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CHAPTER I

HISTORY AND INTRODUCTION

Investigators in the field of experimental cancer research all seem to agree that one of the outstanding effects of tumor transplantation on the host is a lowered activity of liver catalase. This observation was first made by Rosenthal (1912) in mice and by Brahn (1916) in cancerous patients. Since 1941 this phenomenon has been confirmed repeatedly by many investigators using different types of tumor and hosts.

Greenstein, Jenrette, and White (1941) found that rats carrying Hepatic Tumor #31 have one-tenth as much liver catalase as normal animals. Greenstein and Andervont (1942) also studying different strains of mice with spontaneous, methylcholanthrene-induced sarcoma, and many transplants of tumor tissues found that the decrease in liver catalase was progressive with the growth of the tumor. The slow-growing tumors did not show this phenomenon. They also found that in case of tumor regression or extirpation of the tumor mass, catalase returns to normal values.

Greenstein, Andervont, and Thompson, (1942) studied the effect of Hepatoma 31 and Jensen sarcoma on Osborne-Mendel and Buffalo rats and also studied some systemic effects of transplanted and spontaneous

tumors on different strains of mice. They found that kidney catalase of tumor-bearing rats and mice became slightly reduced. Kidney catalase of normal mice is but one-half that of the livers of this species while kidney catalase of normal rats is only slightly less than their liver catalase. Erythrocyte catalase of tumor-bearing animals was unchanged in spite of low red blood cell count and hemoglobin concentration.

Weil-Malherbe and Schade (1948) studied the effect of Jensen sarcoma on rats and found that the tumor growth caused lowering of the liver catalase in the host. In extreme cases this reduction reached 5 per cent of the normal value. These same authors studied the effects of two non-specific proteins, sheep serum and peptone on catalase concentration. They found that an injection of sheep serum 11 days after inoculation with tumor caused no inhibition of tumor growth or change in liver catalase concentration whereas an injection of sheep serum 4 days after tumor transplantation caused a slight inhibition of the tumor growth. Injection of sheep serum into normal rats caused increased liver catalase activity with no change in protein concentration of liver extract. Peptone was without effect.

Adams (1950) investigated the effect of subcutaneous growth of sarcoma 37 and of carcinoma 63 in two strains of mice. He showed that normal males, usually possessing higher liver catalase than normal females are more sensitive to the liver catalase reduction response than females when injected with the tumor.

Appleman et al. (1950) using a subcutaneous methylcholanthrene-induced active fibrosarcoma and Jensen rat sarcoma in adult rats of both sexes found that both of these tumors produced a reduction in liver

catalase activity to about 20 per cent of normal in the tumor-bearing rats, while kidney and erythrocyte catalase did not change significantly.

Begg (1951) studied the systemic effects of the solid form of Walker-256 carcinoma and Jensen sarcoma on young male rats. Among other effects, he found that lowering of liver catalase was progressive with the tumor growth, and was reduced to 48 per cent of the normal value when the tumor was 40 mm in size. Decreases in adrenal ascorbic acid and cholesterol, low hemoglobin concentration, and increased weight of liver were other systemic effects of these tumors reported by Begg.

Hargreaves and Deutsch (1952) reported the effects of Jensen rat sarcoma on male and female rats and found that liver catalase is reduced to 60 per cent of the normal value in males and to 35 per cent of normal in the female host.

Begg et al. (1953) investigated the effects of the solid form of Walker-256 carcinoma on young male and female rats. They found that males, which normally possess higher liver catalase, than females, show a greater reduction in liver catalase (66 per cent) than the female rats (45 per cent) when supporting an actively growing tumor.

Liver catalase reduction in tumor-bearing hosts does not seem to be peculiar to mammals. Adams (1953) studied in similar fashion Rous sarcoma in adult chickens and found that tumor-bearing chickens show a significant reduction in liver catalase in about 48 hours after injection of a large dose of homogenized fresh Rous tumor.

Lucke (1954) studied the effects of a kidney carcinoma of the Leopard frog on liver catalase. He found but half as much liver catalase in tumorous frogs as was found in normal untreated animals; while kidney

catalase was reduced to 34 per cent of the normal value.

In regard to the mechanism of reduction of liver catalase in tumor-bearing hosts, two notions exist at the present time. Greenstein (1942, 1943) and others have assumed that liver catalase reduction in tumor-bearing hosts may be due to an actual lowering of synthesis of liver catalase by a toxic agent liberated from tumor tissue. Likewise, Adams (1950), working with mouse sarcoma 37 and obtaining similar results to those of Greenstein, interpreted his findings as evidence that the tumor exerts its influence by releasing some toxic product into the circulation. These interpretations, however, throw no light on the mechanism of catalase inhibition.

Nakahara and Fukuoka (1948) and again in 1951 and 1953 (Fukuoka and Nakahara, 1951, 1953) isolated from human tumor tissues a protein-like, heat-stable fraction, which when injected into mice intraperitoneally, caused a marked reduction in their hepatic catalase. These authors suggested that the tumor-forming agent may exert its function by affecting protein metabolism and, therefore, the synthesis of iron-containing protein enzymes (Fukuoka and Nakahara, 1953). Greenfield and Meister (1951), using a fraction obtained from mouse mammary tumor, found that intraperitoneal injection of such a fraction into normal mice produced a marked but reversible decrease in their liver catalase activity. This effect was noticeable 24 hours after the injection and had disappeared in 72 hours. This tumor fraction was also heat-stable as noted by Nakahara. Neither group could demonstrate an inhibitory effect of this tumor fraction <u>in vitro</u>. Lucke, Berwick and Zeckwer (1952), studied the liver catalase activity of parabiotic rats with one partner

carrying a tumor and found that liver catalase was depressed in both animals. They concluded that the catalase-depressing agent from the tumor tissue is either elaborated in relatively large amounts by the tumor or disappears relatively slowly from the blood stream.

Greenfield and Price (1954) extracted quantitatively the liver catalase of tumor-bearing rats and found that there is actually less catalase in these animals than in normal rats.

Tschudy and Collins (1957), studying δ-amino-levulenic acid dehydrase, an enzyme involved in the synthesis of porphobilinogen, in tumor-bearing mice found that the variations in concentration of this enzyme closely resemble those of liver catalase of the same animals. This finding may only suggest the possibility of reduction of catalase synthesis in tumor-bearing animals. This, as the authors point out, is only a conjecture and should be further investigated.

The second idea considers the lowering of liver catalase of tumor-bearing animals to be due to an inhibition of activity of this enzyme by the tumor tissue extract. Hargreaves and Deutsch (1952) found that boiled aqueous extract of tumors, and to a lesser extent the boiled aqueous extract of normal liver and spleen homogenates, caused an <u>in vitro</u> inhibition of crystalline catalase. This fraction also produced inhibition of other iron-containing enzymes. This inhibitory function operates apparently through a reversible combination with the iron porphyrin group, since mixtures of catalase and inhibitors absorb much less light (optical density 0.185) at 405 mμ than does the catalase (optical density 0.205) alone. Alexander (1957), utilizing ethanol-soluble boiled aqueous extract of a tumorous rat liver

homogenate concluded that the catalase inhibition is due to autoxidation of ascorbic acid and sulfhydryl groups in the extract. The exact mechanism of inhibition caused by tumor extracts *in vivo* or *in vitro* still has not been established.

In light of these reports, it is clear that many of these authors have by inference implicated catalase as a factor in tumorigenesis. The problem, as we see it, is whether the low liver catalase levels contribute to tumorigenesis, or whether the progressive growth of the tumor produces low liver catalase, or whether there, in fact, exists any causal relationship between the two.

Our recent knowledge of the effect of adrenal gland hormones on natural resistance of many species to heterologous tumor transplants (Toolan, 1954) and the depressing effects of these hormones on liver catalase (Troop and Stanley, 1956) make the consideration of the possible role of catalase in tumorigenesis not only attractive but imperative.

While the details of study of this problem were being considered, early in the spring of 1956, the author discovered that a colored strain of rats in our laboratory when inoculated with the standard dose of ascitic fluid, did not respond to the inoculation by development of the tumor. On further challenge, it was found that this group of rats was resistant to the solid form as well as to the ascitic form of Walker-256 carcinosarcoma. These rats are presumably descendants of the captive colony of wild Norway rats propagated by the Wistar Institute as described by King (1932). Since there has been no report in the literature concerning resistance of this strain of rats to

Walker-256 carcinosarcoma, the strain was collectively designated OK-R (Oklahoma resistant).

The OK-R strain then seemed to be an invaluable means by which the problem of tumorigenesis and tumor resistance could be more thoroughly investigated. Although there have been some suggestions in regard to liver catalase level and tumorigenesis, (Adams, 1950) no unified study has been completed based on this point.

It was then decided to base our study upon the investigation of the effects of adrenal steroids on liver catalase and tumorigenesis in both resistant and susceptible animals.

With the advent of ascites tumors as a modern tool in experimental cancer research (Warburg, 1956), it was decided that this type in preference to the solid form should be employed. The ascitic form of Walker-256 carcinosarcoma used in our laboratory produces severe symptoms promptly, and thus conveniently establishes a short survival time in the infected host (Kitabchi, 1956; Master's thesis).

CHAPTER II

EXPERIMENTAL PROCEDURES AND RESULTS

The animals used in the first part of these experiments were Holtzman albino rats of the same age and sex and were maintained on Purina Laboratory Chow and water ad libitum.

The tumor used was the ascitic form of Walker-256 carcinosarcoma. Transplantation was effected by withdrawal of ascitic fluid from the abdomen of an infected host. The fluid was mixed in vitro with an antibiotic[1] and subsequently injected intraperitoneally in a standard dose of the fluid containing live tumor cells into the new host.

Liver catalase was determined by the perborate method of Feinstein (1949). The animals were killed by disjoining the cervical vertebrae; the livers were excised and immediately washed in a M/15 phosphate buffer solution (pH 6.8), blotted dry and weighed. They were then frozen for at least eight hours and were used for catalase assay not later than 36 hours after freezing. A two-gram sample of each liver by wet weight was taken. Eighteen milliliters of cold buffer solution were added to each sample and the mixture was homogenized in a Waring blendor for exactly one minute. Three one-ml aliquots of this homogenate were placed in 3 aluminum dishes and their dry weights were

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[1]Terramycin hydrochloride, Chas. Pfizer & Co.

determined. To the fourth aliquot of the homogenate was added enough triply distilled water to make a 1:500 dilution. Of this dilute homogenate, 0.5 ml was used for each determination of the liver catalase activity as follows:

A 1.5 per cent solution of tetrahydrated sodium perborate was used as a generator of hydrogen peroxide. This in turn served as substrate for liver catalase. Four 125 ml Erlenmeyer flasks numbered from 1 to 4 were set up for each determination. Eight ml of cold sodium perborate solution were placed in each flask and 1.5 ml of M/15 phosphate buffer (pH 6.8) was added. The flasks were then immersed in a water bath at 37° C and were incubated for exactly 15 minutes; at the end of this period, flasks 1 and 4 received 0.5 ml of triply distilled water while flasks 2 and 3 received 0.5 ml of the 1:500 dilution of liver homogenate. Each flask was then incubated for 5 minutes. The reactions in each flask were then stopped by the addition of 10 ml of a solution of 2 N sulfuric acid. The contents of each flask were titrated with a standard potassium permanganate solution. Flasks 1 and 4 served as control blanks. Hydrogen peroxide values were then established for all four flasks and those values for flasks 2 and 3 were substracted from those for flasks 1 and 4. Thus, the amount of perborate that was destroyed by the catalase alone in flasks 2 and 3 was determined.

