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The Effect of Adrenal Steroid Hormones on the Protein Content of Tumorous Livers

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THE EFFECT OF ADRENAL STEROID HORMONES ON THE PROTEIN CONTENT OF TUMOROUS LIVERS

By

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CHAPTER I
INTRODUCTION

Protein is basic to the structure and function of protoplasm. Enzyme systems which regulate the rate of cellular reactions are primarily composed of protein and many of these enzymatic systems influence the metabolism of less specialized proteins that form the major structural material of protoplasm. It is believed that experimental procedures which modify protein composition of cells may also influence the activity of intra-cellular enzymes. In experimental liver cancer, it has been shown that the activities of a number of oxidative enzymes are modified as the carcinogenic process proceeds (Richmond, 1955, p. 38). Furthermore, it has been demonstrated that endocrine function influences carcinogenic processes. Moon, et al. (1951, p. 539) reported that long-term administration of growth hormone to hypophysectomized rats did not produce the numerous neoplasms that developed in control rats. Hormones of the adrenal cortex are intimately involved in the regulation of protein metabolism, and Umbriet (1951, p. 574) showed that cortisone influences enzyme systems that aid in the degradation of cellular protein. Such studies indicate that more work is needed on the effects of adrenal hormones on protein composition and activity during carcinogenesis.

Symeonidis, et al. (1954, p. 811) have reported that certain modifications of adrenal function inhibit liver tumor formation that occurs after the feeding of the azo dye para-dimethylaminoazobenzene (DAB).

The present study was undertaken to determine whether modification in adrenal function would affect the protein composition of the liver of rats fed the potent azo dye 3'-methyl-4-dimethylaminoazobenzene (3'-Me-DAB). Attempts were made to correlate these findings with changes in enzyme activity that have been reported by others.

CHAPTER II
SURVEY OF LITERATURE

Atypical growths due to azo dyes were first described by Fischer in 1906 (cited by Rusch et al., 1945, p. 267). The injection of Scarlet red into the ears of rabbits resulted in atypical epithelial growths. These growths receded when the chemical was withdrawn. In 1924, Schmidt (Rusch et al., 1945, p. 267) observed a proliferation of the epithelial cells of the liver when feeding Scarlet red to mice. These growths were considered by Schmidt to be both adenomatous and sarcomatous in nature. Yoshida, in 1933 (Rusch et al., 1945, p. 267), conclusively demonstrated the carcinogenicity of azo dyes. Hepatomas were obtained by feeding 1 mg of 2' 3-dimethyl-4-aminoazobenzene per gram of food for several months. In 1937, 4-dimethylaminoazobenzene was reported to be the most active carcinogen for rats (Kinosita 1937, as cited by Sugiura and Rhoads, 1941, p. 3). The diet used in this study was brown rice, carrots, olive oil, and the carcinogen. When wheat bread was introduced into the diet, the tumor incidence dropped markedly. T. Ando, in 1938, found that adding yeast to the diet of dye-fed animals decreased tumor incidence; also, Nakahara et al., found that beef liver added to the diet of DAB-fed animal reduced tumor incidence (Sugiura and Rhoads, 1941, p. 3). These findings of the Japanese were confirmed in this country (Sugiura and Rhoads, 1941, p. 15).

The above findings provided another tool for cancer research. Liver tumors are now easily produced and are markedly affected by dietary ingredients.

As it was apparent that a dietary influence was involved in tumor induction with azo dyes, purified diets were used to study these influences of vitamins, proteins, fats and specific agents on the carcinogenic process.

The protein content of the diet was demonstrated to be a factor in tumor induction of dye-fed animals. Griffin and Baumann, (1949, p. 86) noted that if the dietary protein (casein) was raised to 24% as compared to a 12% control diet the tumor incidence dropped. Other dietary constituents were also observed to have an influence on DAB-fed animals. Corn oil at the 20% level in the diet produced tumors in 53-64% of the rats in six months. Hydrogenated cocoanut oil in the diet reduced the tumor incidence to 8% in the same period of time (Miller et al., 1949, p. 158). Kline (1946, p. 4) showed that the addition of 20% corn oil to the basal diet would produce more tumors than a diet containing 5% corn oil. These findings demonstrated that the amount and kind of fat present in the diet would influence tumor development.

Vitamin content in diets of DAB-fed animals was also studied. Riboflavin was demonstrated to be the most significant in liver tumor inhibition. Kensler et al., (1941, p. 309) first obtained marked inhibition of liver tumors by adding riboflavin to the basal diet. Subsequently, riboflavin became well established as an inhibitor of azo dye carcinogenesis. In addition the role of dietary constituents in liver tumor induction by azo dyes has been demonstrated to exert an effect upon the retention and utilization of riboflavin in the liver. Griffin and Baumann (1946, p. 475) showed that 24% casein in the diet increased

As it was apparent that a [illegible] factor, [illegible] was involved in tumor [illegible] when [illegible] purified diets were [illegible], each of [illegible] vitamins, proteins, fats [illegible] in [illegible] carcinogenic process.

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the retention of riboflavin. Fat in the diet also influences the liver's capacity to retain riboflavin. Retention of riboflavin in the liver is less when 20% corn oil is included in the basal diet than when 5% corn oil is used (Miller et al., 1948, p. 92). Riboflavin retention is increased by the use of hydrogenated cocoanut oil in the diet (Griffin and Baumann, 1948, p. 282). The location of riboflavin in the liver cell became a point of investigation. Price et al. (1950, p. 27) showed a decrease in riboflavin content of the large granule fraction (mitochondria) of liver tumors compared to normal livers.

