THE EFFECT OF ADRENALECTOMY AND HYDROCORTISONE ON THE RATE OF GROWTH OF HARDING-PASSEY MELANOMAS IN MICE

by

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It is an established fact that many animal and human neoplasms can be influenced by the addition or removal of certain drugs, chemicals or hormones resulting in acceleration, inhibition or regression of the tumor growth. Further knowledge in this regard is desired in the study of both human and animal melanomas. Therefore, this experiment was undertaken to determine if the rate of growth of the Harding-Passey mouse melanoma implanted into albino mice was affected by bilateral adrenalectomy or by bilateral adrenalectomy plus daily injections of hydrocortisone.

REVIEW OF THE LITERATURE

In 1925, Harding and Passey discovered a pigmented tumor[1] growing on the ear of a chocolate brown mouse. It was found to be a melanoma which grew upon implantation into other mice. Its behavior is much like other transplantable mouse tumors in that it grows progressively when grafted into other mice, recurs after an incomplete removal, is invasive, and sometimes gives rise to metastases. In 1939 another type of mouse melanoma was discovered growing spontaneously at the base of the tail on a female JAX-dba mouse by Cloudman[2]. This Cloudman melanoma has been grown in the above strain and also in C-mice (albino) from which an amelanotic melanoma (S91A) has been derived by transplanting lighter portions of the tumor[3]. Both of the Cloudman melanomas metastasize more readily than the Harding-Passey and thus kills the animal in a shorter time.

Among other animals in which melanomas have been produced or have arisen spontaneously are: dogs[4]; the axolotl[5]; drosophilae[6]; and certain fish[7]. A melanoma was produced on the skin of a dog by the application of tar once a week for six years. A melanotic neoplastic disease appeared spontaneously in hybrid tropical fish produced by cross-mating platyfish and Mexican swordtails. An unsuccessful attempt was made to produce melanomas in C57blxdba hybrid mice by painting the

skin with 5, 9, 10-trimethyl-1, 2-benzanthracene, although areas of melanin pigmentation were produced as well as atrophy, hyperplasia, inflammation, ulceration, papilloma formation, epidermoid carcinoma and sarcoma[8].

The melanomas occuring in various animals have been studied in great detail microscopically, both in vivo and in vitro. Algire[9] devised an ingenious method of studying melanomas in vivo in the mouse by the use of a transparent chamber implanted in the skin, through which he could see the site of implantation in the subcutaneous tissue and could use magnification up to 500 diameters to study the growth characteristics of the tumor during the first thirty to forty days. After this time the surrounding tissue became necrotic resulting in sloughing out of the chamber. By this method Algire found, with the Cloudman S91 melanoma, that during the first twenty days there was no evidence of tumor growth and the surrounding tissue showed only leucocytes, macrophages and multinucleated cells. After twenty days vascularization of the area occurred and was accompanied by migration and proliferation of the neoplastic cells. He could easily distinguish between the pigment-containing macrophages and the melanoma cells by the uniform size and distribution of the pigment granules in the latter[10].

In vitro tissue culture studies of the Harding-Passey mouse melanoma showed that it is composed of three types of cells; namely,

melanoblasts, macrophages and fibroblasts[11]. There are two types of melanoblasts: small cells with uniform slender dendrites and large cells with stouter dendrites containing knobby swellings. The macrophages, which make up a large portion of the tumor, are differentiated into actively moving sparsely laden cells and swollen cells filled with clumped melanin granules. The fibroblasts are spindle-shaped with oval nuclei and are almost devoid of melanin. Tissue cultures of fish melanomas were obtained in which all cell types characteristic of mammalian melanomas could be identified, i. e., macrophages, fibroblasts and melanoblasts. The morphology and behavior of the melanoblast, including clasmatosis, were identical to that found in mouse and human melanomas. In addition to the above cells, typical melanophores were also observed[12]. The Cloudman melanoma was studied in vitro for thirteen months, resulting in the isolation of the primary tumor cell. Injection of this tumor cell into mice produced an amelanotic melanoma.

By back crossing second generation hybrids of platyfish and Mexican swordtails, Gordon and Smith found that these fish would develop a melanotic disease during the first day of life which was histologically similar to that found in the adult hybrids[13]. The spindle cells in these growths resembled the melanoblasts of mammalian melanomas. These tumors were infiltrative and destructive to adjacent tissue, but no metastases were observed.