The unit of liver catalase activity was expressed as milliequivalents of sodium perborate destroyed per mg dry weight of liver tissue.

Troop (1955), utilizing the perborate method of Feinstein

and a Waring blendor for homogenization of the liver tissue, reported the liver catalase activity per gram of wet weight of the tissue. Since the period of one minute that he used for homogenization of tissue appeared to be too short for complete homogenization, it was decided to determine the dry weight of each sample in triplicate and report the liver catalase activity per mg dry weight of the tissue.

Studies on Susceptibility in Albino Rats

In order to investigate the nature of susceptibility to Walker-256 carcinosarcoma, it was thought that such a study should be focused first on the systemic effects of this tumor. For this purpose four series of Holtzman albino rats were used. This strain of animals has been found to be practically 100 per cent susceptible to both ascitic and nodular forms of our experimental tumors.

Series I consisted of 27 mature females, subdivided into 6 groups, of three animals each, while a seventh group of 9 untreated animals served as controls. Each animal in the experimental group received 1 ml of ascitic fluid previously treated with antibiotic intraperitoneally, whereas the control animals received 1 ml of isotonic saline. Each group was sacrificed at a definite interval following tumor implantation. Groups 1, 2, 3, 4, 5, and 6 were sacrificed 2, 4, 6, 8, 10, and 12 days respectively after inoculation with the tumor fluid. At the time of autopsy, each liver was removed, washed clear of blood in cold phosphate buffer, blotted dry, weighed, and its catalase activity determined as outlined above. Weights of the spleen and adrenal glands were also determined. These weights were then

calculated per 100 grams of body weight and are so reported. The organs were fixed in a 10 per cent solution of formalin and histological sections of the liver, spleen and adrenals were prepared and stained with iron hematoxylin and eosin for later study.

The data from the 27 female rats used in this experiment are given in Table I. The data in Table I show that mature tumor-bearing female rats responded with a small decrease in liver catalase. The only significant reduction occurred by the 8th day after injection of the tumor and even then there was only a 14 per cent reduction in liver catalase.

Since it is known that female rats normally have a lower liver catalase than males, (Hargreaves and Deutsch, 1952), we wondered if this small reduction in tumor-bearing females might not be due to the initial low catalase level based on a sex difference. It was, therefore, decided to contrast this finding with a similar study in males. For this purpose a second series of rats consisting of 6 groups of males were chosen. A group of 12 mature males served as controls. The results of this experiment are given in Table II. It will be noted that mature tumor-bearing male rats showed a greater catalase depression by 48 per cent than did the female group. This reduction was greatest by the eleventh day after inoculation of the tumor fluid.

It has been reported that very little difference exists between the liver catalase activity of normal immature male and female rats. A sex difference in catalase values becomes apparent only as

TABLE I

SYSTEMIC EFFECTS OF WALKER -256 CARCINOSARCOMA ON MATURE FEMALE ALBINO RATS

Sacrificed Days After Inoculation	Number of Animals	Gm. Liver Wt. per 100 gm. Body Wt.	Mg. Adrenal Wt. per 100 gm. Body Wt.	Mg. Spleen Wt. per 100 gm. Body Wt.	Liver Catalase Activity
2	3	3.01	13.9	171	2.21 (± 0.32)a
4	3	2.79	16.3	255	2.13 (± 0.14)a
6	3	3.15	18.4	329	1.89 (± 0.22)a
8	3	3.21	19.1	547	1.93 (± 0.14)b*
10	3	3.29	17.1	680	2.39 (± 0.32)a
12	3	3.06	19.5	619	2.07 (± 0.20)a
Control	9	2.66	15.7	180	2.26 (± 0.28)

*Data assumed to be significant at this level.

a = P 0.05
b = P < 0.05 > 0.02

TABLE II

SYSTEMIC EFFECTS OF WALKER-256 CARCINOSARCOMA ON MATURE MALE ALBINO RATS

Sacrificed Days After Inoculation	Number of Animals	Gm. Liver Wt. per 100 gm. Body Wt.	Mg. Adrenal Wt. per 100 gm. Body Wt.	Mg. Spleen Wt. per 100 gm. Body Wt.	Liver Catalase Activity
3	3	3.01	11.9	223	2.51 (± 0.39)a
6	3	3.13	12.3	357	2.26 (± 0.07)c*
9	3	3.47	15.2	812	2.24 (± 0.49)b*
10	3	3.37	27.1	584	1.15 (± 0.26)e*
11	3	3.10	26.2	566	1.00 (± 0.39)e*
12	3	3.24	19.8	601	1.43 (± 0.01)e*
Control	12	2.88	10.7	199	2.65 (± 0.48)

*Data assumed to be significant at this level.

a = $P > 0.05$ c = $P < 0.02 > 0.01$

b = $P < 0.05 > 0.02$ e = $P < 0.001$

the animals approach maturity (Seabra and Deutsch, 1955).

In the light of the above report, it appeared desirable to study catalase values in a series of immature animals of both sexes after they were inoculated with the ascites tumor. A total of 31 young females and 30 young males were used as indicated in Tables III and IV respectively. The details for this experiment were similar to those outlined above for the adult animals. Tables III and IV show that liver catalase was reduced in both immature male and female rats. The cumulative data reveal the following results from Tables I-IV and Figures 1-4:

a. Liver catalase activity was decreased in all four groups of tumor-bearing rats, although this depression was least in mature females.

b. In all four groups of tumor-inoculated rats there was an increase in weight of spleen, liver, and adrenal glands. This increase was statistically significant when mean absolute values of each organ for each group were calculated.

c. The enlargement of the adrenal glands in the mature tumor-bearing male rats seemed to be inversely related to the change in liver catalase. To determine the significance of such a relationship, the Piersonian correlation coefficient (r) was calculated under the assumption of linearity between the mean value of liver catalase and the mean value of the absolute weight of adrenal glands at each time interval (see Appendices A through D). Only one value of z was found to exceed twice the standard error. This occurred in the mature male group where there was significant inverse relationship between adrenal weight and liver catalase. No such significant relationship seemed to exist

TABLE III

SYSTEMIC EFFECTS OF WALKER 256 CARCINOSARCOMA ON IMMATURE FEMALE ALBINO RATS

Sacrificed Days After Inoculation	Number of Animals	Gm. Liver Wt. per 100 gm. Body Wt.	Mg. Adrenal Wt. per 100 gm. Body Wt.	Mg. Spleen Wt. per 100 gm. Body Wt.	Liver Catalase Activity
3	4	4.26	32.9	580	1.51 (± 0.11)e*
6	5	3.85	28.5	863	1.78 (± 0.20)b*
8	5	4.14	32.2	1577	1.93 (± 0.09)a
10	5	3.98	32.0	962	1.26 (± 0.04)e*
12	5	3.92	35.3	940	1.50 (± 0.21)e*
Control	6	3.21	28.6	240	2.04 (± 0.33)

*Data assumed to be significant at this level.

a = $P > 0.05$
b = $P < 0.05 > 0.02$
e = $P < 0.001$

TABLE IV

SYSTEMIC EFFECTS OF WALKER-256 CARCINOSARCOMA ON IMMATURE MALE ALBINO RATS

Sacrificed Days After Inoculation	Number of Animals	Gm. Liver Wt. per 100 gm. Body Wt.	Mg. Adrenal Wt. per 100 gm. Body Wt.	Mg. Spleen Wt. per 100 gm. Body Wt.	Liver Catalase Activity
3	5	4.26	19.3	488	2.09 (± 0.22)a
6	5	4.13	15.9	727	2.46 (± 0.30)a
8	5	4.56	16.5	1467	1.79 (± 0.28)a'*
10	5	3.91	22.1	693	1.29 (± 0.18)c*
12	5	4.51	17.3	1526	2.32 (± 0.23)a
Control	5	4.02	14.0	272	2.16 (± 0.23)

*Data assumed to be significant at this level.

