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Effects of a Hepatocarcinogenic Diet on Adrenal Glands and Liver Of Rats. (30968)

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There is an increasing interest in the hormonal influences in liver carcinogenesis and the role adrenal glands play in it(1-7). The adrenal glands of rats bearing primary hepatomas induced by azo-dye, show marked histological changes including heavy deposit of lipoid droplets which stain deep red with scharlach R(4). These observations suggested further study of the chemistry of these lipoids of the adrenal glands of rats during azo-dye carcinogenesis.

This paper reports the effect of a low-protein, low-riboflavin diet alone and with an azo-dye carcinogen on the chemistry of the adrenal glands and liver of rats during the induction period of the primary hepatoma and after.

Materials and methods. Rats of the NIH Osborne-Mendel strain were used in all experiments. They were about 3 months old at the start of experiments and males weighed 150-175 g and females 100-125 g. One group of rats was used to study the effects of the hepatocarcinogenic diet and a second group to study the effects of hormonal changes on the chemistry of the adrenal glands and liver.

The diets used in these experiments were: 1) Purina Laboratory Chow Pellets—standard control diet; 2) Semi-Synthetic Diet—low protein, low riboflavin diet as described before(8); and 3) Hepatocarcinogenic Diet—semi-synthetic diet in which 0.06% N,N-dimethyl-*p*-(m-tolylazo) aniline (3'-Me-DAB) was incorporated.

The early effects of hepatocarcinogenic diet on the chemistry of the adrenal glands and liver were studied by feeding hepatocarcinogenic diet to male rats for 2, 5, 7 and 17 weeks. Rats fed on Purina Laboratory Chow pellets and those fed on semi-synthetic diet were used as controls.

The effects of hormonal changes were studied on male and female rats, intact or gonadectomized. A month after the surgical procedures, the rats were fed the hepatocarcinogenic diet for 17 weeks and the experiment terminated 4 weeks later. During the experimental period some animals received implants of hormone pellets. In all there were 6 subgroups according to treatment as listed in Table 2. Hormone pellets were prepared in melted cholesterol and a weighed pellet containing 50 mg of hormone was implanted under the skin at the start of the carcinogenic diet and another 8 weeks later. Diethylstilbesterol and testosterone pellets were implanted in gonadectomized males and females, respectively.

There were 10 rats in each sub-group and all animals were housed singly in partitioned cages, with individual feeding jars and water bottles. The animals were examined every day except Saturday and Sunday and their food intake checked. Rats were killed at the end of the experimental period and each rat examined for macroscopic lesions, and tissues were taken for microscopic and chemical study.

The adrenal glands and livers were analyzed for total fat, "steroids,"* and ascorbic acid. Some samples of adrenal glands were

* This includes cholesterol, corticosteroids and the intermediates found in the adrenal gland and reacting with ferric chloride (11).

TABLE I. Effect of Hepatocarcinogenic Diet on Adrenal Gland and Liver Chemistry of Male Osborne-Mendel Rats. Each value is a mean of determinations on 10 rats. Standard error for each value is within 2% of respective mean.

	Adrenal glands					Liver	
Diet	Time, wk	Ascorbic acid, mg/g	Steroids, mg/g	Fat, mg/g	Weight, mg/100 g body wt	Steroids, mg/g	Fat, mg/g
Purina chow (control)		2.38	29.3	186	11.7	3.23	49.3
Hepatocarcinogenic	2	2.51	35.2	198	12.2	3.11	62.4
"	5	3.13	50.5	257	12.6	3.03	70.1
"	7	3.38	61.6	286	12.8	2.97	80.5
"	17	2.17	52.0	290	15.5	3.85	55.7
Semi-synthetic	6	2.98	37.9	194	13.7	4.15	93.7

