenals of the animals administered MCA and m'MeDAB simultaneously, and he postulated a hormonal relationship to the inhibition through the adrenal.

P U R P O S E

In our previous studies, NGA was found to be inhibitory when given simultaneously with m'MeDAB. The purpose of this experiment was to determine if NGA was effective with delayed administration. This experiment was especially significant since the morphological changes up to carcinoma appear to have some sequential relationship. The definitive knowledge obtainable from this experiment may be presented as follows:

1) Are the changes initiated by m'MeDAB reversible?

2) Does m'MeDAB produce cancer by advancing through continuous injury to successive stages of hyperplasia and benign neoplasia?

3) Is there a correlation between the time of appearance of morphological liver changes with m'MeDAB and the time of initiation of NGA feeding?

MATERIALS AND METHODS

ANIMALS

Three hundred and fifty albino rats of the Sprague-Dawley Strain were used. There were 137 males and 203 females. The animals were housed in individual wire cages, maintained on Purina Laboratory Chow prior to initiation of the experiment and supplied with water from clean drip bottles. The weight of the animals at the beginning of the experiment ranged from 120 grams to 320 grams. They were weighed weekly during the experiment and charts of their weights were maintained.

GROUPS

The animals were divided into groups of 10 to 15 animals and placed on a basal synthetic diet to which 0.06 percent m'MeDAB had been added. After 3 weeks on the carcinogen, 0.067 percent MCA was added to the diet of 15 animals. (Group 3) Groups 4 through 18 similarly were given MCA beginning on the week of the experiment designated by their group number. Two control groups were established, one group received the basic diet alone, and the other received m'MeDAB alone. At weekly intervals a few m'MeDAB animals were killed. The m'MeDAB plus MCA animals were killed at 2 to 3 week intervals in order that the liver changes could be noted, and to see if progressive changes could be determined. The basic diet animals were sacrificed later in the experiment in order to show that the basic diet was adequate for maintainence.

Animals in groups 21 through 32 were maintained on a basic diet for two weeks, and then the procedure listed on Tables I, II, and III was performed. The animals were killed daily in groups 21 through 24, and every other day in groups 25 through 32. The groups are listed on Tables I, II, and III.

TABLE I

GROUP	MALES	FEMALES	TOTAL	DIET OR PROCEDURE	DIED MALES	DIED FEMALES	DIED TOTAL	KILLED MALES	KILLED FEMALES	KILLED TOTAL
CONTROLS										
1	0	13	13	Basic diet	0	0	0	0	13	13
DELAYED MCA ADMINISTRATION										
3	6	9	15	m'MeDAB 3 wks then m'MeDAB & MCA until autopsy	0	0	0	6	9	15
4	4	11	15	m'MeDAB 4 wks then m'MeDAB & MCA until autopsy	1	1	2	3	10	13
5	0	15	15	m'MeDAB 5 wks then m'MeDAB & MCA until autopsy	0	0	0	0	15	15
6	14	1	15	m'MeDAB 6 wks then m'MeDAB & MCA until autopsy	2	0	2	12	1	13
7	1	13	14	m'MeDAB 7 wks then m'MeDAB & MCA until autopsy	0	1	1	1	12	13
8	11	3	14	m'MeDAB 8 wks then m'MeDAB & MCA until autopsy	0	1	1	11	2	13
9	7	7	14	m'MeDAB 9 wks then m'MeDAB & MCA until autopsy	2	0	2	5	7	12
10	7	7	14	m'MeDAB 10 wks then m'MeDAB & MCA until autopsy	3	2	5	4	5	9

TABLE II

GROUP	MALES	FEMALES	TOTAL	DIET OR PROCEDURE	DIED MALES	DIED FEMALES	DIED TOTAL	KILLED MALES	KILLED FEMALES	KILLED TOTAL
12	6	6	12	m'MeDAB 12 wks then m'MeDAB & MCA until autopsy	1	3	4	5	3	8
14	6	6	12	m'MeDAB 14 wks then m'MeDAB & MCA until autopsy	2	3	5	4	3	7
16	0	5	5	m'MeDAB 16 wks then m'MeDAB & MCA until autopsy	0	3	3	0	2	2
18	5	4	9	m'MeDAB 18 wks then m'MeDAB & MCA until autopsy	1	1	2	4	3	7
CONTROLS										
20	30	63	93	m'MeDAB only until autopsy	11	33	44	19	30	49
PRELIMINARY BASIC DIET										
21	10	0	10	Basic 2 wks then MCA & m'MeDAB until autopsy	0	0	0	10	0	10
22	0	10	10	Basic 2 wks then MCA & m'MeDAB until autopsy	0	3	3	0	7	7
23	0	10	10	Basic 2 wks then m'MeDAB until autopsy	0	1	1	0	9	9
24	10	0	10	Basic 2 wks then m'MeDAB until autopsy	0	0	0	10	0	10

T A B L E XXI

GROUP	MALES	FEMALES	TOTAL	DIET OR PROCEDURE	DIED			KILLED		
					MALES	FEMALES	TOTAL	MALES	FEMALES	TOTAL
ENDOCRINE ADMINISTRATION										
25	5	0	5	Basic 2 wks then 8 units ACTH IM daily until autopsy	0	0	0	5	0	5
26	0	5	5	Basic 2 wks then 8 units ACTH IM daily until autopsy	0	0	0	0	5	5
27	5	0	5	Basic 2 wks then Basic & MCA until autopsy	0	0	0	5	0	5
28	0	5	5	Basic 2 wks then Basic & MCA until autopsy	0	0	0	0	5	5
29	0	5	5	Basic 2 wks then 20 mg. Cortisone daily until autopsy	0	0	0	0	5	5
30	5	0	5	Basic 2 wks then 20 mg. Cortisone daily until autopsy	0	0	0	5	0	5
31	0	5	5	Basic & MCA 2 wks then m'MeDAB until autopsy	0	0	0	0	5	5
32	5	0	5	Basic & MCA 2 wks then m'MeDAB until autopsy	0	0	0	5	0	5

DIETS

Separate spoons and jars were used for each diet.

1. Purina Laboratory Chow is manufactured in pellet form by the Ralston Purina Company of St. Louis, Missouri. It contains

Crude Protein, not less than	23%
Crude Fat, not less than	5%
Crude Fibre, not less than	6%
Nitrogen-free extract	44%
Ash, not more than	9%
Bone Meal	1%
Sodium Chloride	0.5%
Magnesium Sulfate	0.2%
Vitamins A, D, B_{12}, Riboflavin, Niacin, Thiamin, and Brewers Yeast.	

2. The basic synthetic diet was formulated by Griffin. (75)

Casein	18%
Glucose Monohydrate	73%
Corn oil	5%
Wesson Salt Mixture containing NaCl, $Ca_3(PO_4)_2$, $MgSO_4$, KCl, FeSi, NaF, KI, $CuSO_4$, KH_2PO_4, $K_2Al_2(SO_4)_2$	4%
Thiamin	60 mgm / 20 Kg.
Riboflavin	40 mgm / 20 Kg.
Pyridoxine	50 mgm / 20 Kg.
Calcium Pantothenate	140 mgm / 20 Kg.
Choline	10 grams / 20 Kg.