a' = $P = 0.05$
a = $P > 0.05$
c = $P < 0.02 > 0.01$

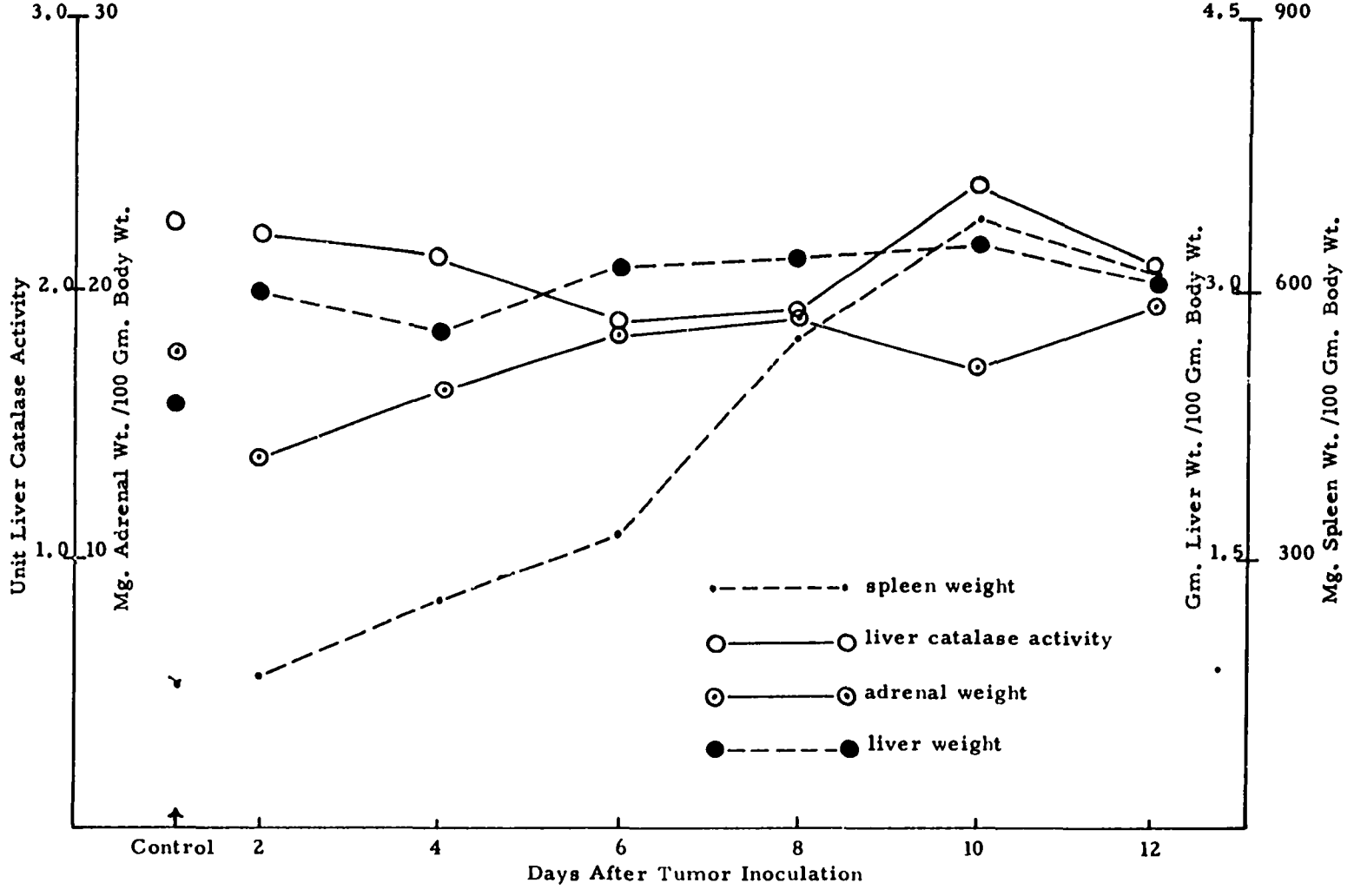


FIG. 1. SYSTEMIC EFFECTS OF WALKER-256 CARCINOSARCOMA ON MATURE FEMALE RATS

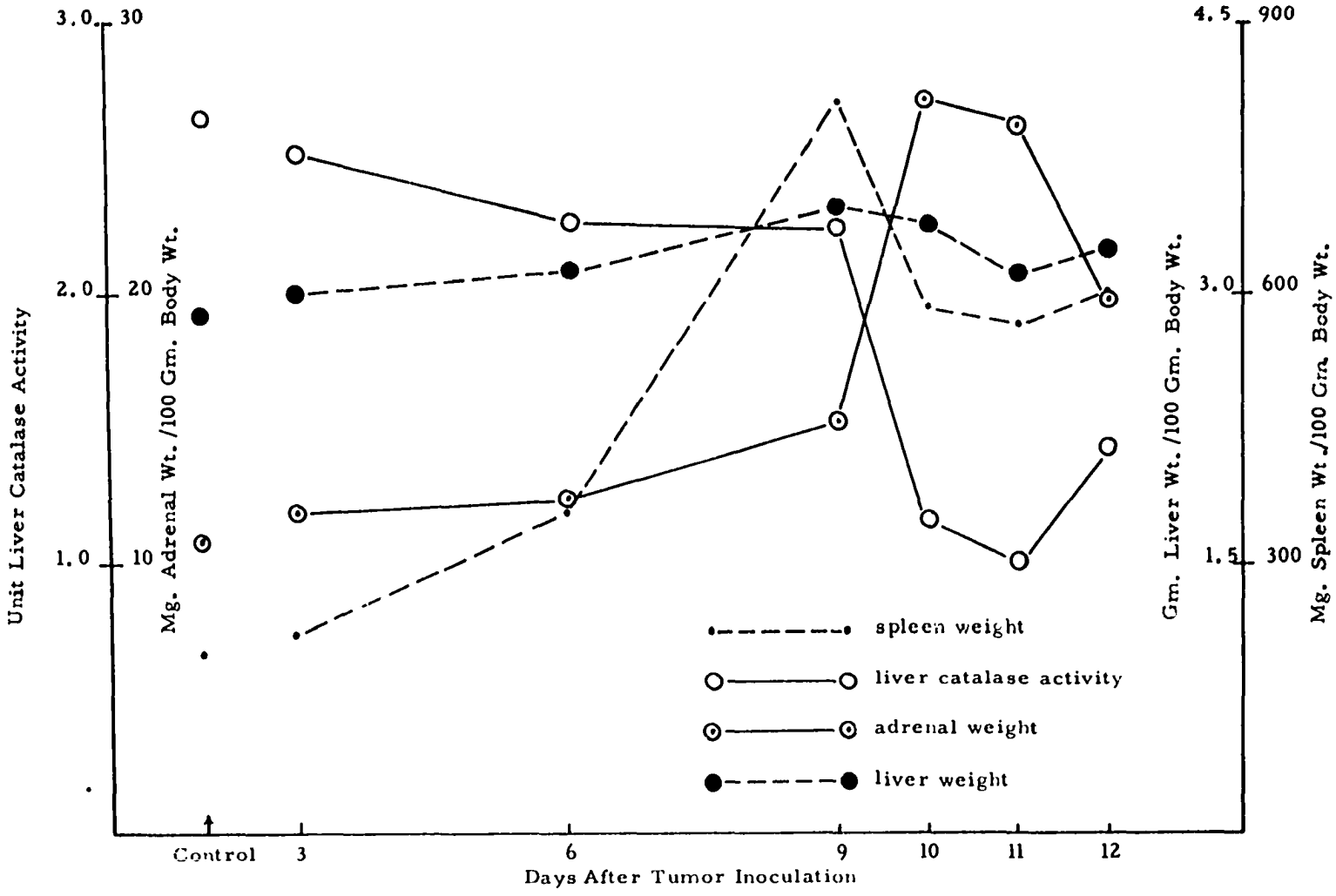


FIG. 2, SYSTEMIC EFFECTS OF WALKER-256 CARCINOSARCOMA ON MATURE MALE RATS

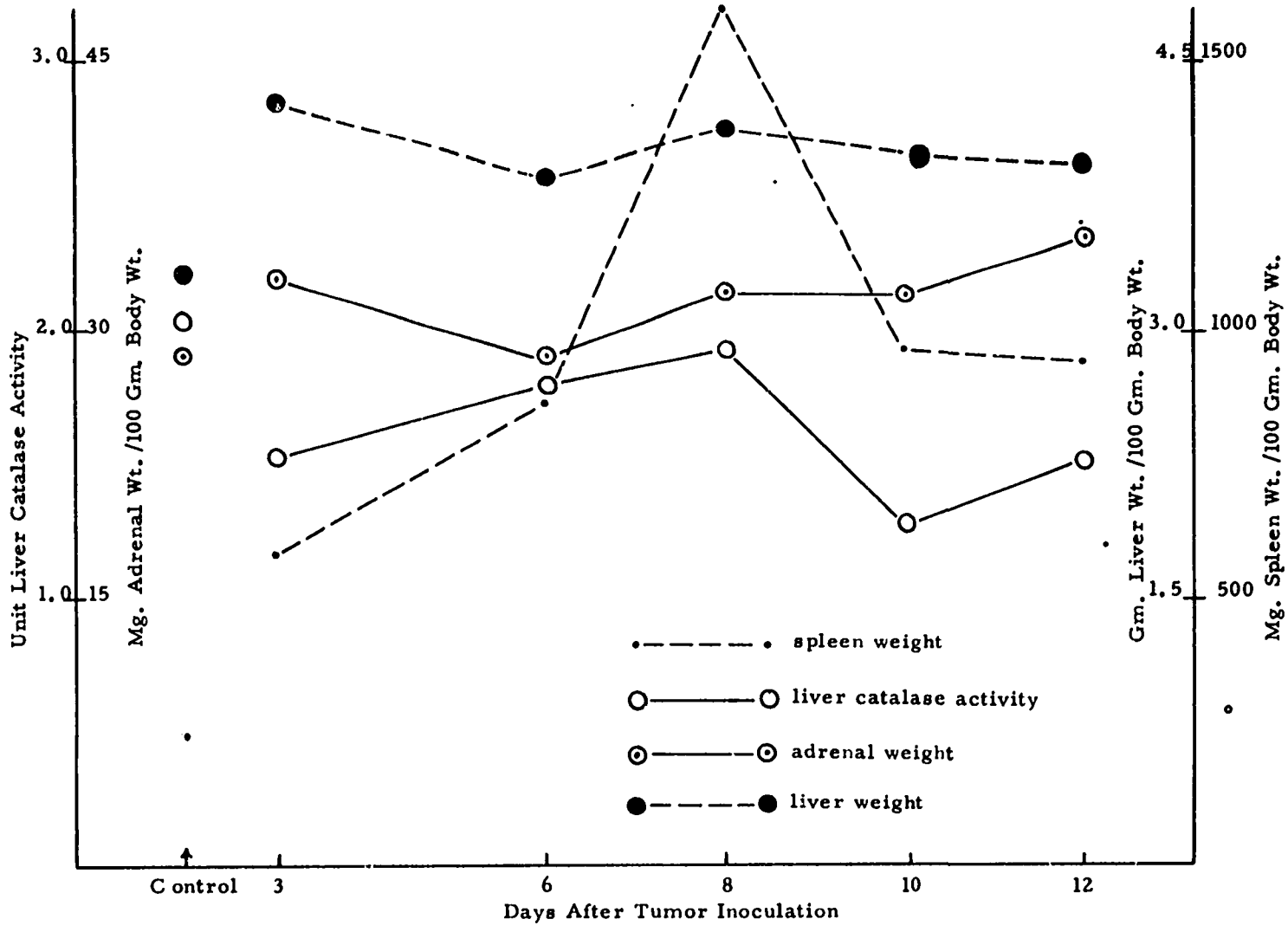


FIG. 3. SYSTEMIC EFFECTS OF WALKER-256 CARCINOSARCOMA ON IMMATURE FEMALE RATS

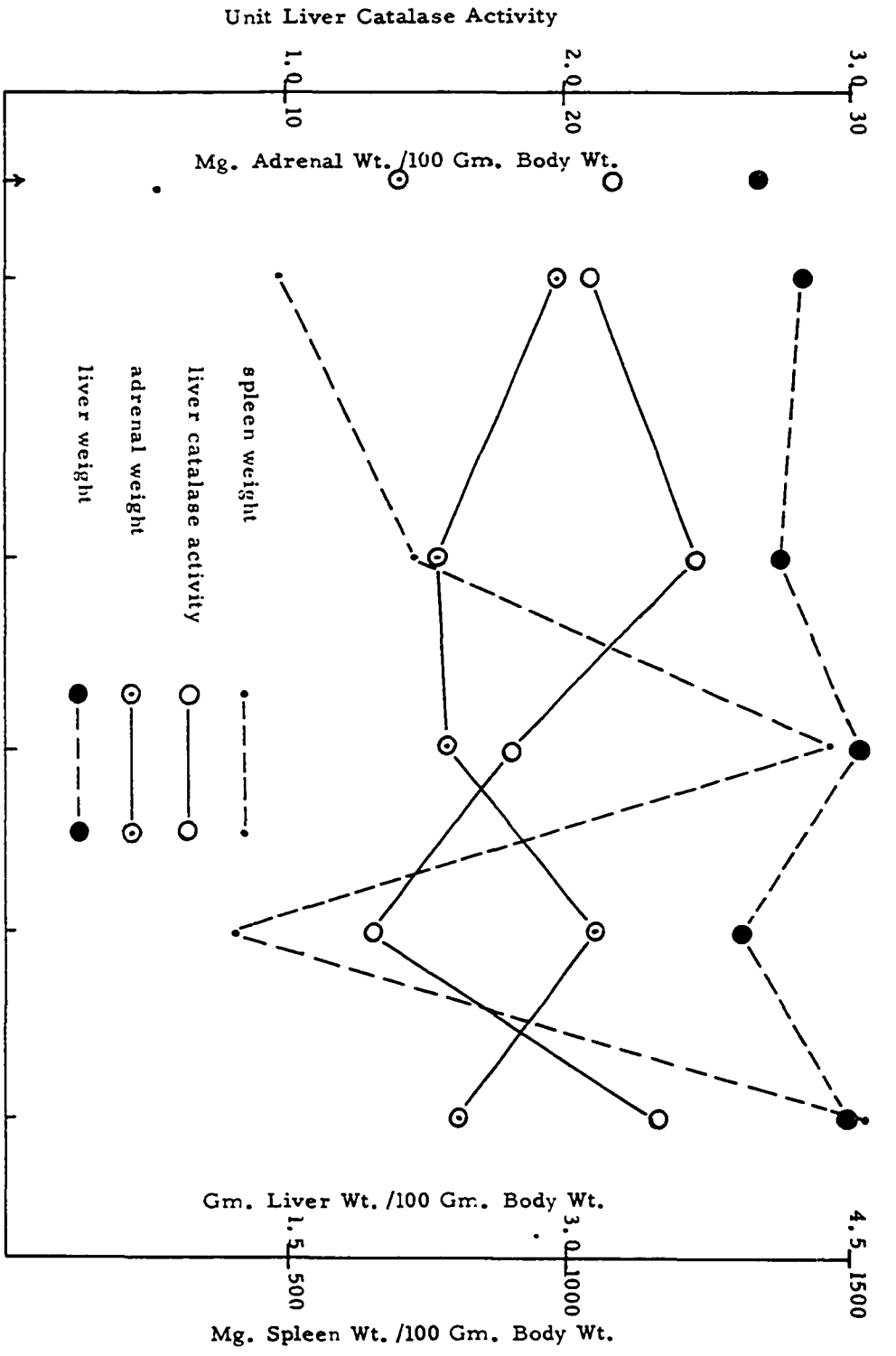


FIG. 4. SYSTEMIC EFFECTS OF WALKER-256 CARCINOSARCOMA ON IMMATURE MALE RATS

in the other three groups. These results were as follows:

Groups	r	z	S.E.$_z$
Mature Male	0.9608	1.9588	0.578
Immature Male	0.8280	1.1880	0.709
Mature Female	0.1180	0.1186	0.578
Immature Female	0.2520	0.2550	0.709

d. A determination of dry weight of spleen, liver, and adrenals in the latter part of this experiment revealed that the spleens of the tumor-bearing and normal animals had similar values for water content. The liver and adrenal glands of the tumorous rats were 2.5 and 3.5 per cent higher in water content respectively. These determinations were performed on a total of ten experimental and 5 control animals.

