blood sterol levels. Their coats improved when treatment was stopped.

Summary. In normocholesterolemic cockerels the bile acid binding polymer, cholestyramine resin (MK-135), lowered plasma cholesterol concentrations when fed in the diet; the hepatic cholesterol synthesis inhibitor, benzmalecene, lowered cholesterol levels when given by injection, but not when fed in the diet. In combination, their effect was additive. In dogs, feeding of cholestyramine resin plus benzmalecene or triparanol (MER-29) had additive cholesterol-lowering effect.

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Phagocytosis of Colloidal Carbon by the Reticuloendothelial System During Hepatocarcinogenesis in Rats. (26896)

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Evidence that the reticuloendothelial system (RES) may be involved in the carcinogenic process has been reviewed by a number of investigators(1,2,3). Several different types of colloids which act directly on the RES have been implicated in the development of tumors(4,5,6). There is also evidence that procedures which stimulate or block the RES will affect the carcinogenic process in animals being fed azo dyes(7,8,9).

It was of interest, therefore, to determine the functional capacity of the RES during the carcinogenic process by measuring the rate of phagocytosis of colloidal carbon. Rates of phagocytosis were determined in rats fed the carcinogens 3'-methyl-4-dimethylaminozobenzene (3'-Me-DAB) or DL-ethionine as well as the basal diets and a weakly carcinogenic azo dye 4'-methyl-4-dimethylaminozobenzene (4'-Me-DAB). A combination of DL-methionine with DL-ethionine was also employed since methionine has been shown to eliminate the liver damage and tumors caused by ethionine(10). Studies with hypophysectomized rats on a 3'-Me-DAB diet were included, since removal of the pituitary gland has been shown to suppress tumor formation in rats fed azo dyes (11). Administration of ACTH or growth hormone will partially restore tumor forma-

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tion in hypophysectomized rats fed diets containing 3′-Me-DAB(12). Combinations of ACTH and insulin have also been shown to restore tumor formation(13).

Methods. Groups of female Holtzman rats were given 0.06% 3′-Me-DAB or 4′-Me-DAB in the semi-synthetic, riboflavin-deficient diet described by Medes *et al.*(14). Basal diets without carcinogen were prepared by the same method and given to a group of control rats. Composition of diets containing 0.6% DL-methionine and/or 0.25% DL-ethionine have been reported by Farber and Ichinose (10). With the exception of one group, all animals on the basal diet were fed *ad libitum*. This group of 12 rats was pair-fed the amount consumed by a similar group receiving 3′-Me-DAB. Since only a slight increase in phagocytosis was noted in the pair-fed animals, the results were included with those obtained under *ad libitum* conditions.

The activity of the RES was determined by slight modifications of the method of Biozzi *et al.*(15). A suspension of carbon (C11/143a)† was injected at the rate of 16 mg/100 g body weight into the femoral vein. The rats were kept under light ether anesthesia during administration of carbon and sampling of the blood. At appropriate intervals blood was collected from the tail vein and a 0.02 ml sample was pipetted into 5 ml hemoglobin diluent (1 g $NaHCO_3$, 50 g KCN, and 200 mg $K_3Fe(CN)_6$ diluted to 1 liter). The concentration of carbon was determined by its absorption at 540 mμ in a Beckman DU spectrophotometer. The blanks were samples of blood obtained prior to injection of carbon. Blood carbon concentration decreased according to an exponential function of time expressed as follows:

$$\log C_1 - \log C_2/T_2 - T_1 = K$$

C_1 and C_2 were the concentrations of carbon in the blood at times T_1 and T_2 respectively. K, which expressed the phagocytic activity of the RES for the injected dose, was called the phagocytic index.

It has been shown that the phagocytic index depends on the weights of the liver and spleen(15). This allows the use of a corrected phagocytic index (α) expressed as:

$$\alpha = \frac{W}{WLS}\sqrt[3]{K}$$

W is body weight and WLS the sum of weights of liver and spleen.

The hypophysectomized rats used in these experiments were Sprague-Dawley females obtained from Hormone Assay Laboratories, Chicago, Ill. One group received daily subcutaneous injections of 0.5 unit of ACTH and 0.0075 unit of insulin,‡ the other group served as a control. Both groups were fed the diet containing 3′-Me-DAB.