One hypothesis advanced to explain the carcinogenic action of the azo dyes involves the depletion of liver protein by a dye binding process (Miller et al., 1947, p. 479). They observed that, preceding tumor formation, protein bound derivatives of the azo dyes are found in the liver; protein bound dyes are found only in species which are susceptible to azo dye carcinogenesis. They suggested that, "In any event p-dimethylaminoazobenzene might initiate the carcinogenic process through sublethal combinations of this dye or its metabolites with critical proteins in normal cells and their descendents. The autonomous tumor may be the eventual outcome of some of the damaged liver cells through a permanent alteration or loss of proteins essential for the control of growth but not for life." This concept is supported by the observation that protein bound dyes are absent in tumors formed during dye feeding.

That the endocrine system may be involved in azo dye carcinogenesis is based upon the observation of Moon, et al. (1951, p. 539). They noted that

the long-term administration of growth hormone to hypophysectomized rats did not produce the numerous neoplasms that developed in control rats. Griffin et al. (1953, p. 79) reported that hypophysectomy inhibited liver tumor induction in rats fed 3'-Me-DAB. Histological examination (Richardson et al., 1953, p. 1029) of completely hypophysectomized rats fed 3'-Me-DAB revealed atrophy of adrenals, testes, prostate, and thyroid. In incompletely hypophysectomized rats, histological examination revealed cirrhosis and concomitant changes associated with 3'-Me-DAB induced tumors. Administration of adrenocorticotropic hormone (ACTH) to hypophysectomized rats receiving 3'-Me-DAB resulted in the formation of liver tumors (Robertson, et al., 1954, p. 551). Various hormones have been given to hypophysectomized rats receiving the 3'-Me-DAB by Richardson et al. (1954, p. 1047). They found that ACTH and growth hormone administered simultaneously were the most effective in restoring the activity of the carcinogen. Gonadotropin and thyrotropin were less effective in restoring this activity. Cortisone, testesterone, and desoxycorticosterone acetate (DCA) were not effective in tumor restoration. In addition they point out that changes in adrenal lipoid of hypophysectomized rats given hormones indicate that pituitary-adrenal relationships may be a prerequisite to the process of tumor induction.

Some influences of adrenal cortical hormones in liver tumor induction using DAB were reported by Symeonidis et al. (1954, p. 811). They indicate that adrenalectomized animals, fed DAB, treated or not treated with DCA, failed to develop liver tumors.

This evidence is contrary to that reported by Griffin et al. (1955, p. 1625). Griffin and his co-workers using 3'-Me-DAB as the carcinogen found that tumors were not inhibited in adrenalectomized animals, with or without treatment with cortisone or DCA. Griffin, et al. (1955, p. 1629) also reported that hypophysectomy did not inhibit tumors produced by the carcinogens benzpyrene, dibenzanthracene, and methylcholanthrene.

It has been established that total adrenalectomy invariably results in death. However, life can be maintained by administration of proper types and quantities of preparations. (Turner, 1955, p. 166). Two types of synthetic preparations were used in the present study, desoxycorticosterone trimethylacetate and cortisone acetate. Desoxycorticosterone trimethylacetate is a chemical modification of desoxycorticosterone, the steroid hormone secreted by the adrenal cortex. Desoxycorticosterone has been studied extensively and plays an important role in the retention of sodium and chloride by the kidney (Turner, 1955, p. 173). Desoxycorticosterone trimethylacetate administered in single small doses has been established to be especially effective in long-term maintenance of totally adrenalectomized animals (Gaunt et al., 1952, p. 530). Cortisone acetate is a chemical modification of the natural steroid hormone (cortisone), and is primarily effective in carbohydrate and protein metabolism; its effects on water and electrolyte metabolism are about one-thirtieth that of desoxycorticosterone (Turner, 1955, p. 173).

CHAPTER III
METHODS AND MATERIALS

In these experiments 72 female Long-Evans rats were housed 6 to a cage in animal quarters with the temperature controlled from 74-76° F.

At the time of operation the rats weighed between 180 and 200 grams. All animals were ovariectomized because it has been reported that female rats are more resistant to liver tumor induction by azo dyes (Rumsfeld et al., 1951, p. 818). Twelve animals, used as controls, were left with the adrenals intact. In experimental animals, the ovariectomy and adrenalectomy were performed at the same time. The adrenalectomized animals received either desoxycorticosterone trimethylacetate (DCT) or cortisone acetate or both hormones subcutaneously throughout the course of the experiment. Dosage, type, and time intervals involved in hormonal treatment of the various groups are shown in Table 1. All animals which received cortisone acetate were injected with 5 mg of DCT at the time of operation. Rats receiving DCT were allowed to select between 5% dextrose solution and tap water. Cortisone acetate treated animals were maintained on a drinking solution which contained dextrose, sodium chloride, and potassium chloride: 25, 4, and 2 grams per liter, respectively.

Animals were fed, ad libitum, a purified diet (Table 2) similar to the one used by Price et al. (1952, p. 193), and modified by Richmond (1955, p. 19). In this study an antibiotic (Tetracycline-Vet, Pfizer) was added to the diet.

After 126 days on the diet the animals were sacrificed by decapitation. Livers were removed, washed in physiological saline, blotted and weighed to the nearest tenth of a milligram. Liver tissue containing tumors was then dissected from the non-tumorous tissue or, as in many cases, the least tumorous tissue. The livers were then dried to constant weight at 110° C. and the percentage water calculated from the wet and dry liver weights.

Livers were ground in a Waring Blender and the lipid content was determined according to a modification of the Saxon fat extraction procedure by Richmond (1954, p. 30).

The nitrogen content of the dry, fat-free livers was determined by a modification of the Kjeldahl procedure as given by Marcali and Riemann (1946, p. 709). Phosphorus was also removed from the sample as suggested by Marcali and Riemann (1948, p. 387); one-half gram aliquots of the tissue was used for these determinations. Protein content was determined by multiplying the nitrogen content by the factor 6.25.