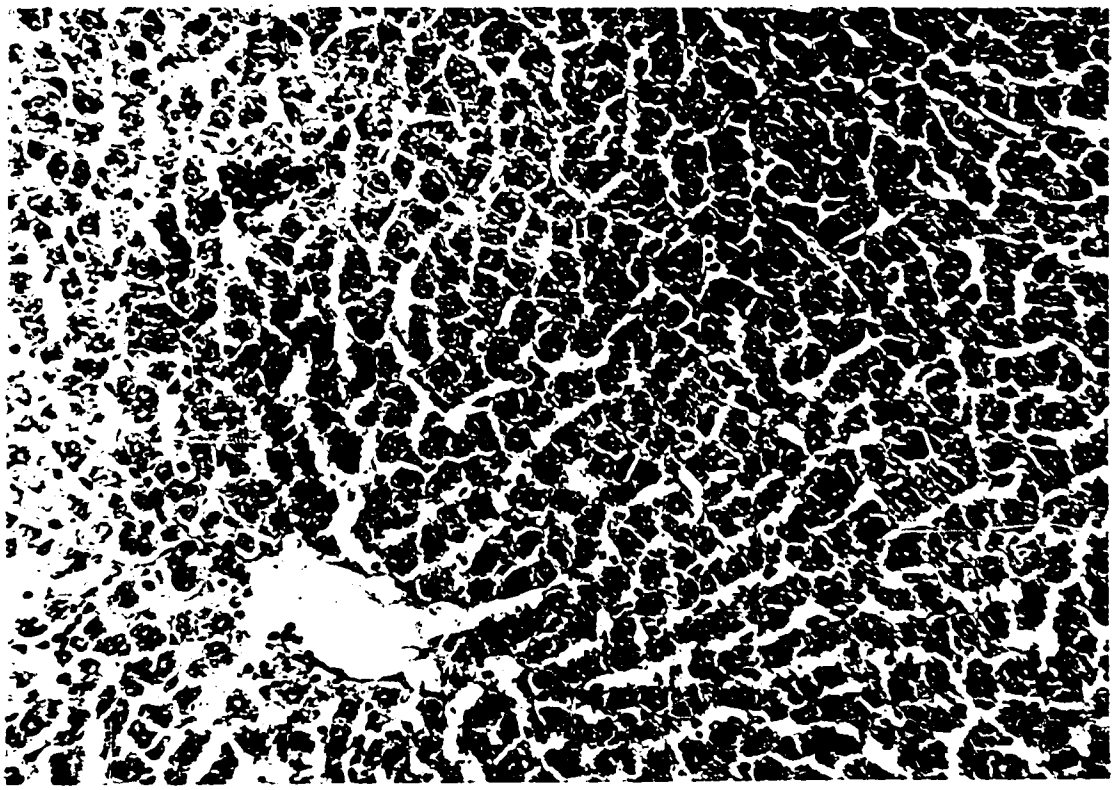
e. When absolute organ weights in each group (Appendices A through D) were plotted as in Figure 1-4, a strikingly similar overall graph was obtained that closely paralleled the changes of the relative weights of the organs when calculated per unit of body weight.

Histological studies of liver, spleen and adrenal glands of animals with the tumor revealed active foci of hematopoiesis in these organs. Hematopoiesis was first detectable, in all of the three organs after the rats had carried the tumor for 8 days. Adrenal glands of both tumorous males and females showed hematopoietic activity in the zona fasciculata. Plates 1, 2, 3, and 4 are photomicrographs of sections of these three organs.

Liver catalase activity of all four groups was subjected to the "t" test and when values for P were equal to 0.05 or less the

Plate 1. Hematopoiesis in liver of tumor-bearing rat (x 163).

Plate 2. Hematopoiesis in liver of tumor-bearing rat (x 473).