Biochemical studies on animal melanomas have brought to light some interesting facets of this problem. In 1944, Greenstein and Algire[14] compared the enzymatic activities of the Cloudman S91 and S91A amelanotic melanoma and found that the S91 tumor possessed not only the tyrosinase system but also a cyanide insensitive system which oxidized p-phenylene diamine. The S91A or amelanotic tumor was found to be almost completely lacking in both these enzymes. The activity of cytochrome oxidase was about equal in both tumors. In 1947, Burk and his co-workers confirmed the above findings by showing that all three mouse melanomas had a much higher percentage of stimulation of oxygen consumption by the addition of p-phenylene diamine than any other tumors previously tested. This stimulation was eliminated by cyanide, which points to cytochrome oxidase as the enzyme present. These workers also reported that these neoplasms showed similar aerobic and anaerobic metabolism, O_2 consumption and respiratory quotients to tumors in general[15]. Then, in 1949, DuBuy, Woods and others isolated melanin granules of the above three tumors by centrifugation and found that all three possessed cytochrome oxidase, succinic dehydrogenase and cytochrome c enzyme activities. However, the granules of the amelanotic S91A melanoma did not possess tyrosinase or dopa oxidase activities, while the S91 tumor displayed dopa oxidase but no appreciable tyrosinase[16]. In the light of later studies by Lerner

and Fitzpatrick and the investigations they have conducted on the mechanism of melanin formation[17] it now appears that tyrosinase and dopa oxidase are one and the same enzyme.

In 1950, Dyer and Ross found that ascorbic acid content of S91 and S91A melanomas was two to three times greater than that found in the Harding-Passey melanoma and other tumors[18]. Ascorbic acid reduces melanin in the skin to a light colored substance and prevents the oxidation of dopa quinone, which is an intermediate step in the production of melanin from tyrosine. Also the S91A melanoma has no tyrosinase activity. It would seem that these two facts might partially explain the lack of pigmentation in certain melanomas.

Other investigators have turned to the field of hormones and their effect on animal neoplasms in an effort to learn more about these tumors. Not only melanomas but many other tumors have been subjected to changes in the hormone environment experimentally. In 1931, Sugiura attempted to influence the growth of transplanted carcinoma, sarcoma and melanoma in mice by the use of aqueous, alcoholic, ether and glycerine extracts of sheep adrenal cortical tissues, without any demonstrable effect[19]. He followed this experiment with a more intense treatment of Harding-Passey melanomas with subcutaneous and intramuscular injections of an aqueous extract of suprarenal cortex. The injections showed no curative, retarding or accelerating influence

upon tumor growth, nor did they prolong the life of the animal[20]. In 1933, Sugiura and Benedict included in their investigations on mouse melanomas an alcoholic extract of the thymus gland, theelol, and an aqueous pituitary extract. None of these had any effect except the pituitary extract which exerted a definite stimulating effect on the melanoma and, to a lesser extent, upon other tumors being tested[21]. So, in 1934, Sugiura injected various amounts of intermedin, a pituitary extract, ranging from 0.05 cc. to 0.3 cc. three times a week over a period of eleven weeks, into twenty young adult mice having seven-day-old Harding-Passey melanomas. The injections had no deleterious effects on the animals and had a slight but distinct stimulating effect on the growth of the tumors[22].

Many other animal tumors have been treated with hormones, notably cortisone, in an effort to alter their rate of growth. Heilman and Kendall tried cortisone on a lymphosarcoma in mice with some success, but the tumor eventually recurred and killed all the animals[23]. Murphy and Sturm tried cortisone and ACTH injections on rat leukemia with increased survival time in every case[24]. Diller and others found that cortisone inhibited mouse sarcoma in females, but had no effect on the tumor in males or on MCA-induced sarcomas[25]; while Brownell, Moore and Jerome found that whole adrenal extract caused necrosis of sarcoma 37, but DOCA, cortisone and hydrocortisone all enhanced its

growth[26]. Higgins and his co-workers found that cortisone restricted the growth of a transplanted rhabdomyosarcoma in mice[27], and Ingle and others produced suppression of a Walker rat carcinoma by administering cortisone[28] as did Gottschalk and Grollman working with mouse mammary carcinoma and high doses of cortisone[29].

Recently another aspect of the action of cortisone has been brought to light. Toolan has found that about ninety per cent of human tumors including melanomas could be successfully transplanted into rats and hamsters which had been treated pre- and post-operatively with cortisone, while no tumors grew in the untreated controls[30]. Along these same lines, Green and Whitely found that tumors could be transplanted from one species of animal to another by similar treatment[31].

Obviously, the next step in this pattern of investigation was the removal of hormonal influence from the tumor environment by surgical methods. Adrenalectomy had been tried alone and in combination with gonadectomy and/or hypophysectomy in animals having various types of neoplastic growths, with equivocal results[32-35]. Although adrenalectomy or hypophysectomy has never been done in animals having melanomas, each has been done separately in humans. Adrenalectomy and orchiectomy was done on three male patients with far advanced melanomatosis with no distinguishable improvement[36]. Surgical hypophysectomy was performed on a 32-year-old man with

metastatic melanoma. The patient survived nine weeks and developed pan-hypopituitarism one month post-operatively. The operation had no effect on melanoma growth, although unusual degenerative changes were found in metastases to the liver and spleen[37].