CHAPTER IV
RESULTS

A. Maintenance of adrenalectomized animals with adrenal steroids

After one week on a stock diet (Purina Chow) adrenalectomized rats treated with cortisone acetate were transferred to a purified diet containing 3'-Me-DAB. Previous experiments had indicated that cortisone acetate alone will not maintain adrenalectomized rats and it was deemed advisable to supplement cortisone treatment with salt solutions. Therefore, animals were allowed to choose between tap water and a mixture of sodium chloride and potassium chloride (0.5% each); they selected salt solution, became edematous, and several animals died. In attempts to improve their health, survivors were allowed to select from solutions of dextrose (5%), sodium chloride (1%), and potassium chloride (1%). Dextrose and sodium chloride were selected in larger quantities than potassium chloride. This regime ameliorated the edema and slightly improved the appearance of the animals. On the basis of this information, the animals were allowed to drink a mixture of dextrose, sodium chloride, and potassium chloride (25, 4, and 2 grams per liter respectively) for a number of weeks. Some rats did not recover their lost weight and, because of poor condition and high mortality, it was deemed advisable to inject a steroid that had good life-maintaining properties. Therefore, 50 days after adrenalectomy, DCT was again injected.

After DCT injection the rats were allowed to choose, as drinking fluid, between dextrose and tap water. The rats selected dextrose solution, drank little water, gained weight, and were in good physical condition when sacrificed. Figures 1, 2, and 3 show the relationship of the hormonal treatment to weight changes.

Desoxycorticosterone trimethylacetate, a long-acting adrenal steroid known to be good for life maintenance of adrenalectomized animals fed a stock diet, did not appear very effective in maintaining adrenalectomized rats fed a purified diet containing the carcinogen. Of twelve rats given 25 mg of DCT each at one injection, six died during the first month. The six survivors were then injected again with 25 mg of DCT andallowed to choose between dextrose solution and water. They selected dextrose, drank little water, but improved in health and were in good condition when sacrificed.

Many animals receiving cortisone acetate died during the course of the experiment. Those which had received large doses (50 mg per month) of cortisone revealed, upon autopsy, pulmonary infections, adhesions in the pleural cavity, and a general edema of tissues. Water content of the livers of these animals was high (78%).

B. Macroscopic appearance of the livers

All control livers were tumorous, exhibiting vesicular bodies and nodules. In most cases these abnormal structures were diffusely scattered throughout the substance of the liver, but in a few cases the posterior surface of the liver was more affected than were other areas.

Tumorous livers of animals receiving DCT were different in appearance from control tumorous livers. They were darker in color, appeared more compact, and lacked vesicular bodies.

Tumorous livers of group II, the group receiving the highest amount of cortisone acetate relative to DCT, were pale and contained numerous vesicular bodies. Other groups, i.e., those groups receiving a low ratio of cortisone acetate to DCT, had livers similar in appearance to control livers.

Table 3 gives the tumor incidence in the various groups. It can be seen that those groups which received DCT alone, or a low ratio of cortisone acetate to DCT, tended to show a lower tumor incidence. All animals receiving a high dose of cortisone acetate (Group II) had tumorous livers.

C. Liver analysis

Data on the analyses of experimental and control livers are given in Table 4. The difference of two means under comparison was considered to be significant when the p value was .05 or less.

a. Percentage of solids, water, and lipid in tumorous livers

Treatment of adrenalectomized rats with adrenal steroids increased the percentage of solids above the control value, and this increase was significantly greater in groups III through VI ($p < .01$). There was little or no correlation between percentage of solids and treatment with different ratios of DCT

to cortisone acetate

Those livers which had significantly higher percentage of solids also showed a decrease in percentage of water ($p < .05$). Other experimental groups studied showed no differences in percentage of solids when compared to control values. The percentage of lipid in the livers was not changed by hormonal treatment.

b. Protein content of the tumorous livers

The protein content in the livers was determined by dividing the total grams of protein by the total weight of the liver. The values are expressed either as percentage of the wet weight or as percentage of the fat-free dry weight. Treatment of adrenalectomized rats with 100 mg or more of adrenal steroids (groups I, II, and III) resulted in increases in the percentage liver protein in the dry fat-free livers, and these differences were highly significant in groups I and II ($p < .01$). Administration of less than 100 mg of the steroid hormones failed to elevate the percentage of liver protein, and the probable effect of small doses (52-62 mg) was to decrease the percentage of liver protein. There appeared to be no correlation between the ratios of the two steroids used and the response obtained. On a wet weight basis the percentage of protein was significantly elevated in only the group treated with DCT alone.

Areas of the diseased liver were classified grossly as "tumorous" and"non-tumorous." Comparison of percentage of protein from these areas revealed no differences.

CHAPTER V
DISCUSSION

The results of this investigation indicate that adrenalectomized rats fed a purified carcinogenic diet cannot be maintained routinely with desoxycorticosterone trimethylacetate or cortisone acetate. Supplementation of steroid treatment with special salt solutions and dextrose solutions, when done selectively, will allow for long-term survival under such dietary conditions. It was impossible to maintain adrenalectomized rats for long periods of time with cortisone acetate alone, but small doses of DCT and special drinking fluids containing dextrose, sodium chloride, and potassium chloride did permit long-term maintenance under these conditions. Reasons for this may be related to the "protein sparing action of dextrose" and the replacement of urinary loss of sodium and potassium (Eversole, 1956, p. 60).

It was of some interest that the adrenalectomized rats fed the carcinogenic diet and treated with DCT showed improvement in general physical condition when they were allowed access to dextrose solution. The reason for this is not clear, but is probably related to the fact that normal rats lose weight during the first month of feeding the carcinogenic diet. In other words the glucose treatment may have had a sparing effect on body protein catabolism under these carcinogenic and dietary conditions.