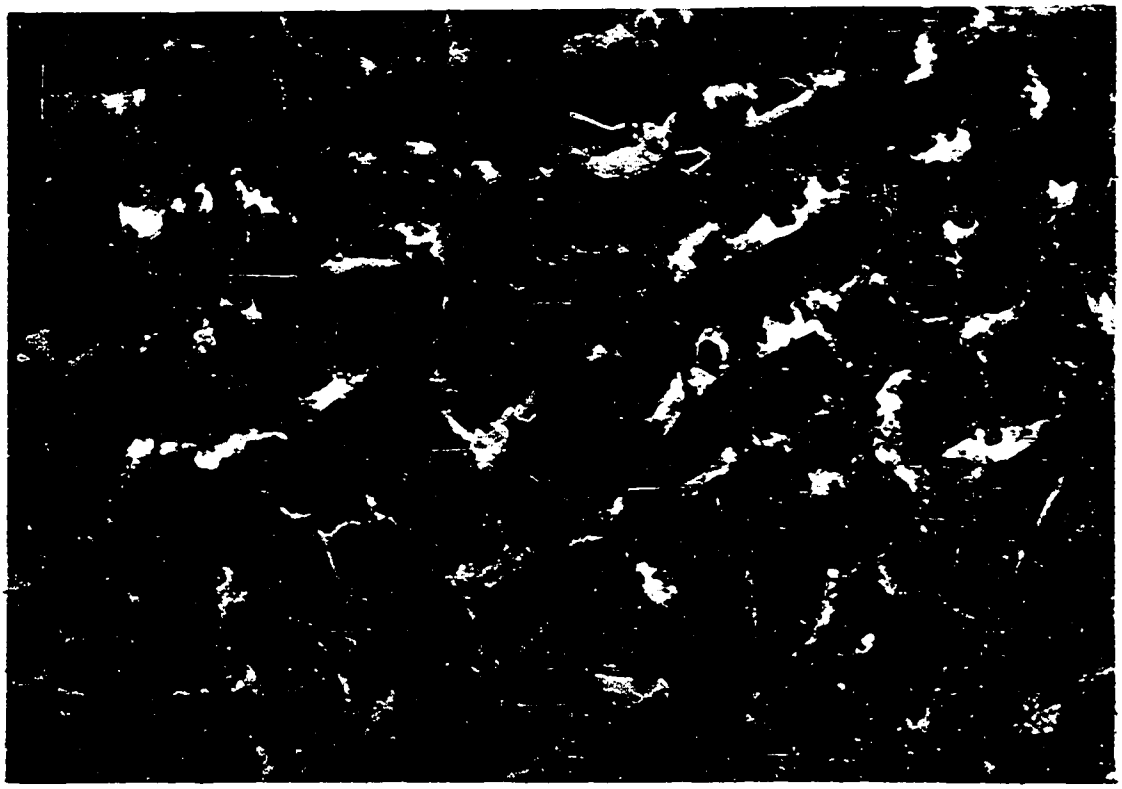
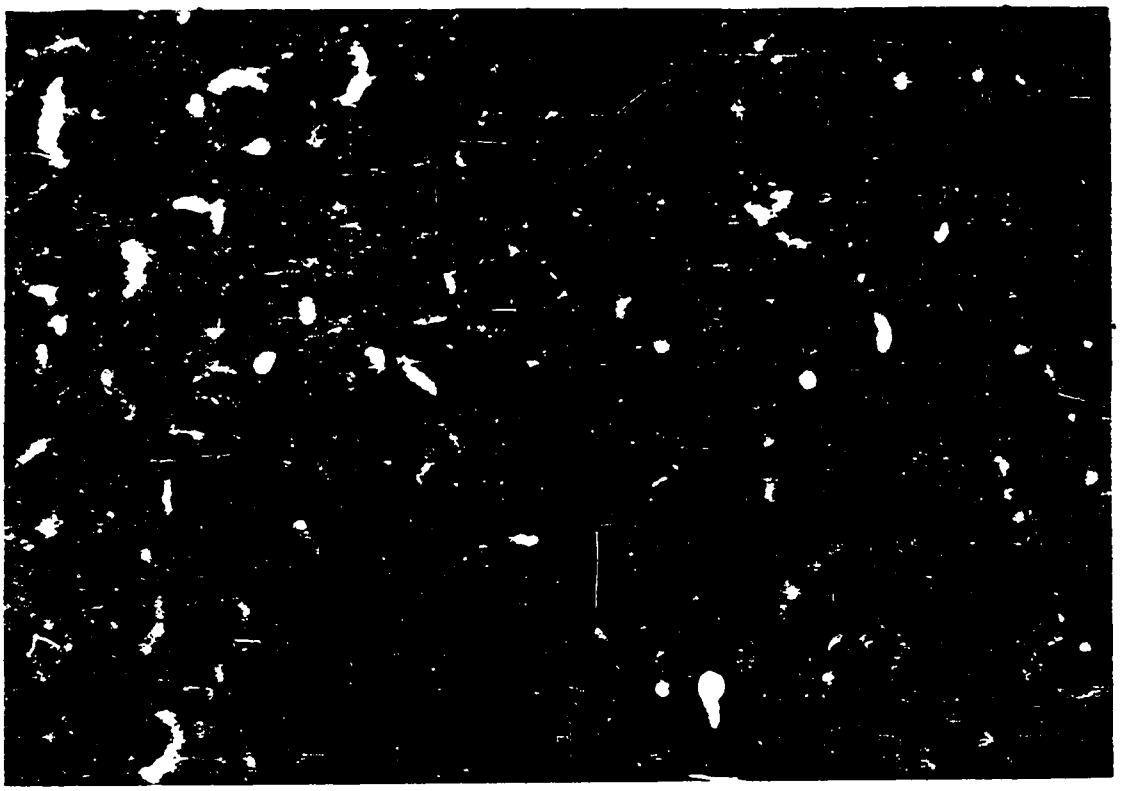
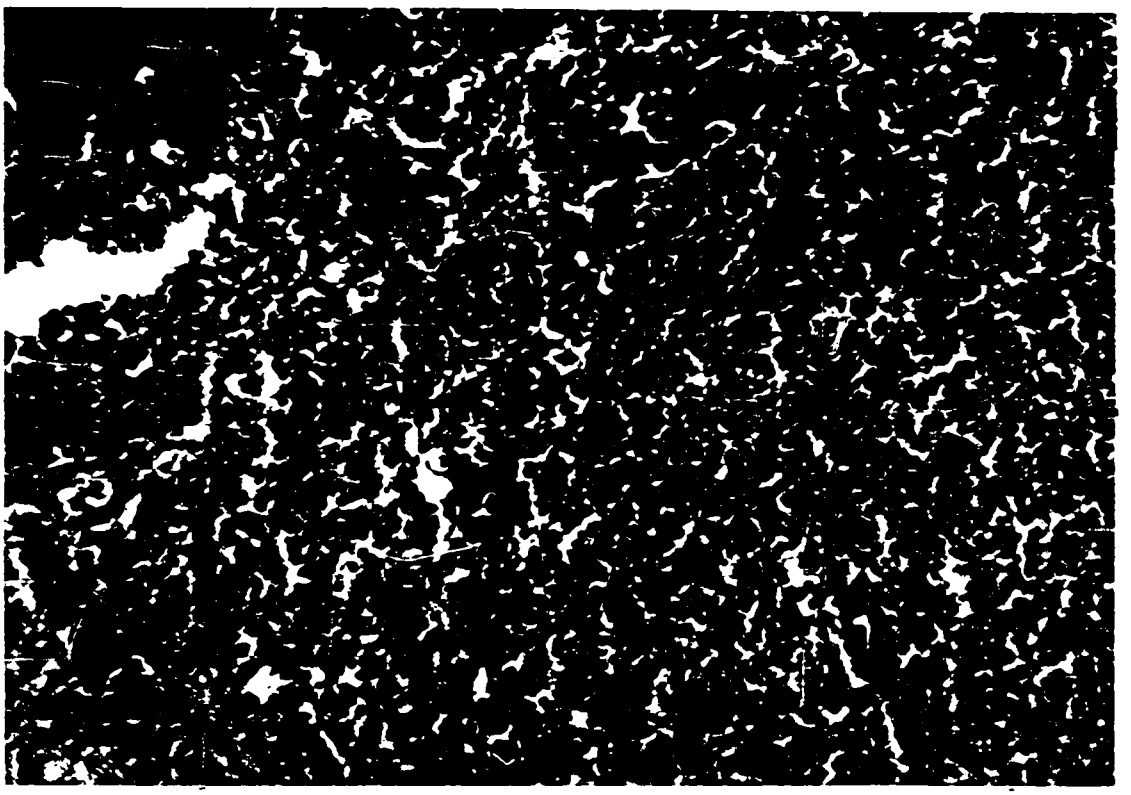


Plate 3. Hematopoiesis in adrenal cortex of tumor-bearing rat (x 473).

Plate 4. Hematopoiesis in spleen of tumor-bearing rat (x 239).





difference was considered to be significant.

The enlargement of adrenal glands has been observed previously by Begg (1951) in a group of rats carrying a solid form of this tumor. Our study supplements his work by supplying the progressive changes in relation to liver catalase.

Other investigators have studied the effects of adrenalectomy on growth rate of the solid form of Walker-256 carcinoma (Talalay et al., 1952), and on Ehrlich mouse ascites tumor (Watson, 1958). These two investigators, however, have reported opposite effects of adrenalectomy on tumor growth. Talalay found that adrenalectomy inhibits the growth of the experimental Walker tumor, while Watson found that growth of the Ehrlich tumor was stimulated by removal of the adrenals.

A study was, therefore, undertaken to establish: (1) the role adrenalectomy on growth rate of the ascitic form of Walker-256 carcinosarcoma as estimated by development of ascites, and (2) the role of adrenalectomy on liver catalase of tumor-bearing rats. For this experiment three groups of mature animals were used.

Groups 1 and 2 were adrenalectomized and sham operated respectively. Each rat in groups 1 and 2 was injected with 1 ml of the ascitic tumor fluid intraperitoneally 4 days after the operation while the animals in group 3 served as controls and received 1 ml of isotonic saline. Table V gives the results of this experiment. Liver catalase of groups 1 and 2 was decreased by 14 and 16 per cent respectively as compared to that of the unoperated control group. While these differences were significant as compared with control values, the adrenalectomized and non-adrenalectomized tumor-inoculated animals showed no

TABLE V

EFFECTS OF ADRENALECTOMY AND TUMOR INOCULATION ON MATURE FEMALE ALBINO RATS

Treatment	Number of Animals	Gm. Liver Wt. per 100 gm. Body Wt.	Mg. Adrenal Wt. per 100 gm. Body Wt.	Mg. Spleen Wt. per 100 gm. Body Wt.	Liver Catalase Activity
Adrenalectomized Tumor-Injected	5	2.41	---	224	2.13 (± 0.16)*
Sham Operated Tumor-Injected	4	2.99	22.1	246	2.08 (± 0.18)*
Untreated Control	3	2.75	15.9	171	2.48 (± 0.07)

*Significant at 0.05 level of probability

significant difference in their liver catalase or in the rate of tumor growth.

These findings raised the question whether adrenalectomy alone would cause any significant change in liver catalase in otherwise normal animals. Troop and Stanley (1956) had shown that adrenalectomized rats had the same liver catalase values as did the control group. The result of our own study also confirmed this finding of the above investigators.

The recent reports on the effect of adrenal steroids on resistance to heterologous tumor transplants in rats and hamsters (Toolan, 1954) along with the inhibitory effect of these hormones on liver catalase (Troop and Stanley, 1956) and on growth rate of Ehrlich ascites mouse tumor (Watson, 1958) pose the question as to whether liver catalase and tumor growth are in fact casually related.

A similar paradox in the reports of the use of cortisone on growth rate of tumors exists in the literature (Toolan, 1954; Watson, 1958). In order to compare our results with those mentioned above, our experiment was planned, making use of the Walker ascites tumor. For the purpose of this experiment, 16 immature male rats were divided into 4 groups as shown in Table VI. Each rat in group 1 received 5 mg of cortisone[1] daily for 13 days prior to tumor inoculation; following tumor inoculation, each rat received 2.5 mg of cortisone daily for 7 days. Rats in group 2 received the same dose of cortisone but without tumor inoculation. Rats in group 3 received

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[1]Cortone acetate, Merck and Co.

TABLE VI

EFFECTS OF CORTISONE ON TUMOR-BEARING MALE ALBINO RATS

(Four Animals in Each Group)

Treatment	Gm. Liver Wt. per 100 gm. Body Wt.	Mg. Adrenal Wt. per 100 gm. Body Wt.	Mg. Spleen Wt. per 100 gm. Body Wt.	Liver Catalase Activity	Development of Ascites
Cortisone	4.34	8.9	167	1.81 (±0.24)*	None
Cortisone Plus Tumor	4.64	14.7	551	1.15 (±0.40)**	ascites was present at autopsy (7 days)
Saline Plus Tumor	3.72	25.1	383	1.11 (±0.25)**	ascites was present at autopsy (7 days)
Controls	3.28	16.1	205	1.98 (±0.10)	None

* Not significant at 0.05 level of probability.

** Significant at 0.001 level of probability.

injections of saline and 1 ml of tumor fluid on the same day that the other two groups were inoculated with the tumor, while rats in group 4 served as controls and received an equivalent amount of saline instead of cortisone. Data in Table VI reveal that although cortisone in the amount injected caused a reduction in liver catalase and a decrease in adrenal weight, it caused no significant change in the rate of ascites development as compared to that of an untreated tumor-bearing control group.

Studies on Resistance in OK-R Rats

During the spring of 1956, a number of colored rats were brought to this laboratory. Some of these animals were inoculated in routine fashion for maintaining the tumor in stock. It was soon discovered that these animals were highly resistant to Walker-256 carcinosarcoma in both nodular and ascitic form. These animals have been designated OK-R (Oklahoma resistant) as outlined earlier. The OK-R strain seemed to be an invaluable tool by which some of the phases of tumor resistance in relation to catalase could be studied. A preliminary study of catalase in these resitant rats showed that their levels were higher by 15 per cent than those of the Holtzman albino strain (susceptible) in use also in our laboratory. Table VII shows the result of this study.