3. The m'MeDAB diet was made by adding 6.0 grams of m'MeDAB to 10 Kg. of basic diet giving a 0.06 percent mixture. The food was mixed in large barrels with a spoon.

4. The m'MeDAB plus NCA diet was prepared by adding 0.2 grams of NCA to 3000 grams of the m'MeDAB diet. This gave a 0.067 percent mixture of NCA.

HORMONE INJECTIONS

1. ACTH was manufactured by the National Drug Company. It contained 20 units per cc. The animals received 0.4 cc, which was injected into the back muscles.

2. Cortisone acetate was manufactured by Merck and Company. It contained 25 mgm per cc. The animals which were to receive this preparation received 0.8 cc. This was injected into the back muscles.

TECHNIQUE OF AUTOPSIES

The animals were killed at the intervals stated by placing them in a bottle with a sponge soaked with commercial ether. The animal was then weighed, and a note was made as to the appearance and the physical condition of the animal. Two and one half cc of blood were drawn from the inferior vena cava and were placed in test tubes containing a measured amount of dried oxylate. A blood smear was taken from the fresh blood, and a bone marrow smear was taken from the femur and prepared with Giemsa stain. The specific gravity of the blood was determined by the copper sulfate method described in The Laboratory Manual of the United States Army. (76)

At the time of autopsy two small sections of the liver were taken from portions of the liver which suggested pathology. The

interior portion of the lobes were used so as not to get too much of the capsule on the slide. The small tissues of liver were crushed and smeared on the slide with a spatula and were immediately placed in a Koplan Jar of Vandegrift's solution. They were allowed to remain for 15 minutes, and then they were transferred to a Koplan Jar containing 20 to 30 percent Dioxan in 70 percent isopropyl alcohol. They were left in the alcohol for approximately 15 minutes, and then they were placed in a Koplan Jar containing 70 percent isopropyl alcohol. The length of time they were left in the isopropyl alcohol was not crucial, but they were usually stained the same day with Eosin, Orange G, and Hematoxylin. The sections were then mounted in Canada Balsam.

The pituitary, thyroid, parathyroid, trachea, esophagus, lungs, stomach, pancreas, liver, spleen, kidneys, adrenals, ovaries, tubes, uterus, and testes were studied grossly and microscopically. Fat stains were made of one adrenal. The animal weight and the weights of the adrenals, pituitary, and liver were recorded. Animals which died during the course of the experiment were not included in the final analysis, because many of the above procedures could not be performed on the dead animals.

The tissues were fixed immediately with Vandegrift's Fixative, (77) and they were then stained with Eosin, Orange G, and Hematoxylin.

Vandegrift's Fixative

Ethyl alcohol 95%	80.0 cc
Formalin, full strength	12.0 cc
Glacial Acetic Acid	4.5 cc
Picric Acid	4.0 Gms
Mercuric chloride	0.2 Gms
Urea	0.5 Gms

OBSERVATIONS AND RESULTS

DESCRIPTION OF PATHOLOGIC CHANGES WITHIN THE LIVER

In order to understand more clearly the different changes which occur in the liver of the experimental animals, a brief description is given, and appropriate illustrations are presented.

The earliest change consists of cirrhosis. In its mild or early stage the only gross change is a slight paller. Histologically one sees a proliferation of fibroblasts and a deposition of collagen beginning in the portal areas and extending out through the interlobular spaces. There is usually no distortion of the lobule until the cirrhosis has advanced to a moderate degree, at which time the cirrhosis is readily detectable grossly.

Bile duct proliferation as a morphologic change is to be distinguished from cirrhosis, since it does not usually occur with the mild stages of the latter. Bile duct proliferation accompanies the later stages of cirrhosis.

Usually, though not always, while the bile duct proliferation is becoming more severe, benign hepatomas appear. These are distinguished by a delicate capsule of fibrous tissue. The parenchymal cells comprising them have a distorted, non-lobular arrangement. They are larger, less uniform, and stain more palely than normal cells. Their nuclei are slightly enlarged and have prominent nucleoli. There is no peripheral bile duct proliferation about these benign hepatomas.

Grossly the benign hepatomas cannot be distinguished from large areas of hyperplasia in cirrhosis or from carcinoma. The carcinoma

never occurs without accompanying cirrhosis, which is usually severe, but occasionally may be mild. Bile duct proliferation is also present. Hepatic carcinomas are rarely unaccompanied by benign hepatomas. The carcinomas may be of two general types, those arising from bile ducts and those from the parenchymal or cord cells.

EFFECTS OF THE BASIC DIET

The animals on the basic diet were followed until the thirty-sixth week. The growth of the animals progressed, although it was somewhat slower than in animals fed the laboratory chow. The liver tissue could not be distinguished from that of normal laboratory animals. No significant differences were noted in the organ weights, hemoglobin, hematocrit, plasma proteins, and the fat content and distribution in the adrenal glands. The only tissue change was the chronic lung disease seen in the majority of the laboratory animals. This, however, was of no greater incidence than in the colony controls.

EFFECTS OF THE m'MeDAB DIET

There was an initial weight loss during the first 2 weeks, but after this time the animals began to gain weight. Between the third and the ninth week a considerable number of animals rapidly became debilitated and died. Liver changes were minimal during this time, and death was usually attributable to severe pulmonary disease. In those animals sacrificed on schedule, milder pulmonary disease was present. In these latter animals no significant differences were noted in the organ weights, blood work, or adrenal studies.

At the sixth week, the first interval of observation after initiation of m'MeDAB, all animals showed cirrhosis of mild to severe

degree. Bile duct proliferation was seen in the majority of animals, and benign hepatomas were common. An unexpected finding was a carcinoma in one animal sacrificed at the sixth week.

After the ninth week all the animals but one showed benign hepatomas. Carcinomas were found sporadically up to the eighteenth week. At this time, and thereafter, all animals had hepatic cancers except two, one at the twenty-seventh week and one at the thirty-ninth week.

The carcinomas most commonly metastasized to the splenic lymph nodes and to the lungs. Other sites of metastases were the pancreas, spleen, and omentum.

Liver smears revealed cellular changes compatible with cancer in all animals found to have cancer on histologic sections.

EFFECTS OF DELAYED ADDITION OF MCA TO ANIMALS ON m'MeDAB

MCA Given After 3 to 5 Weeks on m'MeDAB:

No carcinomas were found. Benign hepatomas occurred, but their appearance was delayed. Cirrhosis occurred in all animals, but it was of milder degree than that observed in the m'MeDAB controls.

MCA Given After 6 to 10 Weeks on m'MeDAB:

Carcinomas were found in groups 6, 8, 9, and 10, but the appearance was delayed, and the incidence was lower than those animals receiving the m'MeDAB diet without MCA. In group 7 no carcinomas occurred. Benign hepatomas were found in all of these groups, but they were delayed. Cirrhosis appeared in all groups, but the severity was less than that found in the m'MeDAB animals.