examined for corticosteroids. Total fat was extracted from the tissues with a boiling mixture of ethanol and ethyl ether, the extract evaporated to dryness and weighed. This was taken as a rough measure of the material in the adrenal glands which stains as lipoid droplets in histological preparations. "Steroids" were determined by a modification of ferric chloride method for serum cholesterol (9) as adapted by Knobil *et al*(10) to adrenal gland study. Since this method develops colors with other steroids(11) and adrenal gland contains, besides cholesterol corticosteroids and all the intermediates, the generic name "steroids" is used here to designate the material determined. Ascorbic acid was determined by a modification of Roe and Keuther method(12) as adapted by Schwartz and Williams, Jr.(13). Corticosteroids were estimated by Blue tetrazolium reduction method (14).

Results. The effect of the duration of hepatocarcinogenic diet on adrenal gland and liver chemistry is given in Table I. The adrenal glands of the rats fed hepatocarcinogenic diet show a gain in weight of about 9% at the end of 7 weeks and 30% at the end of 17 weeks over comparable values for control rats. Ascorbic acid, fat, and steroid concentration of the adrenal glands of these rats show gradual increase from 2nd week on the diet, and at the end of 7 weeks are about 40, 54, and 110% higher, respectively, than corresponding values for control animals. At the end of 17 weeks on the diet, when rats have palpable liver tumors, adrenal fat and steroid values are still 55 and 75% higher respectively and corticosteroids 30% higher than those for corresponding control animals. The rats on semi-synthetic diet also show a higher trend in all the values for the adrenal glands.

The livers of rats on hepatocarcinogenic diet show a gradual drop in steroid and a sizable rise in fat values for the first 7 weeks and a reversal of this trend at the end of 17 weeks of diet. The livers of rats on semi-synthetic diet show higher values both for steroid and fat. Values for the liver ascorbic acid and those for liver weight per unit body weight show no significant difference from comparable control values, and are not included in the Table.

The effects of hormonal changes on the adrenal gland chemistry and on the incidence of hepatoma are given in Table II. The adrenal ascorbic acid and steroid values are higher in intact male than those in intact female rats and their respective hepatoma incidence is 100 and 44.5%. Gonadectomy lowers these adrenal ascorbic acid and steroid values in males and raises them in females. Gonadectomy also increases hepatoma incidence over that in intact females. Hormonal implants in gonadectomized males and females have a tendency to move the adrenal steroid value closer to that in their intact counterpart. This also lowers the hepatoma incidence in females and brings it nearer to that in intact female.

Liver steroid values are lower and fat values higher than those for tumor tissue in the same animal. The hormonal changes in the rats has very little effect on these values either in liver or in tumor.

Discussion. Present study confirms by

TABLE II. Effect of Hormonal Changes on Adrenal Gland and Liver Chemistry and Tumor Incidence in Male and Female Rats Fed Hepatocarcinogenic Diet for 17 Weeks. Mean values for 10 rats with each standard error within 2% of respective mean.

Treatment	Adrenal glands		Liver		Tumor		Tumor incidence, %
	C*	Steroids	Steroids	Fat	Steroids	Fat	
	mg/g						
Male							
Intact	2.17	52.0	3.85	55.7	5.73	49.7	100
Gonad X	1.98	26.1	3.46	52.6	4.08	43.3	100
Gonad X + stilbesterol	1.97	30.7	3.01	52.1	3.74	38.6	90
Female							
Intact	1.83	27.8	3.22	50.1	—	—	44.5
Gonad X	2.17	35.4	2.90	47.9	3.74	36.1	80
Gonad X + testosterone	2.27	27.6	3.58	49.4	5.1	35.5	37.4

* Ascorbic acid.

chemical analysis, histological(4) and histochemical(6) findings of high lipoid concentration in the adrenal glands of rats bearing azo-dye induced hepatomas. This increase in adrenal lipoid is detected as early as two weeks after the start of the carcinogenic diet and shows a steady increase to the 17th week when hepatomas become palpable. Furthermore, adrenal steroids are similarly elevated in the carcinogen fed rats and the corticosteroid values of rats fed carcinogenic diet for 17 weeks were significantly (30%) higher than those for control rats.