In regard to liver catalase and tumor growth, Greenstein and Andervont (1942) showed that in general, the greatest fall in liver catalase levels is produced by fast-growing tumors, and in fact, certain slow-growing tumors produce little or no change in liver catalase.

TABLE VII

LIVER CATALASE ACTIVITY OF THE OK-R RATS

Rat Strain	Number of Animals	Liver Catalase Activity
OK-R (Resistant)	5	2.36 (± 0.06)*
Albino (Susceptible)	4	2.05 (± 0.05)

*Significant at 0.001 level of probability

Adams (1950), working with sarcoma 37 and carcinoma 63 in mice suggested that a parallelism exists between the initial depression of liver catalase and the resistance of the animal to the tumor. He showed that sarcoma 37 which grows readily produces a greater catalase depression in 24 to 48 hours than did carcinoma 63 which is a slow-growing tumor. He also pointed out that female mice, which showed less catalase depression than the males, appear to be more resistant to tumor growth.

Although resistance to some types of tumor is known to exist in some strains of mice (Heston et al., 1945), no attempt has been made to determine the possible relationship between catalase and natural immunity to a specific tumor.

One interesting approach to this problem seemed available in the use of our resistant strain of animals. It was decided to determine whether liver catalase in these animals could be lowered in cortisone and, if so, to challenge them with tumor when their liver catalase levels were thus reduced. For this experiment three groups of male OK-R and three groups of male albino rats were chosen. Group 2 received a pretreatment injection of cortisone for 10 days prior to tumor inoculation. The daily dose of cortisone was 10 mg injected subcutaneously. After tumor inoculation, the animals received further injections of 5 mg doses every three days for the next 12 days. The control group of 9 OK-R adult males (group 1) received the same dosage of cortisone but with no tumor injection. Table VIII sets forth the results. It became obvious from this study that although cortisone injection caused a significant drop in liver catalase of the OK-R strain, ascites did not develop in these animals after they were inoculated with the tumor. Three control albino

TABLE VIII

EFFECT OF CORTISONE ON LIVER CATALASE AND SURVIVAL TIME
OF THE "OK-R" TUMOR-BEARING MALE RATS

Group	Treatment	Number of Animals	Liver Catalase Activity	Survival Time of Tumor-Injected Rats
1	Cortisone (CK-R)	9	1.89 (± 0.30)*	--
2	Cortisone Plus Tumor (OK-R)	5	1.95 (± 0.49)**	survived (no ascites)
3	Untreated (OK-R) Control	5	2.51 (± 0.26)a**	--
4	Untreated (Albino) Control	3	2.17 (± 0.19)	--
5	Tumor-Injected (Albino)	3	--	10.7
6	Cortisone Plus Tumor (Albino)	3	--	11.0

*Significant at 0.01 level of probability.
**Significant at 0.05 level of probability.
a**Significantly higher than group 4.

susceptible rats received one injection of the ascitic fluid. These animals died within 9 to 11 days after tumor inoculation. The OK-R rats that were treated with cortisone plus the tumor showed the same reduction of liver catalase as did the animals that received cortisone alone. This indicates that the tumor exerted no potentiating effect in catalase-depression produced by cortisone in the OK-R (resistant) animals.

Since cortisone is also known to inhibit the growth of some tumors (Watson, 1958) and since other substances of a non-hormonal character have been shown to reduce liver catalase, it was decided to use such an agent with the thought in mind that the effect of a reduction in catalase activity on tumorigenesis might become apparent. Amino acids containing sulfur are known to cause *in vitro* (Ceriotti, 1957) as well as *in vivo* (Seabra and Deutsch, 1955) inhibition of liver catalase. Cystine apparently causes a 20-25 per cent reduction in liver catalase when given in doses of 5 mg per day intraperitoneally (Seabra and Deutsch, 1955). This amino acid was selected in this work as an *in vivo* catalase inhibitor.

A total of 42 rats, consisting of 2 series, was selected for this study. Series I was composed of 4 groups of susceptible albino rats as follows:

Group 1 (5)	Saline-injected control
Group 2 (4)	Saline-injected and tumor-inoculated
Group 3 (5)	Cystine-treated and tumor-inoculated
Group 4 (5)	Cystine-treated alone

Series II consisted of 3 groups of twice challenged resistant

(OK-R) rats and were as follows:

Group 1 (8) Cystine-treated and tumor-inoculated

Group 2 (8) Cystine-treated alone

Group 3 (7) Saline-treated alone

A 5 mg daily intraperitoneal injection of cystine in rats of group 3 of series I and group 1 of series II started 6 days prior to the tumor inoculation. On the 6th day, 1 ml of ascitic fluid, our usual dose for the transplantation of the tumor, was injected into these animals and followed by the daily injection of 5 mg of cystine for the next 15 days. Group 4 of series I and group 2 of series II received only injections of cystine, whereas a separate group in each series received an intraperitoneal injection of isotonic saline and served as controls. Rats in group 2 of series I received saline injections plus the usual dose of tumor fluid. All animals were then sacrificed and their liver catalase activity was determined. Tables IX and X show the data on the albino susceptible and OK-R resistant animals. Although tumor development caused a significant reduction in liver catalase in groups 2 and 3 of series I, cystine caused no significant decrease in liver catalase of either susceptible or resistant rats. The OK-R animals, as usual, did not develop the tumor after inoculation while all control animals developed the tumor in the regular time.

TABLE IX

EFFECT OF CYSTINE ON LIVER CATALASE OF ALBINO RATS

Group	Treatment	Number of Animals	Liver Catalase Activity
1	Saline-Injected Control	5	2.32 (± 0.17)
2	Saline-Injected and Tumor-Inoculated	4	1.69 (± 0.40)**
3	Cystine-Treated and Tumor-Inoculated	5	1.78 (± 0.45)**
4	Cystine-Treated	5	2.29 (± 0.12)*

*Not Significant at 0.05 level of probability.

**Significant at 0.05 level of probability.

TABLE X

EFFECT OF CYSTINE ON LIVER CATALASE ACTIVITY OF THE OK-R RATS

Group	Treatment	Number of Animals	Liver Catalase Activity
1	Cystine-Treated and Tumor-Injected	8	2.33 (± 0.16)*
2	Cystine-Treated	8	2.43 (± 0.27)*
3	Saline-Injected Control	7	2.25 (± 0.21)

*Not significant at 0.05 level of probability.

CHAPTER III

DISCUSSION

Several hypotheses have been advanced to explain the cause of liver catalase reduction in tumor-bearing animals. The exact mechanism of this enzyme depression, however, remains unknown.

Greenstein and Andervont (1943) showed that depression of catalase was not due simply to growing tissue within the host since neither pregnancy nor progressive growth of embryonic tissue implants in mice caused any change in liver catalase.

It is unquestionably true that a host supporting a fast-growing tumor faces a terrific demand for nutritive material. When an animal is starved, its liver catalase is depressed. This finding was reported by Miller (1947), who concluded that the reduction in catalase was due to inanition of the animal. He found that not only was liver catalase depressed in starvation, but that this decrease paralleled the decrease of protein in the liver.

Sherman et al. (1950), utilizing tumorous rats carrying the solid form of Walker-256 carcinoma showed that the nitrogen content of the liver did not change significantly as compared to that of the force-fed control. This fact has also been confirmed by Stewart and Begg (1953).

Begg et al. (1953), studied the influence of diet on liver

catalase activity and found that, (1) rats carrying the solid form of Walker-256 carcinoma on an adequate protein diet still lost body weight and showed loss of liver catalase and, (2) non-tumorous rats on a low protein diet showed an actual loss of carcass weight but retained normal catalase values. They concluded that loss of liver catalase in tumor-bearing animals is not due to disturbances in body growth or to liver enlargement.

In our experiments, as indicated in Tables I through IV, it is clear that some small increase in liver weight did occur. Histological sections of enlarged livers of rats carrying the ascitic tumor showed highly active centers of hematopoiesis as early as the 8th day after tumor inoculation (plates 1-2). Liver enlargement may be due to two distinct causes: (a) the anemia in the cancerous rats may be re-activating one of the embryologic hematopoietic organs, and, (b) as pointed out by Yeakel (1948), the enlargement of the liver may be connected with protein anabolism in growth of the tumor.

Enlargement of the spleen is a significant finding in tumor-bearing rats. Hematopoietic foci were also observed in the spleen of animals carrying the tumor for 8 days or longer (plate 3).

Splenomegaly has been reported in mice carrying carcinoma 744 by Antopol et al. (1954). The enlargement was noticeable before the tumor was palpable and hematopoiesis was also reported by these investigators. Dalton (1949) postulated that hematopoiesis was the cause of splenomegaly in male and female hamsters bearing a mixed cell sarcoma. Begg (1955) has reported enlargement of the spleen in rats carrying the solid form

of Walker-256 carcinoma. Most investigators agree that the cause of spleen enlargement must be due to hematopoiesis. Based on our studies on dry weight of the enlarged spleen, liver and adrenal glands there is essentially no difference between the percentages of water content of these normal organs and those of the ascitic rat.

In histological studies of the adrenal glands, foci of hematopoiesis in the zona fasciculata were located (plate 4).

Apparently when an animal is in dire enough need of blood cells all the hematopoietic organs that are usually active during embryonic life may resume their role as blood-forming organs during adult life. Of these organs, the liver and spleen are most prominent. It is interesting, however, that when in this present work an isolated experiment on hemoglobin determination was done, it was found that enlargement of the spleen preceded both the onset of low hemoglobin values and the production of ascites.

From our analysis of adrenal weights in relation to liver catalase, it is clearly shown that depression of liver catalase is indeed inversely related to the weight of the adrenal glands in mature tumor-bearing male rats. Since it is also known that any type of stress produces an increase in weight of adrenal glands and that this may increase the secretion of cortical hormones (Selye, 1937), it is probably safe to assume at this time that the adrenal glands of mature male rats, which normally are smaller than those of the mature female, have undergone an extensive enlargement due to a tremendous stress produced by the growth of the tumor. Adrenal enlargement in the male tumor-bearing rat seems

to be greater than that of the other three groups of rats with the tumor. Initial large adrenals in normal females may be due to estrogen secretion (Greep and Jones, 1950). The adrenal glands of these rats may then be already stimulated to their physiological maximum.

It is interesting, however, to notice that although the adrenal glands in the female rats carrying the tumor did not become much enlarged and liver catalase did not change significantly, nevertheless, full ascites developed in every instance. This might indicate that catalase depression is but a secondary factor in host response.