Pulmonary infection subsequent to cortisone treatment of intact rats has been reported by other investigators (Hoch-Ligetti, 1955, p. 1633). The

dosage used by Hoch-Ligeti was 15 mg of cortisone per week, a dosage which is higher than any used in the present study. These findings are in keeping with the concept that cortisone inhibits the inflammatory process (Turner, 1955, p. 197) and hence the animals are more susceptible to infections.

In studies, the results of which are not shown in tables, those animals which died while receiving cortisone acetate treatment showed a high percentage of water in the liver (78%). Such animals also showed gross edema. Although cortisone may have been a causative agent in the high water content of the livers, dietary factors are probably also involved. Richmond (1954, p. 22) has reported that rats fed a purified diet lacking a carcinogen show a higher percentage of water in the livers than the values reported for animals fed a stock diet.

Symeonidis et al. (1954, p. 811) reported that adrenalectomized and intact rats treated with DCA did not develop hepatomas when fed DAB. These investigators also reported an absence of tumors in adrenalectomized rats that did not receive any adrenal steroid treatment. The findings of this study are not in strict agreement with those of Symeonidis et al. However, in the present study a more potent carcinogen was used and there were differences in the chemical nature and methods of administration of the steroid. Other workers have failed to inhibit liver tumors by treatment with adrenal steroids (Griffin et al., 1955, p. 1629). In the present study failure to obtain 100% tumors in groups receiving DCT alone, and in groups receiving low doses of DCT and cortisone acetate in about equal proportions

would indicate that possible inhibitory effects with 11-desoxy steroids may be dosage dependent. This possibility is now under investigation in these laboratories.

Richmond (1954, p. 23) found that DAB-fed animals showed decreased solid and increased water percentages in tumorous livers. It is generally accepted that the water content of hepatomas is increased (Greenstein, 1943, p. 430). The results of the present study indicate that some undetermined liver constituent increases in the tumorous livers of animals treated with low doses of adrenal steroids.

The values for percentage of protein in the livers of control animals of this study are in agreement with those obtained by Richmond (1954, p. 41). The decrease in percentage protein in the tumorous livers represents a true decrease in concentration of liver protein. The results of the present study indicate that concentration of protein in cancerous liver can be modified by treatment with adrenal steroids.

The effect of DCT favoring the deposition of protein in the tumorous liver is in agreement with the known action of this steroid on normal liver tissue. Symeonidis et al. (1955, p. 557) have shown that rats under prolonged treatment with DCA regenerate a greater liver mass after partial hepatectomy than do untreated rats. This increase in liver mass was shown to be associated with an increase in total protein.

There is an indication that cortisone acetate does not exhibit protein catabolic effects on the liver during azo dye carcinogenesis. The group

treated with 120 mg of cortisone acetate and 29 mg of DCT (group II) showed protein values that were significantly high.

The results of the present study indicate that the changes in per cent protein of the livers in those groups treated with both adrenal steroids (groups II through VI) are dependent upon the total steroids administered to the animals. Attention is invited to the fact that the groups which received the lowest total steroids (groups V and VI) also revealed the lowest protein values.

In this discussion it is pertinent to point out that the interpretation of the results are made from the viewpoint that supplementation of hormonal treatment with special salt or sugar solution had little or no influence on the carcinogenic process or on liver composition. It is believed that changes in liver composition reported here are due to hormonal changes rather than to differences in fluid supplementation.

CHAPTER VI
SUMMARY AND CONCLUSIONS

Under the experimental conditions of this study, it has been found that:

1. Adrenalectomized rats treated with adrenal steroids and fed carcinogenic diets require, for survival, supplementation of hormonal treatment with either special salt or sugar solutions or both.
2. Tumor formation could not be completely suppressed with the amounts of DCT used, but there was some evidence that inhibition may be obtained with larger doses of the steroid.
3. Hormonal treatment does not influence the percentage of lipid in liver tumors.
4. Administration of large doses of DCT alone favors an increase in percentage of protein in tumorous livers.
5. Increases in the percentage of protein in tumorous livers of animals treated with both DCT and cortisone acetate appears to be dependent upon the total dose of the steroid administered.
6. Increased solids, due to treatment with less than 100 mg of steroid, is a result of an increase in some undetermined liver constituent.

LITERATURE CITED

Eversole, W. J. 1956. Water and electrolyte balance in adrenalectomized rats treated with cortisone and hydrocortisone. Fed. Proc., vol. 15, no. 1, pp. 60.

Gaunt, Robert, James H. Leathem, Constance Howell, and Nancy Antonchak. 1952. Some biological properties of different esters of desoxy-corticosterone. Endocrinology, vol. 50, no. 5, pp. 521-530, 4 tbls.

Greenstein, Jesse P. 1943. Tumor enzymology. Jour. Natl. Cancer Inst., vol. 3, no. 4, pp. 419-447, 6 tbls.

Griffin, A. C., and C. A. Baumann. 1946. The effect of certain azo dyes upon the storage of riboflavin in the liver. Arch. Biochem. and Biophys., vol. 11, no. 3, pp. 467-476, 4 tbls.

Griffin, A. C. and C. A. Baumann. 1948. Hepatic riboflavin and tumor formation in rats fed azo dyes in various diets. Cancer Res., vol. 8, no. 6, pp. 279-284, 4 tbls.

Griffin, A. C., C. C. Clayton, and C. A. Baumann. 1949. The effects of casein and methionine on the retention of hepatic riboflavin and on the development of liver tumors in rats fed certain azo dyes. Cancer Res., vol. 9, no. 2, pp. 82-87, 5 tbls.