No review of this subject would be complete without a discussion of some of the various other factors that have been investigated to determine if any effect was exerted on melanoma growth in animals. As early as 1934 Sugiura and Chesley studied the effect of heavy water (deuterium oxide) on the viability of mouse sarcoma and melanoma in vitro. They found that there was no deleterious effect on the tumor over a twenty-four hour period when the solution contained salts of Locke-Ringer solution in isotonic amounts, but the growth capacity was completely destroyed when the tumor was left in heavy water alone at a pH of 7.0 for twenty-four hours[38]. In 1935, Sugiura and Benedict found that prolonged feeding of a magnesium-high diet had no effect on the tumor[39]. However, they discovered that an anemia-producing diet slowed the growth of the tumor but did not change the percentage of tumor "takes" or regressions[40]. Both goitrogenic and high iodine diets slowed the growth of melanomas, but this was thought to be due to the impaired health of the animals on these diets[41]. Some years later Sugiura found that the following factors had no effect on mouse melanoma growth: age variation, castration in either male or female, strain of the animal, and

immersion in a solution of pH 6, 7 or 8. However, the growth is somewhat inhibited by immersion in a solution of pH 5 or 9, and it is completely destroyed by dehydration from a frozen state or in immersion in solution of pH 4 or 10. He also discovered that the growth is not filterable. He noted that no amelanotic Harding-Passey melanomas have been found in all the transplants he had performed in perpetuating the tumor up to the present time (1944)[42].

MATERIALS AND METHODS

Animals:

Ninety-six stock albino female mice obtained from the Roscoe B. Jackson Memorial Laboratory were used. These animals were housed in individual cages throughout the experiment, given one per cent salt water or plain water ad lib, depending on which group they were in, and all were maintained on a diet of Super Meat kibbled dog food.

Tumor Inoculation:

On February 23, 1955, all 96 animals were inoculated with approximately equal sized fragments of a Harding-Passey mouse melanoma obtained from a tumor-bearing stock albino mouse. At the time of implantation this tumor was approximately one centimeter in diameter and was actively growing under the skin on the flank of the donor animal. The tumor was obtained by clamping the throat of the animal, removing the tumor as quickly as possible, and placing it in an ice-cold phosphate-Ringer's solution which was buffered to pH 7. The tumor was divided into small fragments and injected subcutaneously into the left hip of each animal using a No. 14 trocar. All the animals survived this procedure and seemed to suffer no ill effects from it. As there was not enough time to weigh each piece of implanted tumor separately, random samples of the fragments were removed and

weighed after the procedure was concluded. These fragments ranged from four to eight milligrams.

The tumor site of each mouse was inspected at weekly intervals to note any sign of tumor growth; and if the tumor was palpable, it was measured to the nearest millimeter with a vernier caliper.

Adrenalectomies:

Five weeks after implantation of the tumors, bilateral adrenalectomies were performed on 80 of the 96 animals. The operations were performed under ether anesthesia, using an ether cone with the open drop method. It required about 20 to 30 seconds to induce full surgical anesthesia in the animal by this technique with easy control of the depth of anesthesia. Three anesthetic deaths occurred during the early operations.

The posterior thoraco-lumbar region of each animal was clipped as closely as possible, the area cleaned with seventy per cent alcohol, and a midline longitudinal incision approximately one to one and a half centimeters long made in the skin from T-12 to L-5. As the skin was freely movable over the back, it was possible to use the single incision as an operative approach to both adrenals. The kidneys and adrenals were then exposed bilaterally by sharp dissection through the fascia just lateral to the spinal muscles and immediately posterior to the costo-vertebral angle. The adrenal glands were removed by

bringing them to the surface with a pair of small thumb forceps and grasping them with a pair of locking toothed forceps. This was usually sufficient to free the gland and the peri-adrenal tissue. Occasionally severe bleeding would occur at this point, necessitating packing the area with cotton sponges. Usually, however, there was little or no bleeding. The incision through the fascia was closed with a single 00000 silk suture and the skin was closed with several interrupted sutures of the same material.

Control Animals:

The control animals were subjected to the same type of anesthesia and operation as those in the experimental groups with the exception that no adrenalectomies were done.

Groups:

Of the 96 animals inoculated with melanoma, 72 survived surgery. None of the animals that underwent mock surgery died, but 23 of the mice that had undergone adrenalectomies died during the first few days after surgery. The remaining animals were divided into three separate groups.

The first group (Group A) consisted of 29 animals that had been adrenalectomized. These animals were all maintained on one per cent salt water and tap water and kibbled dog food ad lib, and housed in individual cages. The second group (Group B) consisted of 28 animals

previously adrenalectomized, which were maintained on kibbled dog food and one per cent salt water and tap water ad lib. These animals were given subcutaneous injections of one milligram of hydrocortisone daily except Sunday throughout the duration of the experiment. The third group (Group C) were the control animals, 15 in number, whose adrenals were intact. This group was smaller than the other two because it was felt that the mortality rate would be considerably less among these animals than in the other two groups.

Measurements:

The inoculation site of each animal was inspected at weekly intervals, and if growth had occurred, careful measurements were made of the diameter of the tumor using vernier calipers. Measurements were made in two axes at right angles to each other if the tumor was not round.