MCA Given After 12 Weeks on m'MeDAB:

In those animals which had MCA added after 12 weeks on m'MeDAB, no significant differences in the number of carcinomas or benign hepatomas or in the time of their appearance from those on m'MeDAB could be noted. These relationships are shown in Figures 1, 2, 3, and 4.

General Condition of the Animals:

Animals which had MCA added before the tenth week, had a much lower mortality rate than did those on the m'MeDAB diet. As was observed in animals on m'MeDAB, there was a slight loss in weight after the addition of MCA, but after 2 to 3 weeks the animals began to gain weight. The gross appearance of the livers in these groups was remarkable better than those on the m'MeDAB diet.

ADRENAL GLANDS

Many changes in numbers of vacuoles, size of cells, vascularity, and compactness of cells could be noted. There was, however, no consistent change on either fat or hematoxylin and eosin stain that could be correlated to time on the drugs or time at which the MCA was added. The adrenal weights and adrenal weight to body weight ratios showed no consistent trend.

LIVER SMEARS AND HEMATOLOGIC STUDIES

Changes in the individual liver cells in the smear preparations could not be relied upon for a diagnosis of the type of diet the animal had received. The blood work showed no consistent changes which could be correlated to the administration of the carcinogen or the inhibitor.

EFFECTS OF PRELIMINARY BASIC DIET BEFORE ADDITION OF m'MeDAB AND NCA

This experiment was designed to determine any early changes which might occur on the various diets of NCA and m'MeDAB. These animals were then killed at the rate of one a day for ten days, and the experiment was not carried beyond this point. No changes were noted, and these were recorded as normal animals.

EFFECTS OF ACTH AND CORTISONE

ACTH or cortisone was given after twenty animals had been on basic diet for two weeks. These animals were killed at the rate of one every other day for ten days. No changes which were statistically significant could be noted. Animals on cortisone tended to show many vacuoles in the liver cells.

MISCELLANEOUS FINDINGS:

The following changes occurred rarely, and they seemed to bear no relationship to any particular experimental diet.

1. Atrophy of the testes.
2. Parasitic cysts of the liver and kidneys.
3. Inflammations such as brain abscesses, lymphocytic infiltration of the thyroid, pancreatic fibrosis, fibrous pericarditis, perisalpingitis, hydronephrosis, pyelonephritis, otitis media, focal myocarditis, and chronic hypertrophic cystic gastritis.
4. Adenomas of the parathyroid, adrenal, pancreas, and lungs.
5. Sarcoma of the muscle of the thigh.
6. Squamous metaplasia of the uterus.
7. Adenocarcinoma of the breast.

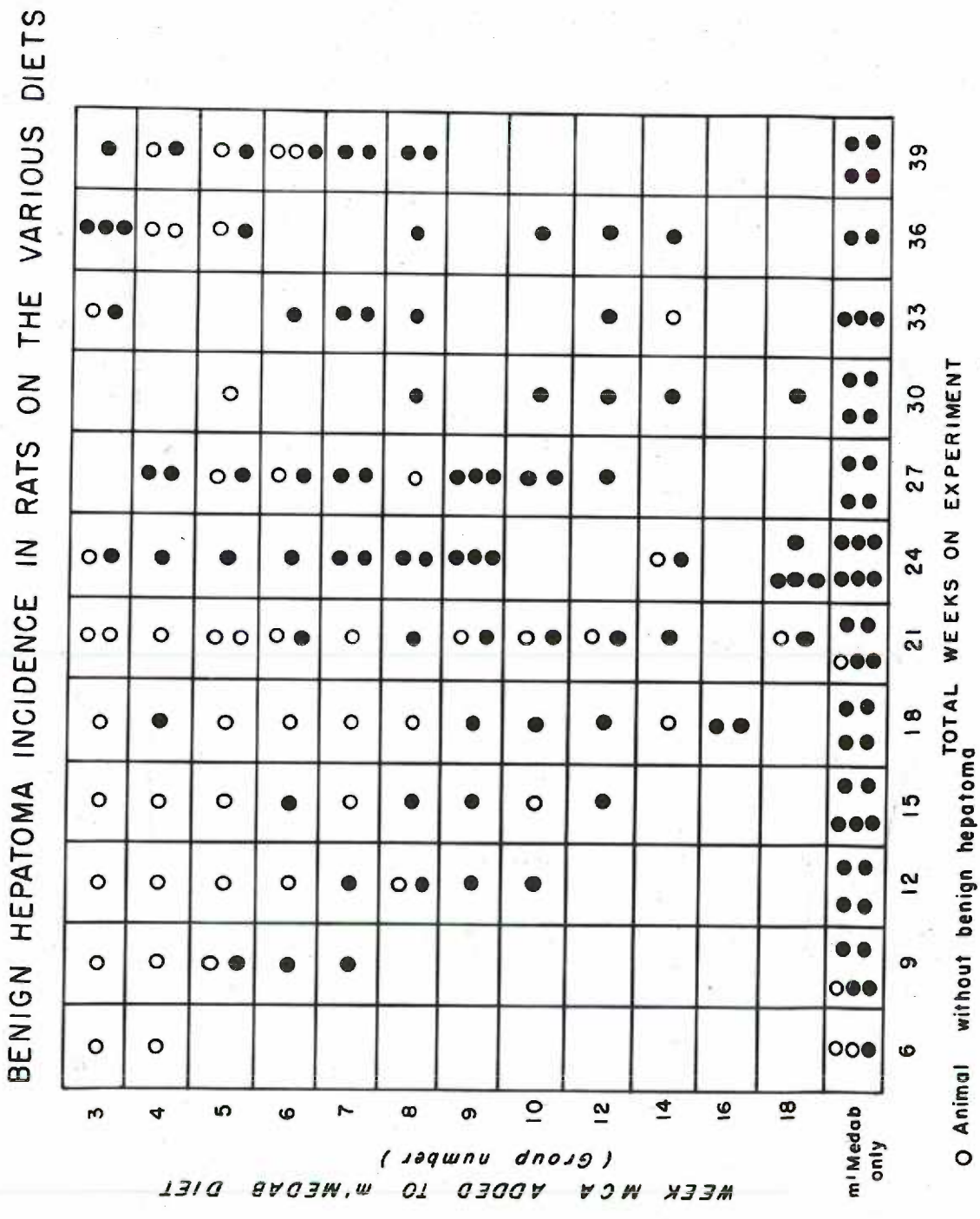

BENIGN HEPATOMA INCIDENCE IN RATS ON THE VARIOUS DIETS
WEEK MCA ADDED TO m'MEDAB DIET
(Group number)
3
4
5
6
7
8
9
10
12
14
16
18
m'Medab only
6
9
12
15
18
21
24
27
30
33
36
39
TOTAL WEEKS ON EXPERIMENT
O Animal without benign hepatoma
● Animal with benign hepatoma

FIGURE 1

Figure 5. 372 male, m'MeDAB 21 weeks, MCA 18 weeks.

Compare the liver of this animal, which has been on m'MeDAB 21 weeks and MCA 18 weeks, with that of the animal below, which has been on basic diet 13 weeks. Also compare it with 366 on the next page which was on m'MeDAB for 29 weeks and has a grossly detectable liver carcinoma.