Griffin, A. C., A. P. Rinfret, and V. F. Corsigilia. 1953. The inhibition of liver carcinogenesis

with 3'-methyl-4-dimethylaminoazobenzene in hypophysectomized rats. Cancer Res., vol. 13, no. 1, pp. 77-79, 1 tbl.

Griffin, C. A., Howard L. Richardson, Charles H. Robertson, Margery O'Neal, and James D. Spain. 1955. The role of hormones in liver carcinogenesis. Jour. Natl. Cancer Inst., vol. 15, no. 5, pp. 1623-1632, 4 tbls.

Hoch-Ligetti, Cornelia. 1955. Effect of cortisone administration on induced and transplanted hepatomas. Jour. Natl. Cancer Inst., vol. 15, no. 5, pp. 1633-1636, 3 figs. 1 tbl.

Kensler, C. J., Kanematsu Sugiura, N. F. Young, C. R. Halter, and C. P. Rhoads. 1941. Partial protection of rats by riboflavin with casein against liver cancer caused by p-dimethylaminoazobenzene. Science, vol. 93, no. 2413193, pp. 308-310, 1 fig. 1 tbl.

Kline, B. E., J. A. Miller, H. P. Rusch, and C. A. Baumann. 1946. Certain effects of dietary fats on the production of liver tumors in rats fed p-dimethylaminoazobenzene. Cancer Res., vol. 6, no. 1, pp. 5-7, 1 tbl.

Marcali, Kalman, and William Riemann III. 1946. Kjeldahl determination of nitrogen; elimination of the distillation. Indust. and Engineer. Chem., vol. 18, no. 11, pp. 709-710, 1 tbl.

Marcali, Kalman, and William Riemann III. 1948. Kjeldahl determination of nitrogen without distillation; application to samples containing phosphorus. Analyt. Chem., vol. 20, no. 4, pp. 381-382, 2 tbls.

Miller, Elizabeth C. and James A. Miller. 1947. The presence and significance of bound aminoazo dyes in the livers of rats fed p-dimethylaminoazobenzene. Cancer Res., vol. 7, no. 7, pp. 468-480, 2 figs. 1 tbl.

Miller, E. C., J. A. Miller, B. E. Kline, and H. P. Rusch. 1948. Correlation of the level of hepatic riboflavin with the appearance of liver tumors in rats fed aminoazo dyes. Jour. Exptl. Med., vol. 88, no. 1, pp. 89-98, 1 fig., 3 tbls.

Miller, Elizabeth C., James A. Miller, R. W. Sapp, and G. M. Weber. 1949. Studies on the protein-bound aminoazo dyes formed in vivo from 4-dimethylaminoazobenzene and its C-monomethyl derivatives. Cancer Res., vol. 9, no. 6, pp. 336-343, 5 figs.

Moon, Henry D., Miriam E. Simpson, Choh Hao Li, and Herbert M. Evans. 1951. Neoplasms in rats treated with pituitary growth hormone V. absence of neoplasms in hypophysectomized rats. Cancer Res., vol. 11, no. 7, pp. 535-539, 5 tbls.

Price, J. M., E. C. Miller, J. A. Miller, and G. M. Weber. 1950. Studies on the intracellular composition of livers from rats fed various

aminoazo dyes. II. 3'-methyl-, 2-methyl-, and 2-methyl-4-dimethylaminoazobenzene, 3'methyl-4-monomethylaminoazobenzene, and 4'-fluoro-4-dimethylaminoazobenzene. Cancer Res., vol. 10, no. 1, pp. 18-27, 8 figs., 6 tbls.

Richardson, H. L., A. C. Griffin, and A. P. Rinfret. 1953. Adrenal histological change and liver tumor inhibition in hypophysectomized rats fed the azo dye 3'-methyl-4-dimethyl-aminoazobenzene. Cancer, vol. 6, no. 5, pp. 1025-1029, 7 figs.

Richardson, H. L., M. A. O'Neal, C. H. Robertson, and A. C. Griffin. 1954. The role of hormones in azo dye induction of liver cancer and the adrenal-lipoid response in hypophysectomized rats. Cancer, vol. 7, no. 5, pp. 1044-1047.

Richmond, Chester R. 1954. Protein metabolism in rats with tumors of the liver. Master's thesis, University of New Mexico, pp. iv + 48, 6 figs., 3 tbls.

Richmond, Donald R. 1955. Respiration, succinoxidase and d-amino acid oxidase studies on livers from rats fed the hepatic carcinogens 4-dimethylaminoazobenzene (DAB) and 3'-methyl-4-dimethylaminoazobenzene (3'-Me-DAB). Doctoral Dissertation, University of New Mexico, pp. vi + 51, 1 fig. 4 tbls.

Robertson, C. H., M. A. O'Neal, H. L. Richardson, and A. C. Griffin, 1954. Further observations on the role of the pituitary and the adrenal gland in azo dye carcinogenesis. Cancer Res., vol. 14, no. 8, pp. 549-553, 2 tbls.

Rumsfeld, H. W. Jr., W. L. Miller Jr., and C. A. Baumann. 1951. A sex difference in the development of liver tumors in rats fed 3'-methyl-4-dimethylaminoazobenzene. Cancer Res., vol. 11, no. 2, pp. 814-819, 4 tbls.

Rusch, H. P., C. A. Baumann, J. A. Miller, and B. E. Kline. 1945. Experimental liver tumors. In Moulton, F. R. Editor. A.A.A.S. research conference on cancer. A.A.A.S., Washington, D. C., pp. 267-287, 1 fig., 3 tbls.

Sugiura, Kanematsu, and C. P. Rhoads. 1941. Experimental liver cancer in rats and its inhibition by rice bran extract, yeast, and yeast extract. Cancer Res., vol. 1, no. 1, pp. 3-16, 10 tbls.