This higher concentration of "steroids" in the adrenal cortex could be due either to the acceleration of steroid production or retardation in its release from the adrenal gland. Former possibility is more attractive for the following reasons: 1) a functioning adrenal gland has been shown to be necessary for azo-dye hepatocarcinogenesis(1,2,3,7); 2) desoxycorticosterone in conjunction with azo-dye diet induces adrenal atrophy(4) and most probably this chemical adrenalectomy protects the rat from hepatocarcinogenesis; 3) the adrenocortical steroids have been found necessary for liver regeneration following partial hepatectomy(16); 4) carcinogenic diet induces hepatic damage, and incidence of hepatoma is proportional to this damage (15). It is suggested that the carcinogenic diet induces mutations in liver cells and causes hepatic damage. The latter in turn stimulates adrenocortical steroid secretion, which promotes regeneration of the liver and accelerates the growth of the mutated hepatic cells into a recognizable tumor mass. This hypothesis of hepatocarcinogenesis is consistent only with most of the experimental evidence for azo-dye carcinogenesis and does not exclude other modes of action with other carcinogens. For example, it fails to explain the failure of adrenalectomy to protect rats against N-2-Fluorenyldiacetamide hepatocarcinogenesis(17).

An early increase in adrenal steroids in rats fed hepatocarcinogenic diet and an association of high adrenal steroids with high tumor incidence and low cholesterol with low incidence in female rats may be suggestive of some relationship between them. Rumsfeld *et al*(18) using a smaller dose of carcinogen, in similar experiments reported lower incidence of tumors in gonadectomized male rats. The dose of carcinogen used in this study proved to be too high to demonstrate the relation of gonadectomy to tumor incidence.

Higher liver fat concentration observed in rats on hepatocarcinogenic diet may have no bearing on the incidence of hepatocarcinoma. Silverstone(19) established that rate of azo-dye carcinogenesis was unrelated to lipid concentration of the liver. However, quality and quantity of fat in the diet could influence the incidence of hepatomas insofar as it influences the riboflavin concentration of the liver (20).

Summary. Rats were fed on hepatocarcinogenic diet (CD) for 2, 4, 7 and 17 weeks. Intact and gonadectomized, male and female

rats, some with and others without hormonal implants were also fed CD for 17 weeks. Total fat, "steroids," corticosteroids, and ascorbic acid were determined on adrenal glands, liver and tumor. Rats fed CD showed rise in fat, "steroid" and ascorbic acid values as early as 2 weeks and continued high through the experimental period. Adrenocorticosteroid values in intact CD fed rats were 30% higher at 17 weeks than those in control rats. Gonadectomy lowered in males and increased in females steroid values over those in comparable intact rats. Stilbesterol and testosterone implants respectively, in these rats, reversed this effect. It is suggested that high adrenal steroid values indicate increased secretion of adrenocortical hormones, which are necessary to promote the growth of altered cells to a recognizable tumor mass.

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Growth of *Mycoplasma gallisepticum* Strain J Without Serum.* (30969)

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The nutritional requirements of the genus *Mycoplasma* are known to be complex(1). All parasitic strains have a protein requirement supplied by serum or serum fraction which is added to defined media(2,3,4). Growth of a saprophyte, *Mycoplasma laidlawii,* has been obtained in a defined medium upon addition of serum fraction, albumin(5, 6), or positively-charged tryptic peptides from ribonuclease(7).

This paper reports the development of media for the growth of the avian parasite, *Mycoplasma gallisepticum* strain J, which permit heavy growth without added serum fraction.

Methods and materials. All work referred to here was done with strain J, kindly given to us by Dr. H. M. DeVolt, Dept. of Veterinary Science, University of Maryland, Col-

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