Figure 6. 2211 female, Basic diet 13 weeks.

Note the resemblance of the liver of this control animal to the animal above which was on m'MeDAB 21 weeks and MCA 18 weeks.

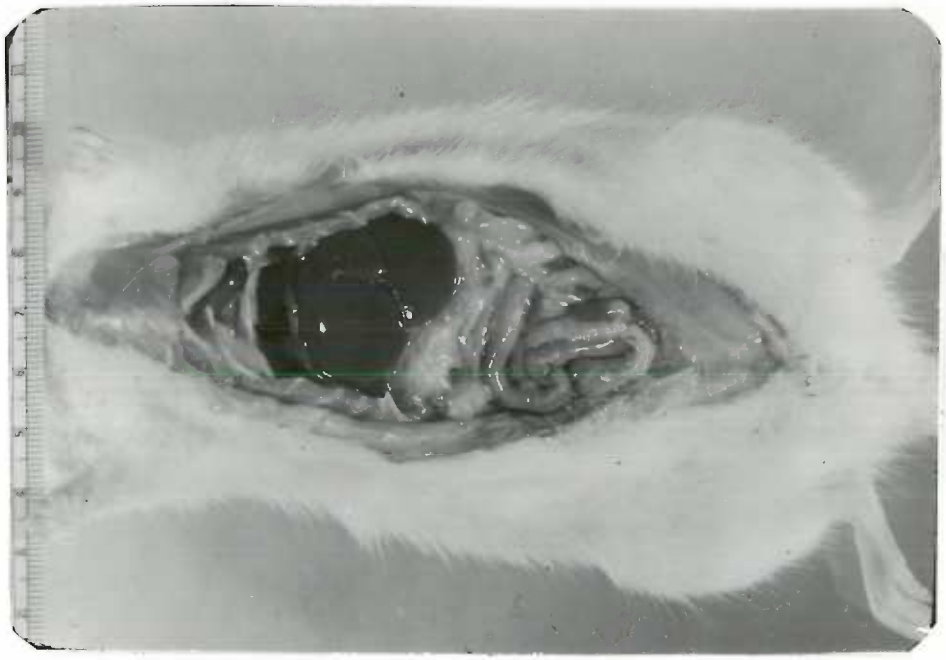


Figure 7. B31 female, m'MeDAB 25 weeks, NCA 19 weeks.

Note the normal appearance of the liver of this animal, which was on m'MeDAB 25 weeks and NCA 19 weeks, as contrasted with that of the animal below, which has a liver carcinoma at the end of 29 weeks on m'MeDAB.

Figure 8. B66 male, m'MeDAB 29 weeks.

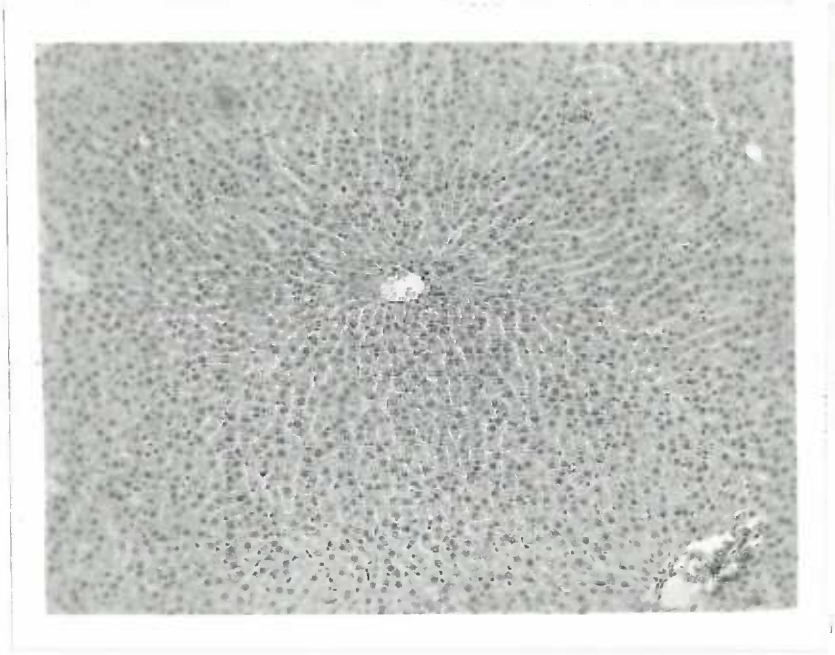
This animal has a readily detectable carcinoma at the end of 29 weeks on m'MeDAB.

Figure 9. B213 female, Basic diet 17 weeks.

This is the appearance of the liver of a normal control animal on a basic diet 17 weeks. (x 100).

Figure 10. B213 female, Basic diet 17 weeks.

This picture shows a smear from the same liver pictured above. (x 400).



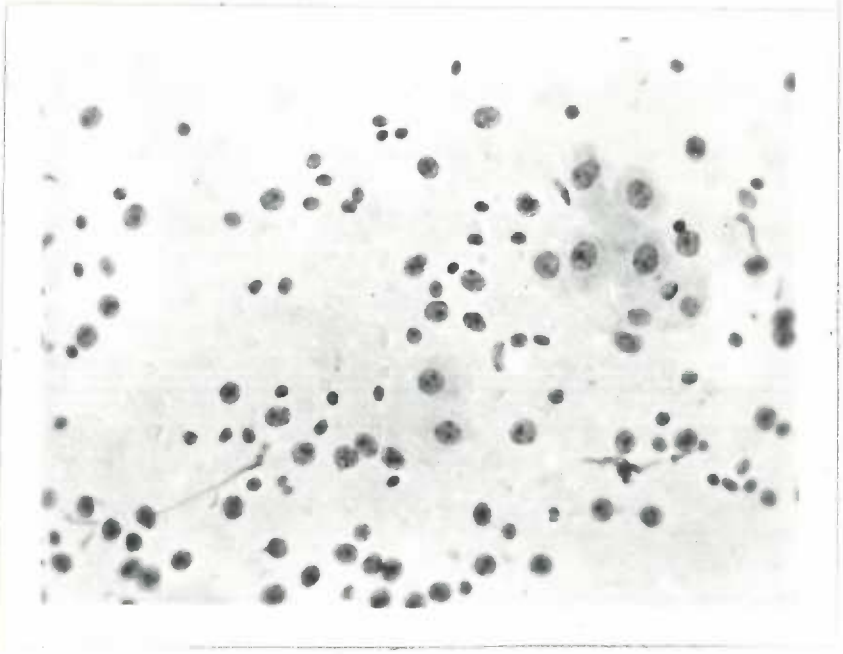
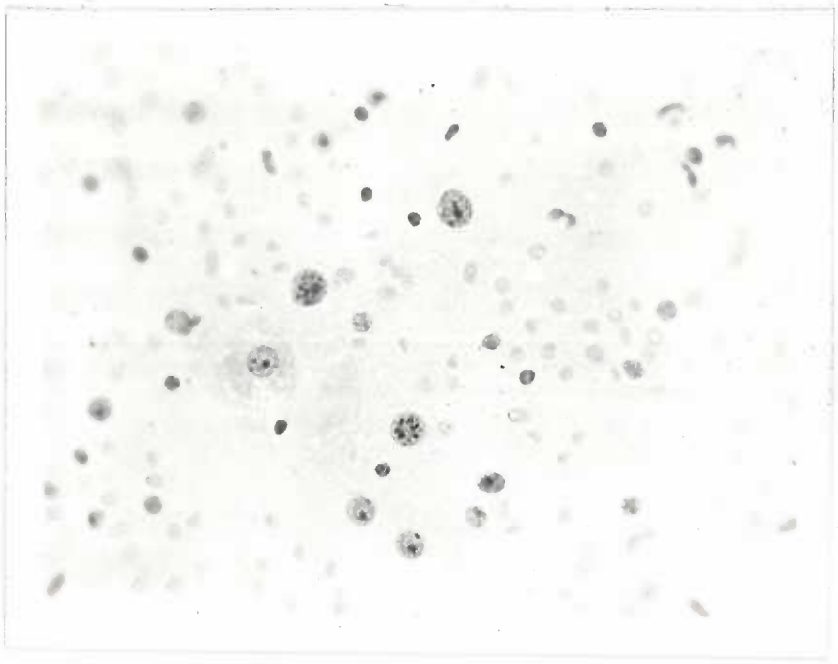