Symeonidis, Alexander, A. S. Mulay and F. H. Burgoyne. 1954. Effects of adrenalectomy and of desoxycorticosterone acetate on the formation of liver tumors in rats fed p-dimethyaminoazobenzene. Jour. Natl. Cancer Inst., vol. 14, no. 4, pp. 805-813, 1 tbl.

Symeonidis, A., A. S. Mulay, and E. G. Trams. 1955. Effect of prolonged pretreatment with desoxycorticosterone on the liver on

hepatectomized rats. Endocrinology, vol. 57, no. 5, pp. 550-558, 4 figs., 3 tbls.

Turner, C. Donnell. 1955. General endocrinology. 2nd ed. W. B. Saunders Co., Philadelphia. pp. xi + 553, 171 figs.

Umbreit, W. W. 1951. The influence of adrenalectomy and cortisone treatment on enzymatic reactions in rat tissues. Ann. New York Acad. Sci., vol. 54, art. 4, pp. 569-574, 2 figs., 1 tbl.

APPENDIX

TABLE 1

Group	Month 1		Month 2		Month 3		Month 4	
	CA mg	DCT mg	CA mg	DCT mg	CA mg	DCT mg	CA mg	DCT mg
I	0	25	0	25	0	25	0	25
II	40	5	40	5	40	5	0	13
III	25	5	25	0	25	13	0	15
IV	20	5	20	0	0	25	0	15
V	15	5	15	0	0	13	0	15
VI	10	5	10	0	0	13	0	15

CA - cortisone acetate

DCT - desoxycorticosterone trimethylacetate

TYPE AND QUANITY OF STEROIDS ADMINISTERED TO ADRENALECTOMIZED ANIMALS EACH MONTH OF THE EXPERIMENT

TABLE 2

Dietary ingredients (per Kg of diet)	grams
Dextrose (Cerelose)	620
Casein (vitamin free)	240
Salt mixture (Osborne-Mendel)	40
Corn oil (Mazola oil)	100
Thiamine chloride	0.003
Riboflavin	0.0015
Pyridoxine hydrochloride	0.0025
Calcium pantothenate	0.007
Choline chloride	0.030
Halibut oil	0.300
3'-Me-DAB	0.580
Antibiotic (Tetracycline-Vet, Pfizer)	0.100

CARCINOGENIC DIET USING

3'-METHYL-4-DIMETHYLAMINOAZOBENZENE (3'-Me-DAB)

TABLE 3

Group	Control	I		II		III		IV		V		VI	
Total Hormone (mg)		CA 0	DCT 100	CA 120	DCT 29	CA 75	DCT 33	CA 40	DCT 45	CA 30	DCT 33	CA 20	DCT 33
Tumors/ Total	10/10	4/6		8/8		2/3		2/2		2/4		1/4	
% Tumors	100%	66%		100%		66%		100%		50%		25%	

TUMOR INCICENCE IN RATS FED 3'-ME-DAB AND TREATED WITH

ADRENAL STEROIDS

TABLE 4

WATER, SOLID, LIPID, AND PROTEIN CONTENT OF LIVERS FROM RATS FED 3'-ME-DAB

Group	Steroid DCT**	Steroid DGA***	% Solids	% Water	% Lipid	% Protein dry wt. fat free	% Protein wet wt.	Number of cases
Controls			25.0 ±1.06	75.0 ±1.06	0.71 ±0.17	65.2 ±0.93	16.8 ±1.49	10 (Solids, water lipid)
						* 66.9 ±2.3		5 (Protein)
P						.90		
I	100	0	26.0 ±0.31	73.2 ±0.37	0.48 ±0.34	73.8 ±1.25	20.9 ±0.84	6
P			.20	.50	.80	<.01	<.05	
II	29	120	25.2 ±0.48	74.5 ±1.3	0.65 ±0.17	70.1 ±0.94	17.7 ±0.96	8
P			.80	>.90	>.90	<.01	.60	
III	33	75	28.9 ±1.08	71.1 ±1.1	0.06 ±0.27	67.5 ±3.3	19.8 ±1.64	3
P			<.01	<.05	.70	.60	.20	
IV	45	60	28.4 ±1.08	71.6 ±1.1	1.9 ±0.1	65.5 ±5	18.4 ±1.2	2
P			<.01	<.02	<.10	>.90	.50	
V	33	30	28.3 ±1.08	71.7 ±0.34	1.5 ±0.14	60.3 ±1.43	16.7 ±1.16	4
P			<.01	<.03	<.20	<.05	>.90	
VI	33	20	30.5 ±1.44	69.5 ±1.44	2.5 ±0.78	59.0 ±1.57	16.9 ±0.3	4
P			<.01	<.005	.10	<.02	>.90	