Results. Liver weights, phagocytic index (K), and corrected phagocytic index (α) after various times on the azo dyes are shown in Fig. 1. Rats on the basal diet had smaller livers per 100 g body weight than those on any of the azo dyes or groups maintained on Rockland chow. The increase in liver weight after 8 weeks on 3′-Me-DAB coincides with the time when nodules first became visible on the liver.

The phagocytic index of animals fed 3′-Me-DAB increased during the first week and was maintained at high activity through the seventh week. After this time a sharp decline in uptake of colloidal carbon was observed. The phagocytic index of the rats receiving the weak carcinogen 4′-Me-DAB or the basal diet remained low throughout the 15-week feeding period.

Rats receiving the carcinogen 3′-Me-DAB showed an increase in the α value through 7 weeks and then a sharp decline. This decline in the K and α after 8 weeks was not due to inability of the RES to respond to stimulation, since intravenous administration of 5 mg Zymosan§(16) to rats fed 3′-Me-DAB for 10 weeks resulted in marked stimulation of the rate of carbon clearance.

Feeding ethionine also stimulated the rate of clearance of colloidal carbon (Fig. 2).

† John Hemschel and Co., Inc., 105 E. 29th St., New York.

‡ACTH from Orgonon Inc., W. Orange, N. J., Protamine Zinc insulin from Eli Lilly and Co., Indianapolis, Ind. Doses used were suggested in a personal communication from Dr. A. C. Griffin.

§ Lot No. 13335S from K and K Laboratories, Jamaica, N. Y.

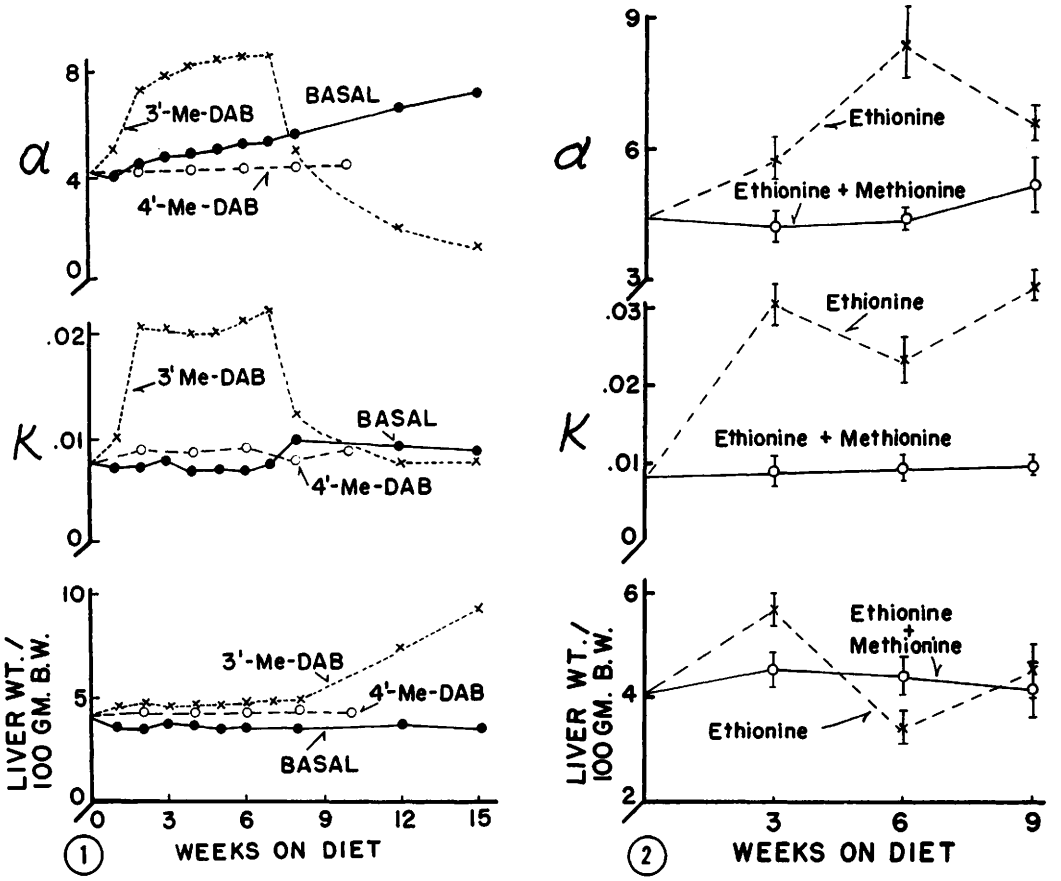

FIG. 1. Liver weights, phagocytic index (K) and corrected phagocytic index (α) of rats fed various azo dyes. Each point is the avg value obtained on from 3 to 6 rats.