Autopsies:

Autopsies were performed on all animals that died in their cages after the first two weeks following adrenalectomy and on all the animals that lived to the end of the experiment. Each tumor site was carefully inspected for any signs of growth, and if any were present the tumor was removed, weighed and saved for microscopic examination[1]. In

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1. A careful search for persistence of adrenal tissue was made in each animal from groups A and B but none was found.

addition, from every animal autopsied the heart, lungs, liver, kidneys and spleen were removed and, along with the tumor, were placed in Vandegrift's Fixative[43] composed of the following:

Ethyl alcohol, 95%	80.0 cc.
Formalin, full strength	12.0 cc.
Glacial acetic acid	4.5 cc.
Picric acid	4.0 cc.
Mercuric chloride	0.2 cc.
Urea	0.5 cc.

All tissues were dehydrated, imbedded and sectioned in the usual manner and stained with hematoxylin and eosin. In addition, special melanin stains were made on some of the tumor slides to identify the pigment present.

TABLE I

Size of the Tumor in Millimeters by Weekly Intervals

Group A (Adrenalectomy)

Animal Number	Weeks after Adrenalectomy 0	1	2	3	4	5	6	Weight of Final Measurements (Grams)
1	3	9	9	12	19	22	25	7.31
7	11	15	17	22	(26)*	(29)*	(33)*	7.99
11	0	3	6	7	7	7	7	0.90
15	0	5	5	11	15	19	21	5.26
29	0	5	7	14	18	24	28	16.36
30	4	11	11	14	22	24	30	16.70
32	0	0	0	5	8	11	14	2.60
35	6	12	12	16	(20)*	(22)*	(26)*	2.17
36	3	8	8	13	18	(21)*	(25)*	3.07
40	5	11	12	18	26	27	32	18.89
44	4	6	9	14	19	23	29	13.43

*Estimate of missing items by Yates method[43].

TABLE II

Size of the Tumor in Millimeters by Weekly Intervals

Group B (Adrenalectomy + Hydrocortisone)

Animal Number	Weeks after Adrenalectomy							Weight of Final Measurements (Grams)
	0	1	2	3	4	5	6	
39	0	3	5	5	6	(6)*	(6)*	0.12
48	4	4	4	(4)*	(5)*	(3)*	(2)*	0.20
50	0	3	3	5	5	6	6	0.07
53	5	6	6	(6)*	(7)*	(5)*	(3)*	0.29
54	0	4	6	7	9	7	(7)*	0.90
55	0	8	8	10	8	(8)*	(8)*	0.55
57	4	8	8	13	12	(12)*	(12)*	0.83
69	5	11	11	13	17	(18)*	(20)*	2.40

*Estimate of missing items by Yates method[43].

TABLE III

Size of the Tumor in Millimeters by Weekly Intervals

Group C (Control)

Animal Number	0	1	2	3	4	5	6	Weight of Final Measurements (Grams)
8	8	13	15	24	41	(49)*	(58)*	16.99
23	7	9	9	9	13	18	23	8.25
24	13	18	21	25	38	(42)*	(51)*	16.80
43	0	6	9	15	17	17	21	5.68
77	0	3	6	7	7	7	7	0.50
94	5	9	9	18	21	27	34	22.45

*Estimate of missing items by Yates method[43].

OBSERVATIONS AND RESULTS

Of the three groups observed, twenty-five animals developed tumors and lived a sufficient length of time after surgery to be included in the data. These animals were distributed amont the three groups as follows: Group A (adrenalectomy + 1% NaCl), eleven animals; Group B (adrenalectomy + hydrocortisone), eight animals; and Group C (control), six animals. The remainder of the animals either did not survive surgery or develop tumors. Tables I, II, and III show the tumor growth of each animal in the three different groups by weekly intervals, and Charts 1, II, and III demonstrate graphically the same information.

Group A:

These animals appeared to survive very well following adrenalectomy. Of the eleven animals in this group three with tumors died during the experiment, with eight animals alive and apparently healthy at the termination. Microscopic examination of the animals that died during the experiment revealed pulmonary edema and bronchopneumonia, but metastatic lesions were not found in any animals of this group.

Group B:

All of the animals of this group, with one exception, succumbed during the experiment. These animals had less tumor growth than either of the other groups as can be seen from TableII and Chart II.

Microscopic examination of the necropsy tissues showed quite marked pulmonary edema and focal areas of bronchopneumonia in all animals. Two of the animals had evidence of bacteriemia, i. e., septic thrombi in the atrium and clumps of bacteria in the collecting tubules of the kidneys.

Group C:

It appears that the two animals that died before the experiment terminated did so as a result of pneumonia from debilitation caused by the large size of the tumors they bore.

Microscopic examination of the tumors from all three groups of animals displayed no variation in cellular structure so any difference found as a result of the changes in tumor environment will be purely on a gross basis, i. e., measurements of the rate of growth. The appearance microscopically was that of a tumor made up of several different kinds of cells, including the typical melanoblasts, macrophages and fibroblasts which are typical of the Harding-Passey melanoma. The most noticable observation was the numerous large, round or oval macrophages packed with dark brown-staining pigment which sometimes obscured the small, inconspicuous nucleus. These made up about one third of the tumor. The true tumor cells were rather fusiform, contained very little pigment, and had prominent

vesicular nucleus, which varied greatly in size and shape between different cells. Mitotic figures were frequently seen in these nuclei. The tumor seemed to have a very adequate blood supply as evidenced by the many vascular spaces present. No definite capsule could be found. Other cells within the tumor included fibroblasts which contained no demonstrable pigment and had a less prominent nucleus than the fusiform cells. No tumor metastases were seen in any organ examined but isolated macrophages containing the dark brown pigment were frequently seen in the liver and rarely in other organs.