Figure 11. B47 female, m'MeDAB 15 weeks, NCA 7 weeks.

This is a microphotograph of a liver smear illustrating the drug effect nuclei seen in animals which have been on the experimental diet for a few weeks. Compare the large size of these nuclei with those in Figure 10 which are from an animal on basic diet. (x 400).

Figure 12. B66 male, m'MeDAB 29 weeks.

This is a microphotograph of a liver smear showing the bizarre nuclei of carcinoma cells. (x 400).



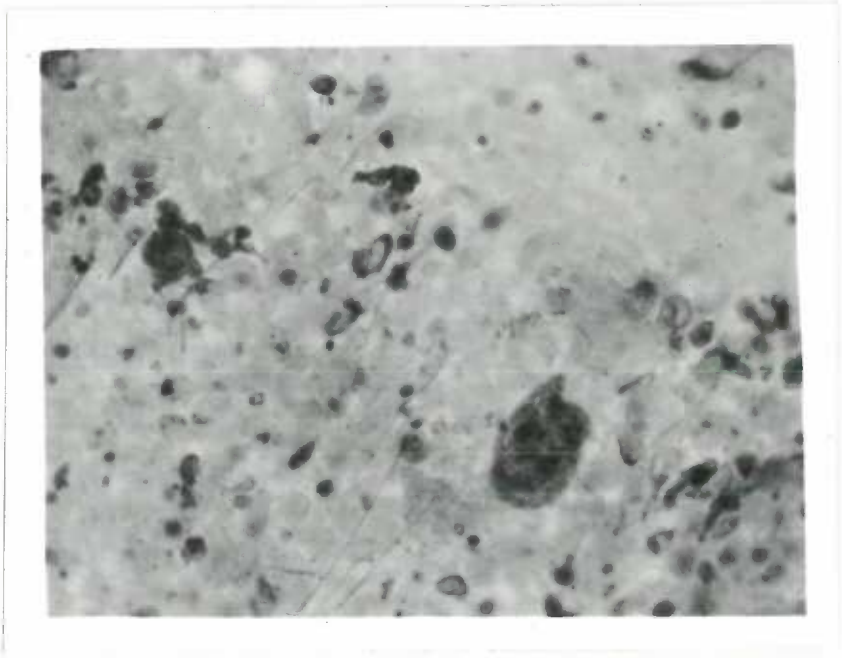
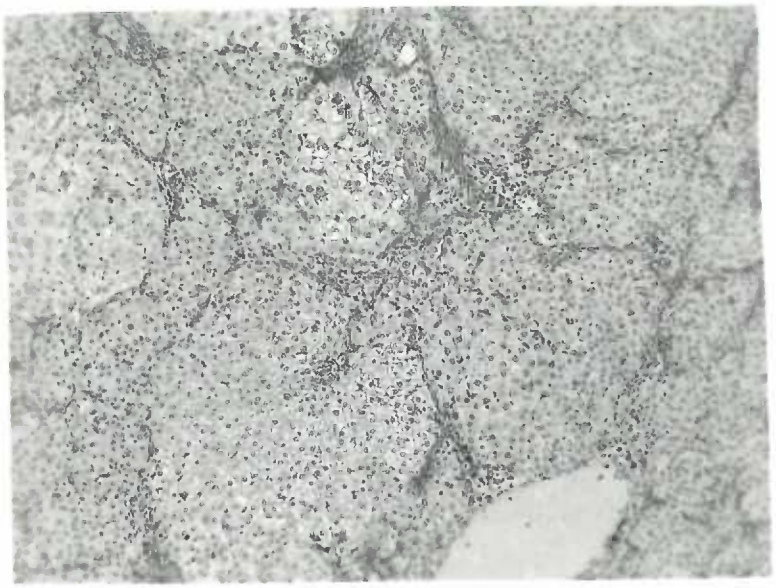


Figure 13. B34 female, m'MeDAB 11 weeks, MCA 4 weeks.

This section of liver shows a moderate amount of cirrhosis with benign hepatomas. (x 100).

Figure 14. B34 female, m'MeDAB 11 weeks, MCA 4 weeks.

This is a higher power view of the same liver section showing the hepatoma cells in more detail. (x 400).



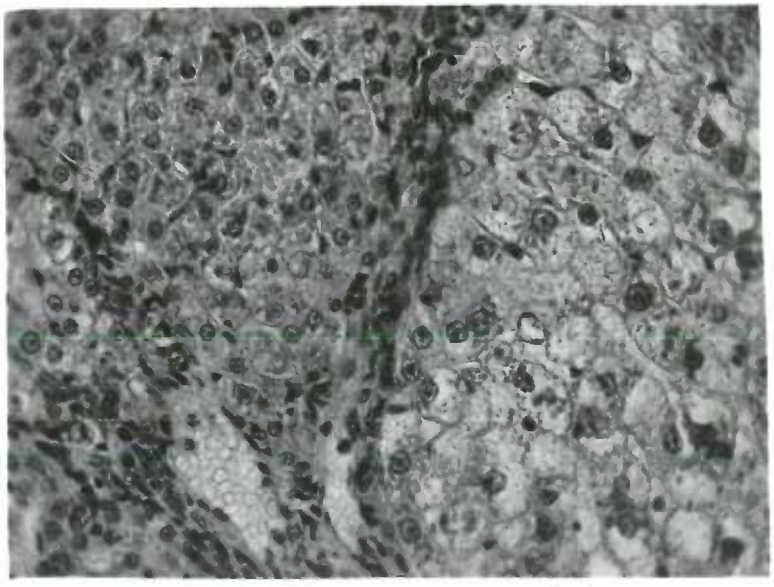


Figure 15. B86 female, m'MeDAB 6 weeks, MCA 2 weeks.