With the fact in mind that adrenalectomy does not affect liver catalase of the otherwise normal rat (Troop and Stanley, 1956), we proceeded to determine the effects of tumor growth on liver catalase of adrenalectomized rats. The results of this experiment, as shown in Table V, indicate that liver catalase was equally depressed in both adrenalectomized and non-adrenalectomized tumorous animals.

The experiment on the effect of cortisone-treatment on the growth rate of this tumor and on liver catalase revealed that although this steroid caused a reduction in liver catalase, there was no change in the rate of ascites formation. This may indicate that the level of liver catalase is not necessarily directly related to tumor development. Although the overall effect of cortisone treatment resulted in low liver catalase activity, this result was not statistically significant because of large variations in response of different animals.

Cortisone plus tumor-inoculation only gave as much decrease in liver catalase as did tumor inoculation alone. This may point to the

fact that the tumor by itself will lower this enzyme maximally.

The mechanism by which cortisone decreases liver catalase is not known. It is known, however, that rats treated with cortisone exhibit hypoferemia (Hamilton et al. (1951). Since catalase is a heme-containing enzyme, decrease of iron in the blood may, in some way, cause reduction of liver catalase. Cortisone may also exert its effect simply through its catabolic action.

While this work was in progress a paper by Troop (1958) presented a new hypothesis in regard to the hormonal mechanism of the regulation of liver catalase in normal rats. He proposed that liver catalase may be controlled by the ratio of mineralocorticoid to glucocorticoid rather than by the absolute value of either. If this assumption is correct, one may assume that in tumor-bearing rats in which liver catalase is low, this ratio may be upset by increased values of glucocorticoids. The loss of weight normally present in tumor-bearing rats may then be explained on the basis of exaggerated gluconeogenesis due to overstimulation of the adrenal cortex. This hypothesis needs to be substantiated.

In the second part of this work, the mechanism for tumor resistance was sought. The first approach to this problem was focused around liver catalase activity since in a preliminary study of normal OK-R (resistant) animals, a significant 15 per cent higher level in liver catalase was observed. Furthermore, these animals maintained this higher value even after being challenged twice with the standard dose of tumor inoculum. It was found that although cortisone injection caused a significant decrease in liver catalase of the resistant strain, no tumor

developed.

It is known that cortisone may exert an inhibitory effect on the growth rate of solid tumors (Watson, 1958). However, our experiment, as indicated in Table VII, showed that cortisone exerted no significant effect, either stimulation or inhibition on formation of ascites in the albino rats. This has also been indicated in Table VIII (groups 5 and 6).

Injection of cystine into both susceptible albino and resistant OK-R rats caused no significant change in liver catalase. Cystine has been reported to be a catalase inhibitor, (Seabra and Deutsch, 1955). It is assumed that failure of cystine to give catalase depression in our experiments may be due to its insolubility in water or saline and, therefore, it might not have been injected in sufficient quantity to cause depression of liver catalase. In any case, the cystine-injected OK-R (resistant) rats did not take the tumor, while the albino (susceptible) animals developed the tumor in every case.

The possibility of a natural or acquired antibody in the OK-R rat was considered as an additional factor in producing resistance in OK-R animals. Sekla and Barvic (1956) reported the isolation of a strain of animals resistant to Walker-256 carcinoma among some black and hooded rats in their colony. This is a different factor from the one present in our OK-R strain, because the rats in Sekla's colony took the nodular type of tumor which grew for a few days and regressed later. Their resistance factor apparently is carried in the blood stream and can be transferred to susceptible Wistar rats by way of serum injection. A more recent report by Sekla (1958) states that their resistance factor is also capable of being milk-borne. We have not investigated these possibilities in our

animals.

A new, non-specific antibody, designated "properdin" by Pillemer (1954) also remains to be investigated. According to Pillemer, rats that show a high degree of resistance to parasites in general have a higher titre of properdin than any other species studied. It is possible that the OK-R rats may have an even greater titre of properdin in their serum than do the normal susceptible animals.

From studies on systemic effects of Walker-256 carcinosarcoma in susceptible animals, and on the effect of this tumor in the OK-R rat, and also in the light of the work of other investigators, a suggestion may be offered in regard to tumorigenesis and liver catalase depression. The development of a tumor may produce such an insult to the host that in an attempt to compensate, all homeostatic mechanisms are activated to a maximal capacity. This insult is indeed greater than either starvation or injury alone. It is rather a result of both. The tumor mass as a nitrogen trap (Mider 1951), not only deprives the host of its nutritive materials, but it also exerts other debilitating effects, hence a tremendous activation of the defenses of the body ensues. This then may bring about, by way of the adrenal cortex, a depression of liver catalase which is still greater than that found in starvation. Liver catalase levels, therefore, may not be directly related to the potentiality of a host to develop or to resist a tumor, but the reduction of this enzyme may be an inevitable consequence of tumor growth.

CHAPTER IV

SUMMARY AND CONCLUSIONS

1. The systemic effects of the ascitic form of Walker-256 carcinosarcoma were studied in mature and immature male and female rats. The animals received an initial dose of the tumor fluid and at a certain interval they were sacrificed and their liver catalase activity determined. Weights of adrenals, liver, and spleen per 100 grams of body weight were calculated.

a. Liver catalase activity was found to decrease in all groups of rats. The drop was most noticeable between the 8th to the 12th day after tumor inoculation. Tumor-bearing mature females showed less change in liver catalase than did mature males or immature animals of both sexes.

b. The adrenal glands became enlarged beginning on the third day after tumor implantation. In mature male groups the increase in absolute weight of adrenals bore a significant inverse relationship to the liver catalase activity of the tumor-bearing mature male hosts. Foci of hematopoiesis were noted in the zona fasciculata of the adrenal cortex of the tumor-bearing rat from the 8th day after implantation until the time of autopsy.

c. Liver weight was increased in all animals inoculated with

the tumor but this increase was of a low magnitude. The liver also showed hematopoiesis at about the 8th day after tumor inoculation.

d. Spleen enlargement was also observed. This increase was noticeable in all the four groups of rats. Foci of hematopoiesis were also noticeable from the 8th day after tumor inoculation until the time of autopsy. Significant enlargement of the spleen in all four groups preceded both hematopoiesis and production ascites.

2. Adrenalectomized tumor-bearing rats showed no greater decrease in liver catalase than did the adrenalectomized non-inoculated animals.

3. Although cortisone injection caused a drop in activity of liver catalase, this drop was not statistically significant. Cortisone did not change the growth rate of ascites tumor as estimated by the formation of ascites in susceptible animals.

4. A colored strain of rats was found to be resistant to both ascitic and nodular forms of Walker-256 carcinosarcoma. This strain has been designated OK-R (Oklahoma resistant). In a preliminary study, it was found that the OK-R strain showed a significant 15 per cent higher liver catalase activity than the susceptible albino strain. The following studies were performed to clarify the possible role of catalase in relation to tumor resistance in the "OK-R" animals:

a. Cortisone, a known catalase-depressing agent, was given to the OK-R rats in an attempt to lower the liver catalase. The animals were then challenged with the tumor. It was found that even though liver catalase was lowered in the OK-R rats by 25 per cent, all of the animals

maintained resistance to the tumor.

b. Cystine, a non-hormonal catalase depressant in the doses given, failed to decrease liver catalase of either the susceptible albino or the resistant OK-R rats.

5. The hypothesis that the reduction of liver catalase of the tumor-bearing rat is a primary effect of hormones of the adrenal glands is discussed in light of this work and that of others.

6. From these studies it is concluded that liver catalase activity bears no direct relationship to tumorigenesis in the albino (susceptible) strain or to resistance in the OK-R (resistant) rats, and that cortisone, even though it markedly reduces liver catalase, has no effect on resistance in the OK-R strain. Cortisone in our hands did not inhibit the development of ascites in susceptible animals.

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APPENDIX A

DATA ON SYSTEMIC EFFECTS OF WALKER 256 CARCINOSARCOMA ON MATURE FEMALE RATS

Days After Inoculation	Final Body Wt. (gm)	Liver Wt. (gm)	Adrenal Wt. (mg)	Spleen Wt. (mg)	Liver Catalase
2	390	10.8	50.0	526	2.20
2	380	11.6	52.0	665	1.89
2	362	11.6	54.8	733	2.54
4	375	11.3	61.9	982	2.11
4	355	9.3	56.0	886	2.13
4	365	10.0	60.8	920	2.14
6	340	11.5	62.0	1276	2.17
6	340	10.2	62.0	1012	1.74
6	345	10.6	64.8	1082	1.75
8	350	11.7	64.9	1862	2.08
8	345	10.5	74.0	2066	1.97
8	325	10.6	56.0	1662	1.74
10	355	11.3	67.0	3034	2.27
10	365	11.7	58.4	2545	2.75
10	355	12.4	58.0	1728	2.16
12	360	11.4	78.4	2306	1.95
12	350	11.0	72.0	1961	1.98

APPENDIX A (Continued)

Days After Inoculation	Final Body Wt. (gm)	Liver Wt. (gm)	Adrenal Wt. (mg)	Spleen Wt. (mg)	Liver Catalase
12	340	9.8	68.8	2227	2.29
Control	355	10.2	42.8	620	2.16
Control	345	9.0	46.0	512	2.17
Control	360	9.3	57.0	771	2.47
Control	345	8.5	60.8	504	2.11
Control	325	8.7	50.0	538	2.35
Control	350	9.7	49.8	735	2.28
Control	350	9.6	64.0	767	2.31
Control	360	9.3	57.0	596	1.73
Control	335	8.9	61.6	591	2.75

APPENDIX B

DATA ON SYSTEMIC EFFECTS OF WALKER-256 CARCINOSARCOMA ON MATURE MALE RATS

Days After Inoculation	Final Body Wt. (gm)	Liver Wt. (gm)	Adrenal Wt. (mg)	Spleen Wt. (mg)	Liver Catalase
3	335	10.6	37.8	708	2.65
3	355	10.4	46.0	832	2.42
3	335	9.8	37.8	752	2.47
6	425	13.5	46.0	1697	2.19
6	420	12.5	56.0	1596	2.31
6	445	14.4	55.9	1312	2.27
9	380	13.6	57.0	3216	2.20
9	405	13.6	72.8	2838	1.78
9	415	14.4	52.0	3684	2.75
10	375	12.5	88.6	2258	0.88
10	370	14.3	106.0	2494	1.16
10	370	10.8	108.0	1759	1.40
11	410	12.0	93.0	2768	0.97
11	395	12.0	101.0	1861	0.91
11	330	11.0	100.0	1825	1.15
12	350	11.0	82.0	2059	1.43
12	415	14.3	63.5	2632	1.42

APPENDIX B (Continued)