Statistical Analysis:

Regression lines were calculated for each group of animals, and the results are shown in Chart IV. The significance of these lines will be tested below. The general formula for the regression line is:

$$\tilde{Y} = \bar{Y} + b(X-\bar{X})$$

where $\bar{Y}$ represents the rate of growth, $\bar{X}$ the time and b the slope of the line. To test the hypothesis of linearity of regression and the deviations from the regression line it was first necessary to compute an analysis of variance which was done in accordance with Table IV. Tables V, VI, and VII are the analyses of variance for the three groups. It can be concluded from the analyses that in all three cases there is a significant regression coefficient relating the growth of the tumor to time and that the points determined fit a log growth-time line. In other

words, the regression lines are linear, and the deviations from these lines are not significant.

In order to determine the dependence of the variable Y (tumor growth) on the variable X (time) it was necessary to test the hypothesis that tumor growth is independent of time, i. e., that the regression slope B = O.

Test for Independence:

Hypothesis: B = O. Level of significance = 0. 01

$$t_o = \frac{(b - O)\, s_x\, (N - 1)^{1/2}}{s_{yx}}$$

Control Group:

$$t_o = \frac{(9.16 - 0)\ 0.985\ (44)^{1/2}}{9.964} = 6.2$$

(df = 36)

$$|t_{0.01}| \geq 2.43$$

Adrenalectomy, Salt H_2O Group:

$$t_o = \frac{(7.05 - 0)\ 0.985\ (76)^{1/2}}{5.58} = 10.8$$

(df = 63)

$$|t_{0.01}| \geq 2.37$$

Adrenalectomy, Hydrocortisone Group:

$$t_o = \frac{(2.12 - 0)\ 0.985\ (55)^{1/2}}{3.75} = 4.2$$

(df = 36)

$$|t_{0.01}| \geq 2.43$$

Since the t_o values are significant at the 0.01 significance level the hypothesis that B = O is rejected and the variable Y is found to be dependent on the variable X.

The final step in statistical evaluation of the data was the comparison of the regression lines. This was done in the following manner.

Comparison of the Regression Lines:

(a) Hypothesis: The three regression lines are equal.

Level of significance = 0.05.

$$F = \frac{S\frac{SSxy}{SSxx} - \frac{\text{within } SSxy}{\text{within } SSxx}}{\frac{k-1}{S(y_1 - y_e) + S(y_1 - y_e) + S(y_1 - y_e)}}$$

$$F = \frac{\frac{580.49}{36.87} - \frac{518.34}{70.521}}{\frac{6}{\frac{465.76}{1211}}} = 22.10$$

(df = 6/1211)

Since F is larger than 2.16 the hypothesis that the three regression lines are equal is rejected.

(b) Hypothesis: The regression of Group C (control) is equal to the regression of Group A (adrenalectomy + salt water).

Level of significance = 0.01

$$t_o = \frac{b_1 - b_2}{s'(1/Sx_1^2 + 1/Sx_2^2)\,1/2} = \frac{2.11}{3.85(0.269)} = 2.03$$

(df = 99) $\quad |t_{0.01}| \geq 2.47$

Since the t_o is less than 2.47 the hypothesis is accepted and one can assume that these two regressions are equal.

(c) Hypothesis: The regression of Group C (control) is equal to the regression of Group B (adrenalectomy + hydrocortisone).

Level of significance = 0.01

$$t_o = \frac{b_1 - b_2}{s'(1/Sx_1^2 + 1/Sx_2^2)\,1/2} = \frac{7.04}{0.906(0.269)} = 28.85$$

(df = 99) $\quad |t_{0.01}| \geq 2.48$

Since the t_o value is greater than 2.65 the hypothesis that the control and the hydrocortisone treated animals have equal regressions is rejected.

TABLE IV

Analysis of Variance Procedure

Source of Variation	Sum of Squares	Degrees of Freedom
Linear Regression (L)	$b^2(SSX_{ij}^2 - \frac{(SSX_{ij})^2}{N})$	1
Deviation from Regression	Difference (T - L)	k - 2
Random Sampling (Error)	$SSY_{ij}^2 - S\ \frac{T_y^2}{n_i}$	n - k
Total (T)	$S(\frac{T_{yi}^2}{n_i} - \frac{T_y^2}{N})$	k - 1

TABLE V

Analysis of Variance (Control)

Source of Variation	Sum of Squares	Degrees of Freedom	Mean Squares	F	P
Regression	2962.7	1	2962.7	21.28	Significant
Deviation	62.06	5	12.41	0.09	Not significant
Error	4934.37	31	139.17		
Total	3583.3	37			