This picture shows the bile duct cysts seen in many of the animals after cirrhosis became advanced. (x 100).

Figure 16. B101 female, m'MeDAB 11 weeks, MCA 2 weeks.

This is an illustration of the fatty metamorphosis frequently seen accompanying the cirrhosis. (x 100).

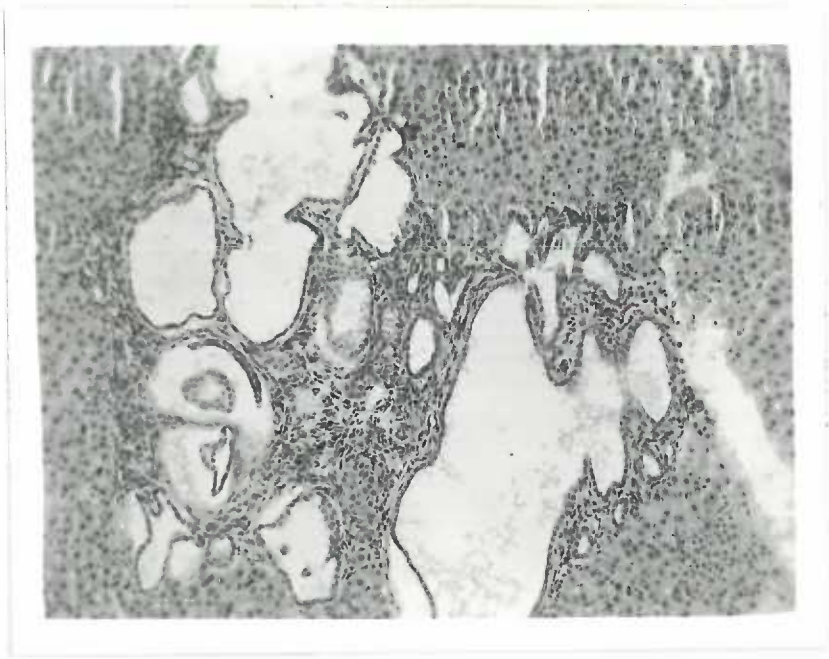


Figure 17. B37 female, m'MeDAB 33 weeks, MCA 26 weeks.

Note the contrast of the mild cirrhosis in the liver of an animal on m'MeDAB 33 weeks and MCA 26 weeks with the carcinoma of the liver in an animal on m'MeDAB 34 weeks. (x 100).

Figure 18. B64 male, m'MeDAB 34 weeks.

This picture shows an anaplastic carcinoma of the liver in an animal on m'MeDAB 34 weeks. (x 100).

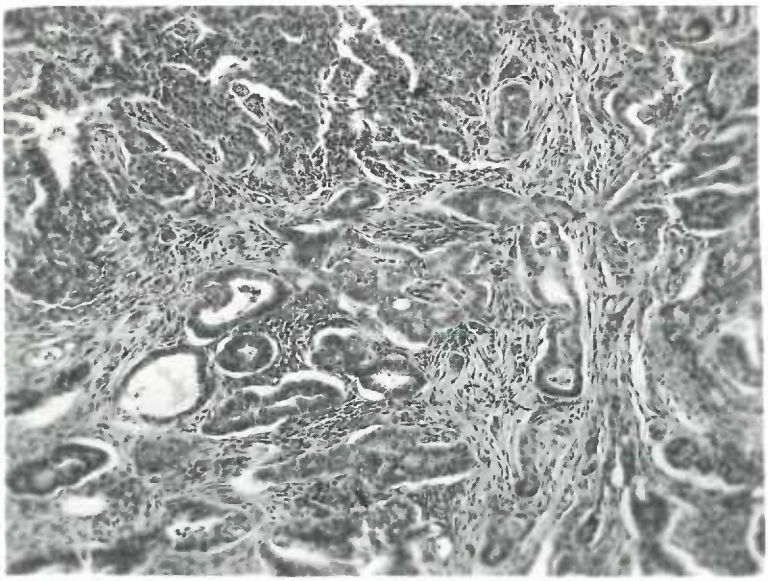
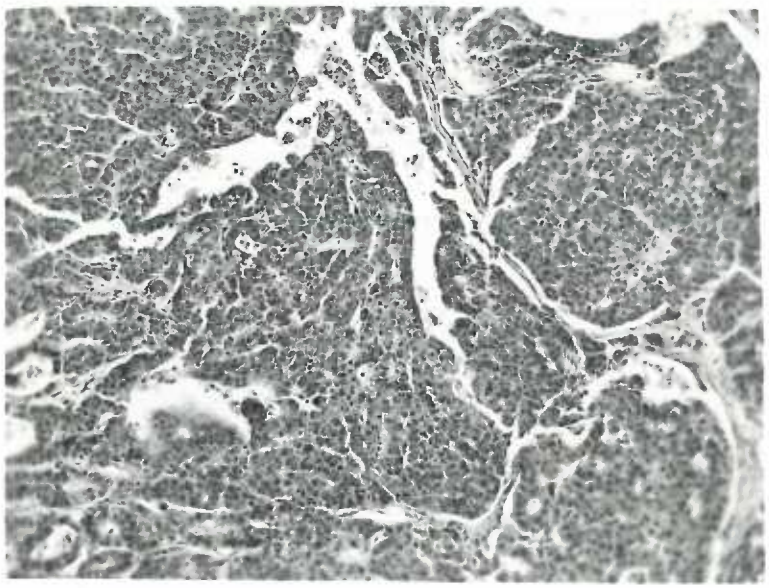


Figure 19. B156 female, m'MeDAB 24 weeks, MCA 10 weeks.

This figure shows an anaplastic carcinoma of the liver. (x 100).

Figure 20. B156 female, m'MeDAB 24 weeks, MCA 10 weeks.

This is a higher power view of the same section of liver as that shown above, giving more cytologic detail of the carcinoma cells. (x 400).