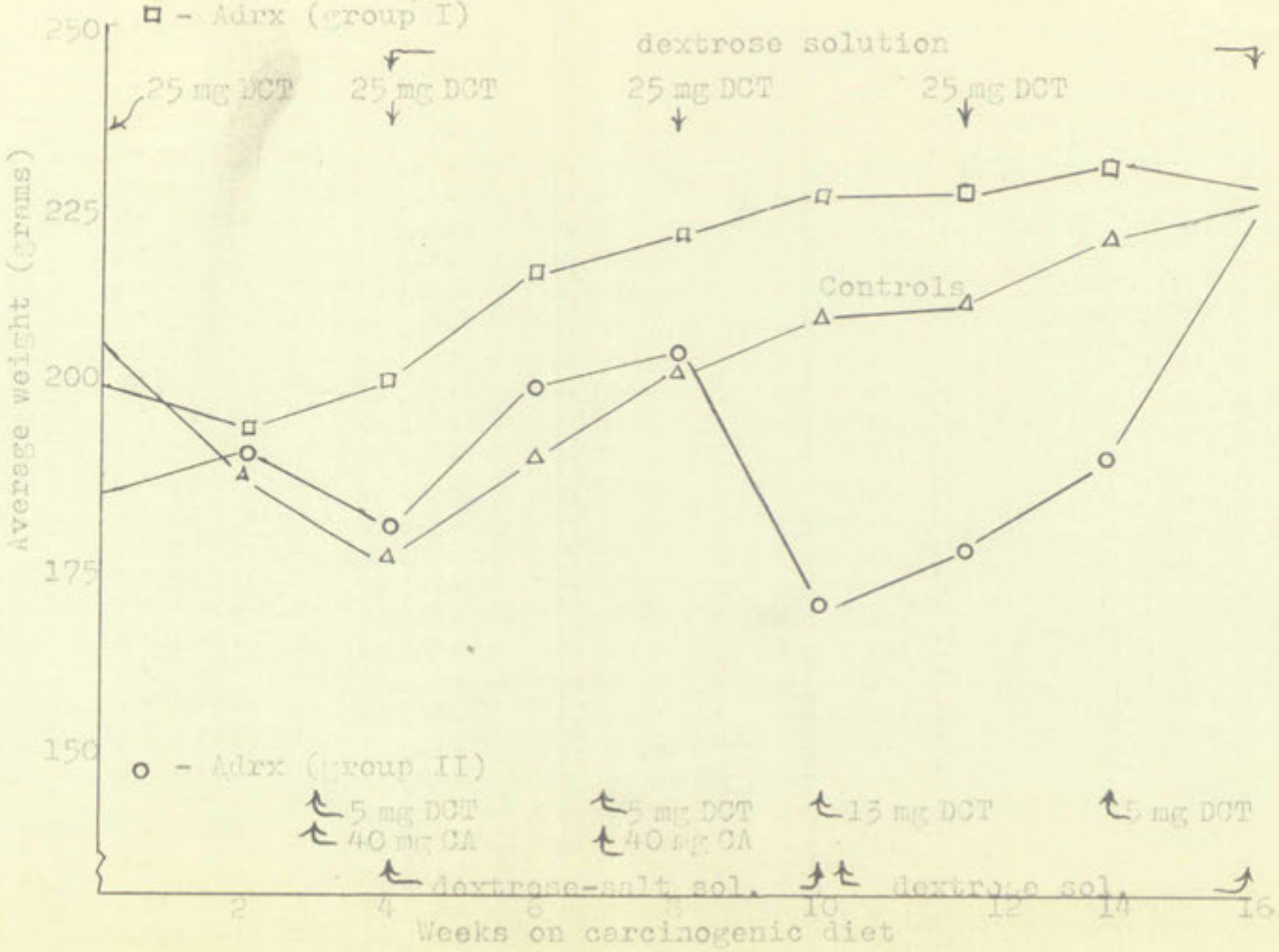
* "Non-tumorous area"

** desoxycorticosterone trimethylacetate

*** cortisone acetate

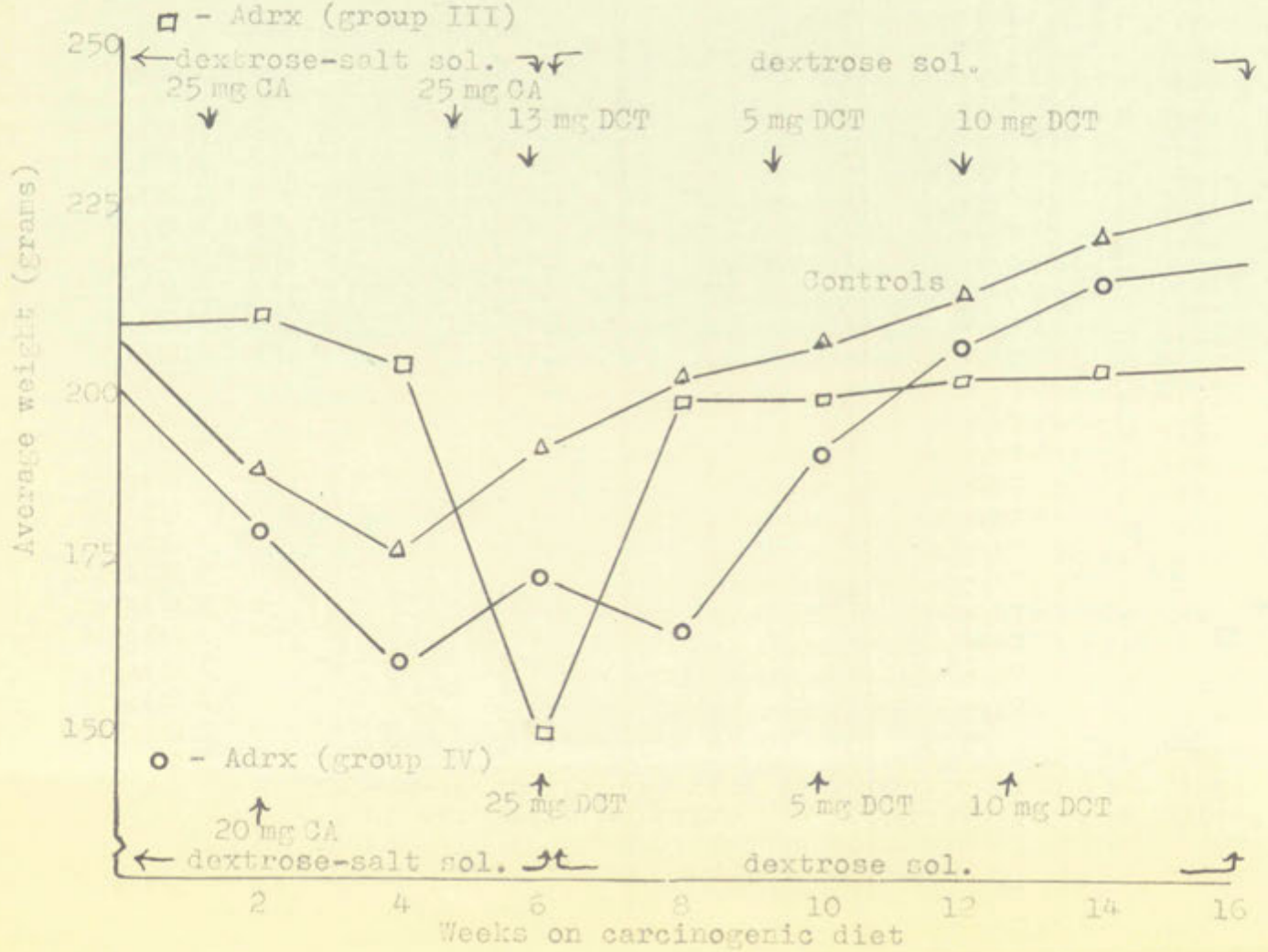
The effects of desoxycorticosterone trimethylacetate (DCT), cortisone acetate (CA), and drinking solutions on the growth of adrenalectomized rats (Adrx) fed 3'-Me-DAB.