For 0.05 Significance: $F_{1, 31} = 4.16$ $F_{5, 31} = 2.56$

TABLE VI

Analysis of Variance (Adrenalectomy + Salt Water)

Source of Variance	Sum of Squares	Degrees of Freedom	Mean Squares	F	P
Regression	3207.6	1	3207.6	92.40	Significant
Deviation	380.3	5	76.06	2.19	Not significant
Error	4934.37	62	34.7		
Total	3587.9	68			

For 0.05 Significance: $F_{1,62} = 3.99$ $F_{5,62} = 2.28$

TABLE VII

Analysis of Variance (Adrenalectomy + Hydrocortisone)

Source of Variation	Sum of Squares	Degrees of Freedom	Mean Squares	F	P
Regression	211.44	1	211.44	7.88	Significant
Deviation	23.14	5	4.63	0.173	Not significant
Error	778.01	29	26.82		
Total	234.58	35			

For 0.05 Significance: $F_{1,29} = 4.18$ $F_{5,29} = 2.54$

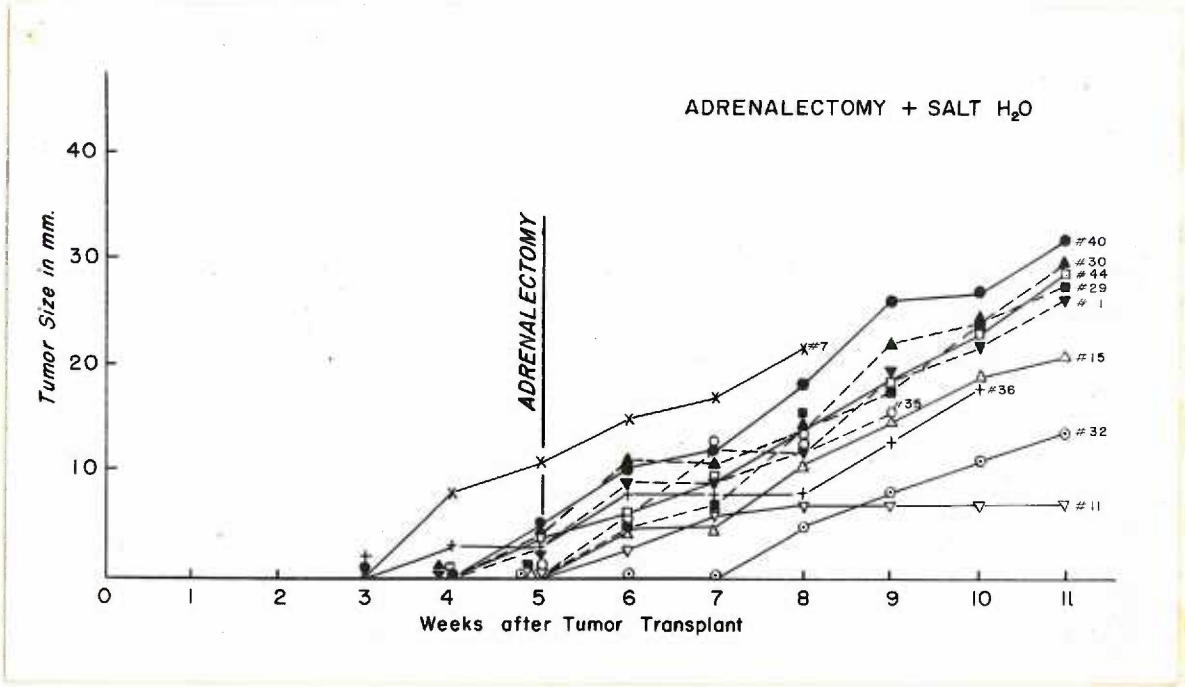


CHART I

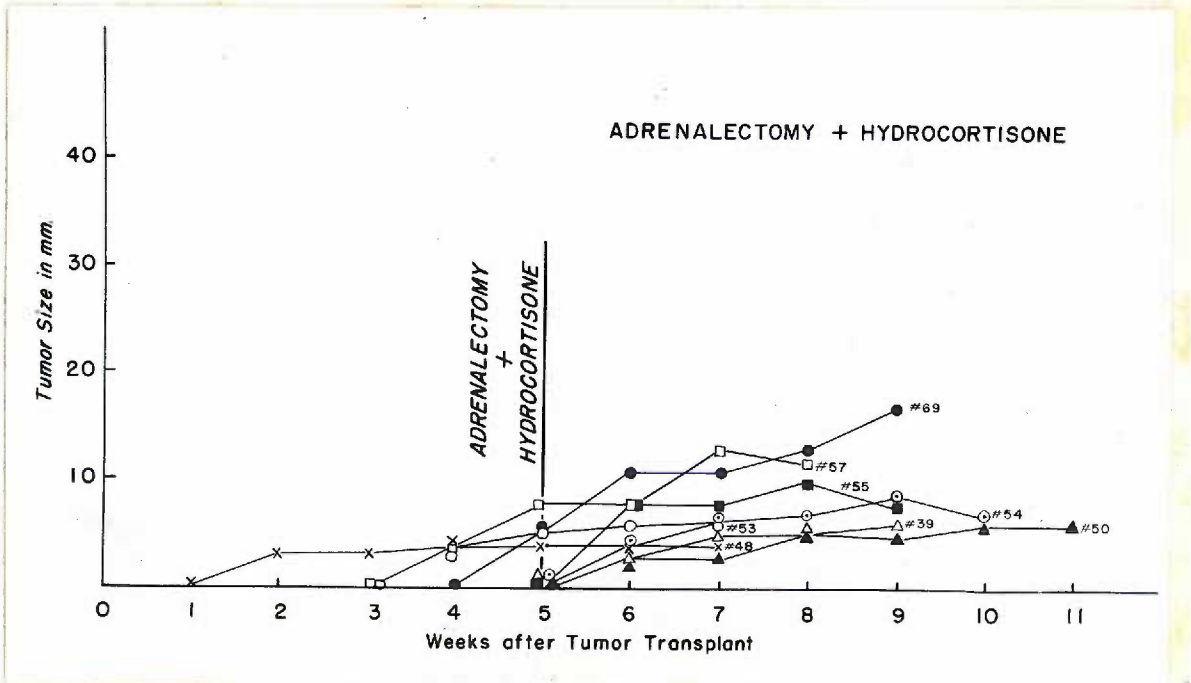


CHART II

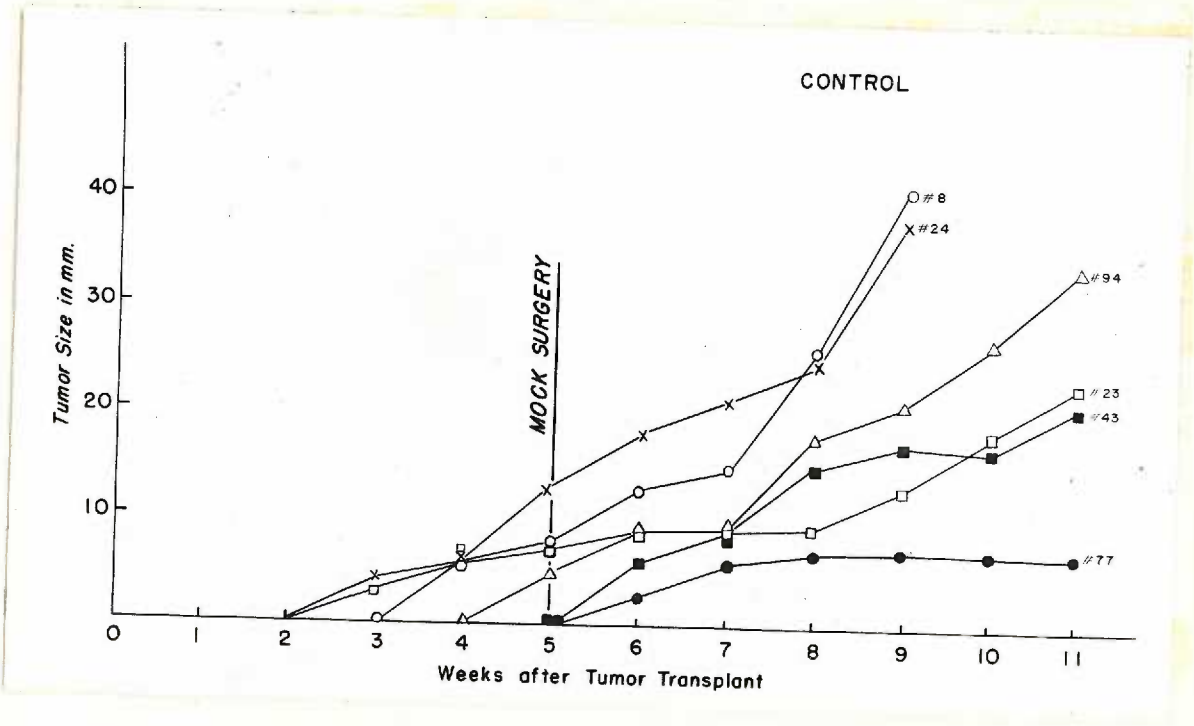

CONTROL
Tumor Size in mm.
40
30
20
10
0
1
2
3
4
5
6
7
8
9
10
11
Weeks after Tumor Transplant
MOCK SURGERY
#8
#24
#94
#23
#43
#77