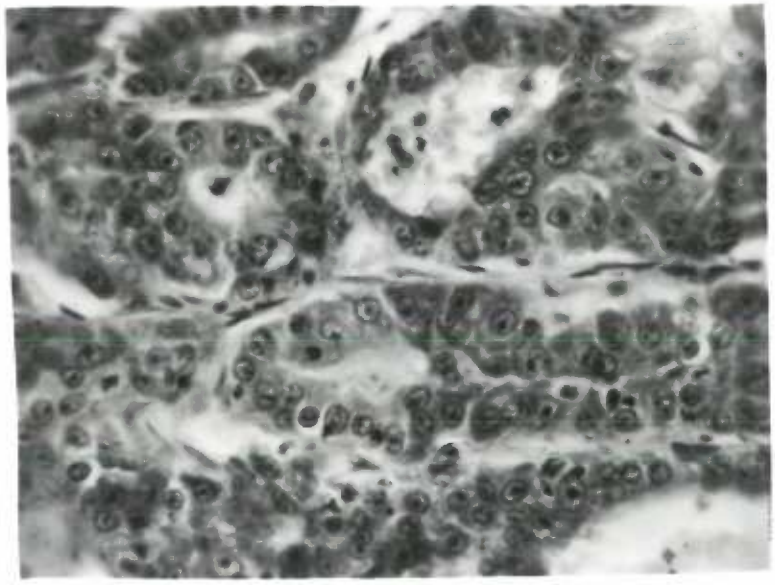
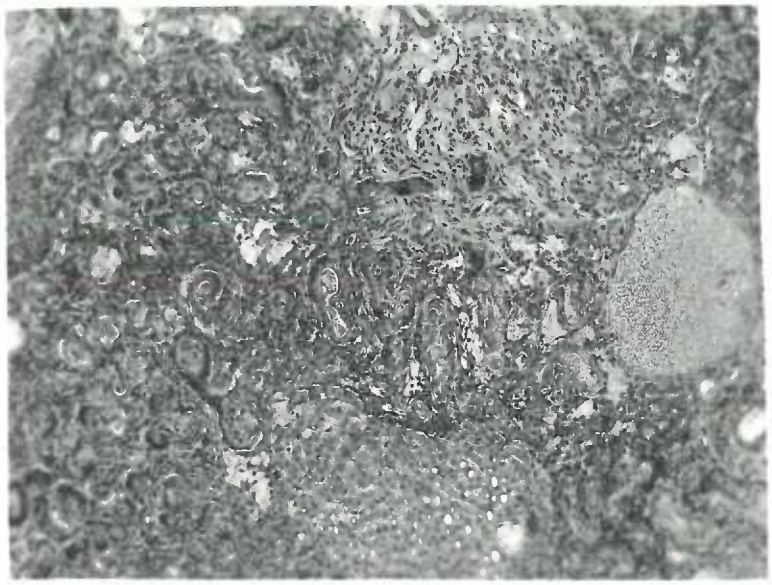


Figure 21. B55 male, m'MeDAB 39 weeks, MCA 31 weeks.

This is a microphotograph of a cholangiofibrosarcoma, a rather uncommon finding in this series of animals. (x 100).

Figure 22. B70 female, m'MeDAB 30 weeks.

This is a microphotograph of an adenocarcinoma of the liver. (x 100).



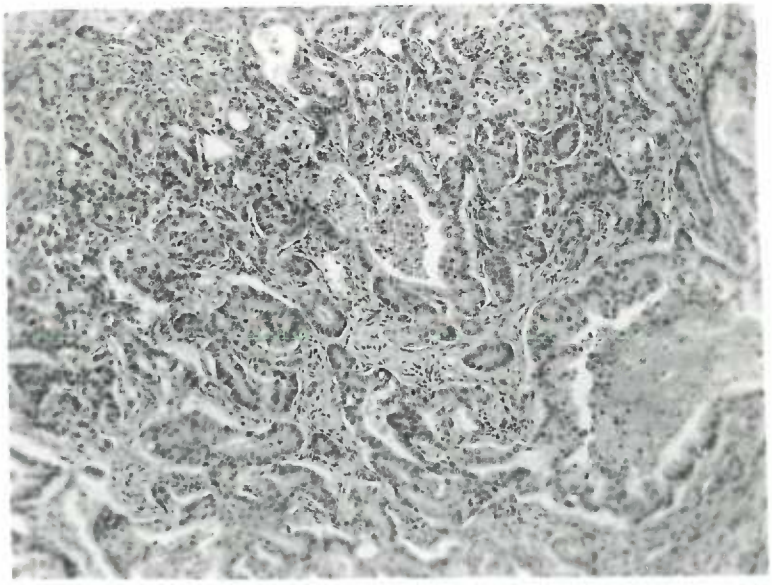
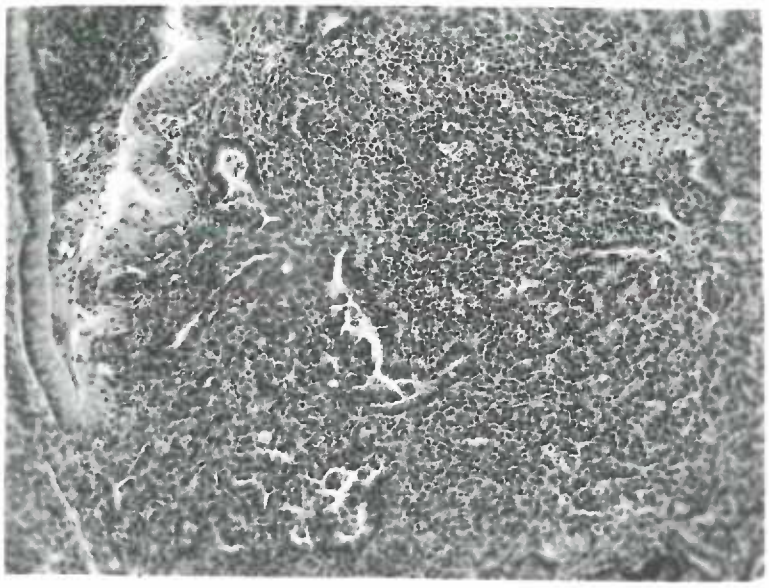


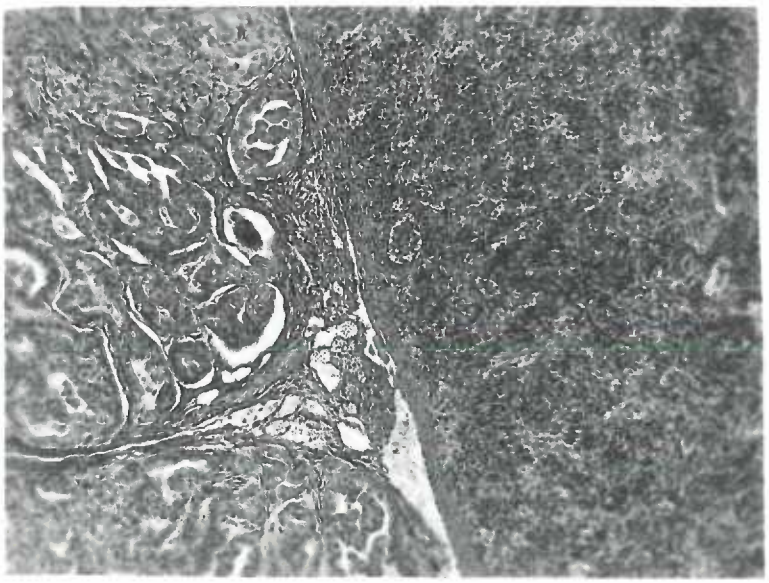
Figure 23. B2k3 male, m'MeDAB 23 weeks.

This photograph shows a metastatic liver carcinoma in the lungs. (x 100).

Figure 24. B2k3 male, m'MeDAB 23 weeks.

This photograph shows a carcinoma of the liver attached to the capsule of the spleen. This was probably the result of direct extension through the peritoneal cavity. (x 100).