Days After Inoculation	Final Body Wt. (gm)	Liver Wt. (gm)	Adrenal Wt. (mg)	Spleen Wt. (mg)	Liver Catalase
12	400	12.5	83.2	2327	----
Control	335	10.2	32.0	689	2.46
Control	345	10.0	33.8	556	1.91
Control	355	10.3	42.0	785	2.35
Control	420	13.6	41.0	748	1.95
Control	420	13.4	45.0	891	2.81
Control	430	13.5	42.1	845	2.44
Control	385	10.4	49.0	890	3.33
Control	405	10.4	42.0	746	2.47
Control	370	10.8	41.0	753	2.78
Control	395	10.1	41.8	825	2.83
Control	375	10.5	41.0	716	3.05
Control	405	10.5	47.0	767	3.47

APPENDIX C

DATA ON SYSTEMIC EFFECTS OF WALKER-256 CARCINOSARCOMA ON IMMATURE FEMALE RATS

Days After Inoculation	Final Body Wt. (gm)	Liver Wt. (gm)	Adrenal Wt. (mg)	Spleen Wt. (mg)	Liver Catalase
3	180	8.0	59.0	980	1.61
3	195	8.0	56.0	1400	1.48
3	195	8.1	70.0	1110	1.56
3	205	8.9	70.0	1004	1.37
6	215	7.8	64.0	2317	1.98
6	195	7.5	66.6	1702	1.48
6	195	7.5	59.0	1365	1.92
6	215	8.6	59.0	1958	1.74
6	220	8.6	56.0	1658	1.80
8	250	10.2	86.0	4000	1.82
8	230	10.5	76.0	4200	1.97
8	255	10.5	71.0	3400	1.92
8	243	9.4	71.2	3950	1.89
8	240	9.7	88.0	3600	2.06
10	245	9.7	84.0	2252	1.14
10	240	9.7	75.0	2737	1.58
10	260	9.9	97.0	2058	0.82

APPENDIX C (Continued)

Days after Inoculation	Final Body Wt. (gm)	Liver Wt. (gm)	Adrenal Wt. (mg)	Spleen Wt. (mg)	Liver Catalase
10	250	9.9	62.0	2867	1.48
10	240	9.9	77.0	1948	1.31
12	240	10.8	74.0	2823	1.43
12	260	11.5	87.0	2218	1.53
12	255	9.5	82.0	2090	1.68
12	230	9.3	116.0	1312	1.70
12	245	7.1	72.0	3134	1.18
Control	220	7.1	61.0	499	1.87
Control	225	6.5	67.0	523	2.26
Control	250	---	68.0	520	2.07
Control	220	7.0	53.0	586	2.10
Control	205	6.4	57.5	463	2.04
Control	195	7.1	67.5	544	1.88

APPENDIX D

DATA ON SYSTEMIC EFFECTS OF WALKER-256 CARCINOSARCOMA ON IMMATURE MALE RATS

Days After Inoculation	Final Body Wt. (gm)	Liver Wt. (gm)	Adrenal Wt. (mg)	Spleen Wt. (mg)	Liver Catalase
3	215	9.5	40.0	1170	2.05
3	250	10.5	45.0	1304	1.90
3	245	10.5	51.0	1180	2.43
3	230	9.8	43.0	1038	2.17
3	230	9.5	47.0	1018	1.91
6	265	11.2	42.0	1795	2.99
6	255	10.5	45.0	1990	2.24
6	270	11.5	36.0	1846	2.35
6	255	10.0	40.4	2162	2.46
6	280	---	47.6	1815	2.30
8	270	12.5	43.0	4100	2.06
8	280	12.3	48.0	4100	1.82
8	295	13.7	43.0	5100	1.82
8	290	12.3	55.0	3038	1.32
8	280	13.7	44.0	4300	1.94
10	285	11.4	62.0	1846	1.86
10	280	11.0	61.0	1899	0.75

APPENDIX D (Continued)

Days After Inoculation	Final Body Wt. (gm)	Liver Wt. (gm)	Adrenal Wt. (mg)	Spleen Wt. (mg)	Liver Catalase
10	295	11.0	71.0	1623	0.85
10	310	11.0	74.0	2239	1.00
10	315	13.6	59.0	2725	1.97
12	305	14.0	48.2	4500	2.61
12	315	15.0	62.0	4500	2.07
12	330	14.5	48.1	4900	2.16
12	320	14.5	48.9	4700	2.51
12	305	13.0	43.8	5400	2.26
Control	310	11.9	35.0	748	2.21
Control	310	13.2	54.0	858	2.08
Control	285	11.7	43.0	818	1.95
Control	270	10.0	33.0	842	2.53
Control	240	10.0	34.0	585	2.01

APPENDIX E

DATA ON EFFECTS OF ADRENALECTOMY COMBINED WITH TUMOR INOCULATION ON MATURE FEMALE RATS

Treatment	Final Body Wt. (gm)	Liver Wt. (gm)	Adrenal Wt. (mg)	Spleen Wt. (mg)	Liver Catalase
Adrenalectomy and Tumor	365	8.3	---	618	2.25
Adrenalectomy and Tumor	320	8.3	---	880	2.27
Adrenalectomy and Tumor	340	8.0	---	721	2.10
Adrenalectomy and Tumor	375	8.5	---	806	2.09
Adrenalectomy and Tumor	290	7.5	---	728	1.93
Sham Operation and Tumor	350	10.0	74	670	2.13
Sham Operation and Tumor	300	10.2	78	830	2.17
Sham Operation and Tumor	310	9.1	67	775	1.89
Sham Operation and Tumor	325	9.0	64	861	2.13
Control	365	8.0	52	578	2.46
Control	275	10.0	55	524	2.45
Control	370	9.0	50	605	2.53

APPENDIX F

DATA ON EFFECTS OF CORTISONE ON TUMOR-BEARING IMMATURE MALE RATS

Treatment	Final Body Wt. (gm)	Liver Wt. (gm)	Adrenal Wt. (mg)	Spleen Wt. (mg)	Liver Catalase
Cortisone Injection	205	8.7	16.4	281	1.95
Cortisone Injection	160	6.1	12.0	224	2.08
Cortisone Injection	180	9.0	16.8	301	1.63
Cortisone Injection	130	5.6	14.2	290	1.60
Cortisone and Tumor	175	7.4	32.4	887	1.10
Cortisone and Tumor	140	6.6	16.0	802	1.30
Cortisone and Tumor	180	8.8	25.0	1252	1.25
Cortisone and Tumor	140	6.6	21.0	599	0.96
Saline and Tumor	285	8.4	71.0	623	0.88
Saline and Tumor	235	9.8	55.0	1452	1.51
Saline and Tumor	250	10.5	70.0	1101	1.02
Saline and Tumor	260	9.2	63.0	664	1.02

APPENDIX F (Continued)

Treatment	Final Body Wt. (gm)	Liver Wt. (gm)	Adrenal Wt. (mg)	Spleen Wt. (mg)	Liver Catalase
Control	305	10.3	41.6	669	1.84
Control	260	8.3	42.0	491	2.07
Control	250	8.0	41.8	600	2.03
Control	230	7.7	41.4	392	1.98

APPENDIX G

DATA ON LIVER CATALASE ACTIVITY OF FEMALE "OK-R" AND FEMALE ALBINO RATS

Strain	Liver Catalase Activity
OK-R	2.35
OK-R	2.36
OK-R	2.27
OK-R	2.46
OK-R	2.37
Albino	1.83
Albino	2.14
Albino	2.04
Albino	2.20

APPENDIX H

DATA ON EFFECTS OF CORTISONE ON LIVER CATALASE AND TUMOR DEVELOPMENT IN MALE "OK-R" AND MALE ALBINO RATS

Treatment	Liver Catalase	Remarks
Cortisone (OK-R)	1.49	---
Cortisone (OK-R)	1.73	---
Cortisone (OK-R)	1.98	---
Cortisone (OK-R)	1.97	---
Cortisone (OK-R)	2.04	---
Cortisone (OK-R)	2.19	---
Cortisone (OK-R)	2.08	---
Cortisone (OK-R)	1.36	---
Cortisone (OK-R)	2.18	---
Cortisone and Tumor (OK-R)	1.16	No ascites formed
Cortisone and Tumor (OK-R)	2.25	No ascites formed
Cortisone and Tumor (OK-R)	2.16	No ascites formed
Cortisone and Tumor (OK-R)	1.78	No ascites formed
Cortisone and Tumor (OK-R)	2.38	No ascites formed
Untreated (OK-R)	2.87	---
Untreated (OK-R)	2.43	---
Untreated (OK-R)	2.51	---

APPENDIX H (Continued)

Treatment	Liver Catalase	Remarks
Untreated (OK-R)	2.16	---
Untreated (OK-R)	2.59	---
Untreated (Albino)	2.20	---
Untreated (Albino)	1.97	---
Untreated (Albino)	2.43	---
Tumor-Inoculated (Albino)	---	Ascites developed in 10 days
Tumor-Inoculated (Albino)	---	Ascites developed in 11 days
Tumor-Inoculated (Albino)	---	Ascites developed in 11 days
Cortisone and Tumor (Albino)	---	Ascites developed in 10 days
Cortisone and Tumor (Albino)	---	Ascites developed in 11 days
Cortisone and Tumor (Albino)		Ascites developed in 12 days

APPENDIX I

DATA ON EFFECT OF CYSTINE ON LIVER CATALASE OF FEMALE ALBINO RATS

Treatment	Liver Catalase Activity
Cystine	2.22
Cystine	2.40
Cystine	2.12
Cystine	2.38
Cystine	2.34
Cystine and Tumor	1.06
Cystine and Tumor	2.12
Cystine and Tumor	2.12
Cystine and Tumor	1.57
Cystine and Tumor	2.02
Saline and Tumor	1.91
Saline and Tumor	1.10
Saline and Tumor	1.94
Saline and Tumor	1.79
Saline	2.15
Saline	2.47

APPENDIX I (Continued)

Treatment	Liver Catalase Activity
Saline	2.47
Saline	2.13
Saline	2.38

APPENDIX J

DATA ON EFFECT OF CYSTINE ON LIVER CATALASE OF "OK-R" FEMALE RATS

Treatment	Liver Catalase Activity
Cystine	2.92
Cystine	2.45
Cystine	2.47
Cystine	2.76
Cystine	2.27
Cystine	2.16
Cystine	2.06
Cystine	2.33
Cystine and Tumor	2.37
Cystine and Tumor	2.57
Cystine and Tumor	2.21
Cystine and Tumor	2.20
Cystine and Tumor	2.26
Cystine and Tumor	2.42
Cystine and Tumor	2.13
Cystine and Tumor	2.48

APPENDIX J (Continued)

Treatment	Liver Catalase Activity
Saline	2.50
Saline	2.26
Saline	2.12
Saline	2.51
Saline	2.31
Saline	1.91
Saline	2.12