Figure 1. Controls and groups I & II.
(see table 4 for liver analyses)



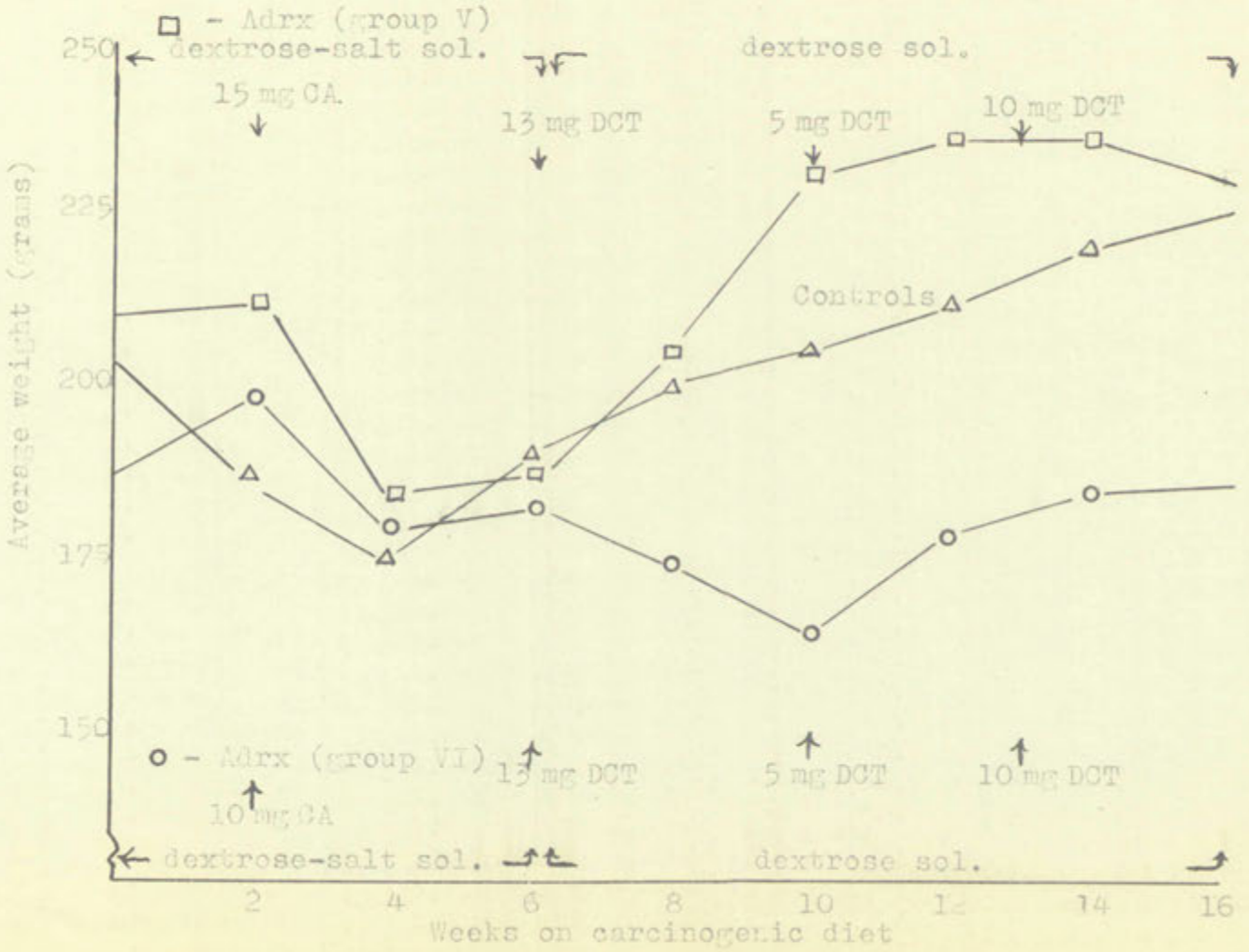
The effects of desoxycorticosterone trimethylacetate (DCT), cortisone acetate (CA), and drinking solutions on the growth of adrenalectomized rats (Adrx) fed 3'-Me-DAB.

Figure 2. Controls and groups III & IV.
(see table 4 for liver analyses



The effects of desoxycorticosterone trimethylacetate (DCT), cortisone acetate (CA), and drinking solutions on the growth of adrenalectomized rats (Adrx) fed 3'-Me-DAB.

Figure 3. Controls and groups V & VI.
(see table 4 for liver analyses)



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