FIG. 2. Liver weights, phagocytic index (K) and corrected phagocytic index (α) of rats fed ethionine or ethionine plus methionine. The brackets represent stand. error of the mean value obtained on 4 animals.

When methionine was included in the diet along with ethionine, liver weights and carbon clearance rates remained near normal levels.

Hypophysectomized rats which were fed the diet containing 3'Me-DAB had about the same rate of clearance of colloidal carbon as animals on the basal diet (Table I). When insulin and ACTH were administered to hypophysectomized rats fed 3'-Me-DAB phagocytic activity was again increased.

Discussion. An early increase in the phagocytic index was consistently noted in rats receiving the carcinogens 3'-Me-DAB or ethionine. This increase was not noted in animals fed the basal or weakly carcinogenic diets. It has been shown previously that the effects of ethionine in hepatocarcinogenesis can be partially reversed by methionine(10). The effects of ethionine on phagocytosis of colloidal carbon are also reversed by methionine (Fig. 2). Likewise, it is of interest that the decreased tumor formation after hypophysectomy and the increase after administering insulin and ACTH are paralleled by corresponding changes in the rate of carbon clearance.

The increased phagocytic index between the first and eighth weeks on a diet containing 3'-Me-DAB corresponds quite well with the preneoplastic period. Foci of tumor nodules were consistently noted in all animals after the eighth week of dye feeding and in agreement with others(17,18) tumor incidence at 4 to 6 months was 100%.

Early stimulation of the RES in rats re-

TABLE I. Effect of Hypophysectomy and Administration of ACTH and Insulin to Hypophysectomized Rats on Phagocytic Activity of Animals Fed Diets Containing 3'-Me-DAB.

Wk on diet	Hypophysectomized rats: 3'-Me-DAB	Hypophysectomized rats: 3'-Me-DAB, insulin and ACTH*
	Phagocytic index—k	
1	.014 (2)†	.006 (2)
2	.007 (2)	.016 (2)
3	.008 (2)	.013 (2)
4	.007 (2)	.014 (2)
5	.005 (1)	.016 (2)
6	.007 (2)	.033 (2)
7	.010 (1)	.031 (1)

* Daily subcut. injections of .5 unit of ACTH and .0075 unit of insulin.
† No. of animals is shown in parentheses.

ceiving carcinogenic diets and elimination of the carcinogenic potency of azo dyes by splenectomy(9) or injections of trypan blue (8), thorotrast or iron oxide(7), suggest an essential role of the RES in hepatocarcinogenesis. Further work will be necessary to establish and clarify the function of the RES in this complex process.

Summary. An early stimulation in the clearance of colloidal carbon by the reticuloendothelial system was noted in rats fed 3'-methyl-4-dimethylaminozobenzene or DL-ethionine. No stimulation was observed in animals receiving the basal diet or 4'-methyl-4-dimethylaminozobenzene. Elimination of the carcinogenic potency of the azo dye by hypophysectomy and of ethionine by methionine abolished the stimulation of phagocytic activity by these carcinogens. Administration of ACTH and insulin restored phagocytic activity in hypophysectomized rats fed diets containing 3'-methyl-4-dimethylaminozobenzene.

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Effect of Age, Temperature and Inoculum Size on Non-Infective Vaccinia Virus Yields in Eggs.* (26897)

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Ratios of 2 biological properties of viruses, such as the ratio of infectivity to hemagglutination units, have been used frequently in studies of the growth characteristics of some viruses. With such technics von Magnus(1) and others(2,3) have demonstrated the appearance of incomplete influenza virus in certain growth situations wherein the proportion

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