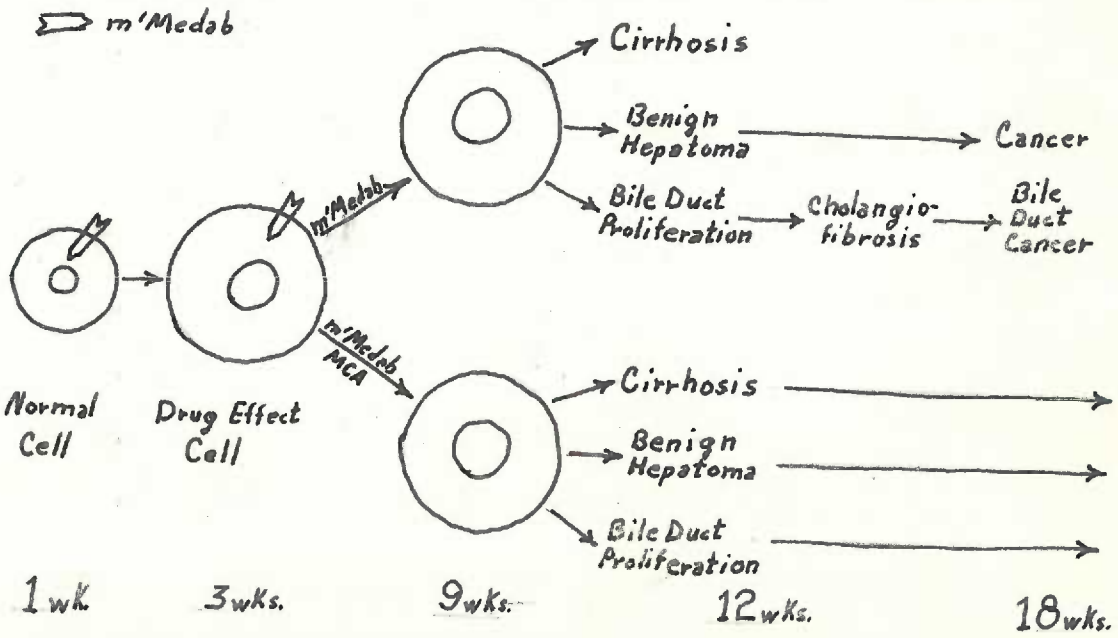




DISCUSSION

This experiment, while dealing primarily with the inhibition by MCA upon m'MeDAB induced carcinomas, also revealed that the other morphologic changes in the liver were inhibited. There is a sequential relation in the development of the cirrhosis, bile duct proliferation, benign hepatomas, and carcinoma. The effect upon the earlier changes is less than that upon the later changes. Thus, cirrhosis always occurs, but it is of a milder form, bile duct proliferation tends to be less marked, benign hepatomas were delayed in their appearance, and carcinoma could be prevented by addition of MCA up to the fifth week and definitely inhibited by addition up to the tenth week.

The time of administration of MCA may be roughly correlated with the time at which the various changes might be expected from m'MeDAB alone. From this correlation it is evident that MCA will not reverse a process that already has been established, but will delay the progression of this stage. This may be schematically presented in the following manner:



The mechanism through which MCA will alter the toxic effects of m'MeDAB is of fundamental importance, since it may afford some means of preventing carcinomas. There are no clues as to the exact mechanism which is involved. Consideration of the mechanisms can be divided into a number of groups, and some of these possibilities can be excluded as a result of this study. Some of these possible mechanisms are:

1. m'MeDAB is neutralized by MCA in the digestive tract by chemical alteration.
2. MCA competes for position on the liver cell and does not allow the m'MeDAB to act.
3. MCA causes some hormonal response which inhibits the induction of the carcinoma.
4. MCA acts directly on the cell and suppresses the carcinoma.

Cortell (47) observed that the m'MeDAB must be given a minimum of 69 days before the dye is stopped if hepatic carcinoma is to occur with any regularity in the following 150 days. For carcinoma to develop in all animals, they must be continued for 30 weeks on the diet. In our study the MCA had no effect on the inhibition of tumors if added after the tenth week of the m'MeDAB diet. Therefore, the m'MeDAB must be acting on the liver and must not have been chemically altered within the intestinal tract. From this it would be reasonable to conclude that MCA does not neutralize m'MeDAB when added earlier than the tenth week.

Since the MCA is apparently excreted through the liver, and m'MeDAB is metabolized or bound to protein in this organ, one might suspect that the site of inhibitory action could be within or upon the liver cell.

One possibility might be the competition for position on the liver cells. Since the m'MeDAB has been acting 5 weeks before the MCA is added, it would seem unlikely that the MCA would compete for position on the cell. Yet, inhibition of the carcinomas is 100 percent at this time.

There is little evidence for or against the hormonal cause of inhibition.

The most likely speculation is that the MCA acts upon the liver cells independently of the m'MeDAB and suppresses the evolution from what may be broadly termed the "precancerous changes." It would appear that this effect is upon the bile duct and hepatic parenchymal cells. This action can, therefore, scarcely be specific for one cell type. From this lack of specificity on any specific cell one would suspect there was some effect on the growth of hepatic tissue.

MCA has been known to slow growth temporarily. It could affect the liver cells in this manner. The m'MeDAB could cause the uncontrolled growth by stimulating the cells through injury. But the MCA does not slow the growth of the liver cancer. The present study indicated no diminution in the incidence of carcinoma within the cancer-time phase of m'MeDAB.

While the present study has eliminated some of the possible mechanisms of action, it is obvious that the exact mechanism of MCA inhibition awaits further experimental study. Some of the information of value would be obtained by the following suggested experiments:

1. The determination of protein bound dye levels in the liver during MCA administration.

2. The determination of the ability of tissue slices of benign hepatomas and carcinomas to metabolize m'MeDAB.

3. The determination of the ability of MCA to aid the liver in metabolizing m'MeDAB.

4. The determination of the exact effect of shorter periods of feedings of m'MeDAB.

5. The determination of the hormonal effects of inhibition by castration, adrenalectomy, and hypophysectomy.

6. The determination of the effect of "protecting" the liver with MCA , and then adding the m'MeDAB.

C O N C L U S I O N S

1. It is confirmed that in the majority of animals, m'MeDAB alone causes cirrhosis at 6 weeks, bile duct proliferation at 9 weeks, benign hepatomas at 12 weeks, and carcinoma at 18 weeks.

2. MCA will inhibit the m'MeDAB induced carcinoma completely, if given 3 to 5 weeks after m'MeDAB has been given.

3. MCA will inhibit carcinomas to some extent, if given 6 to 10 weeks after m'MeDAB has been given.

4. After 10 weeks MCA has no effect in inhibiting the carcinoma.

5. MCA inhibits benign hepatomas before 6 weeks to some extent, but has no effect after the animal has been on the m'MeDAB 6 weeks.

6. MCA has no apparent toxic effect on the animals.

7. MCA does not completely inhibit the production of cirrhosis, but it causes a less rapid and less extensive progression of the process.

8. Liver smear changes can be demonstrated.

9. No demonstrable changes occur in the adrenals or in the plasma proteins, hemoglobin, or hematocrit.

10. MCA probably inhibits by some specific cellular action, and some speculations are made as to the mechanisms through which MCA inhibits the carcinogenic activity of m'MeDAB.

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