CHART III

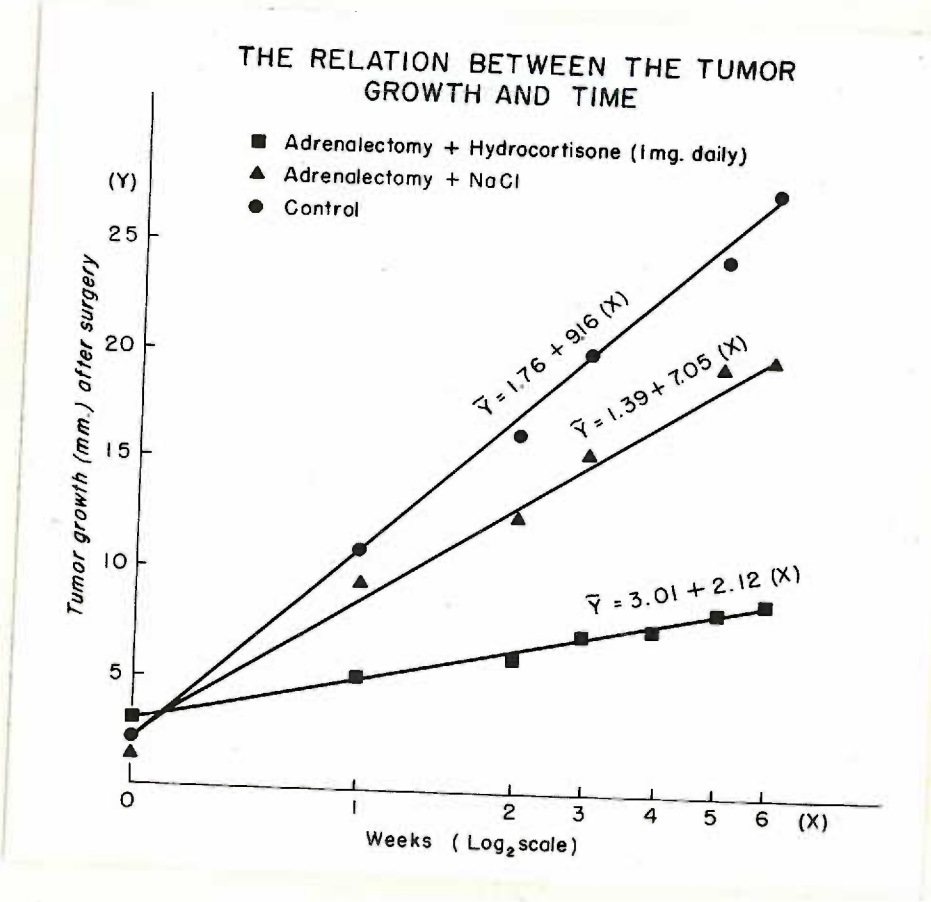

THE RELATION BETWEEN THE TUMOR GROWTH AND TIME
Adrenalectomy + Hydrocortisone (1mg. daily)
Adrenalectomy + NaCl
Control
(Y)
25
20
15
10
5
0
Tumor growth (mm.) after surgery
$\bar{Y} = 1.76 + 9.16\,(X)$
$\bar{Y} = 1.39 + 7.05\,(X)$
$\bar{Y} = 3.01 + 2.12\,(X)$
1
2
3
4
5
6
(X)
Weeks (Log_2 scale)

CHART IV

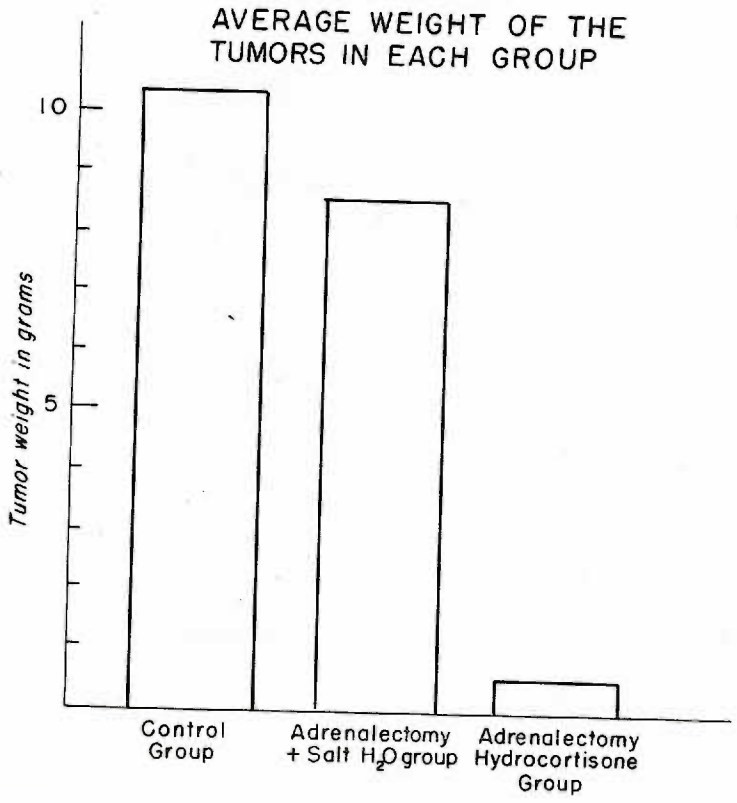

AVERAGE WEIGHT OF THE TUMORS IN EACH GROUP
Tumor weight in grams
10
5
Control Group
Adrenalectomy + Salt H_2O group
Adrenalectomy Hydrocortisone Group

CHART V

Figure 1:

Comparison of tumor size in an adrenalectomized animal (right) and control animal.

Figure 2:

Low-power photomicrograph of melanoma from Group A animal.

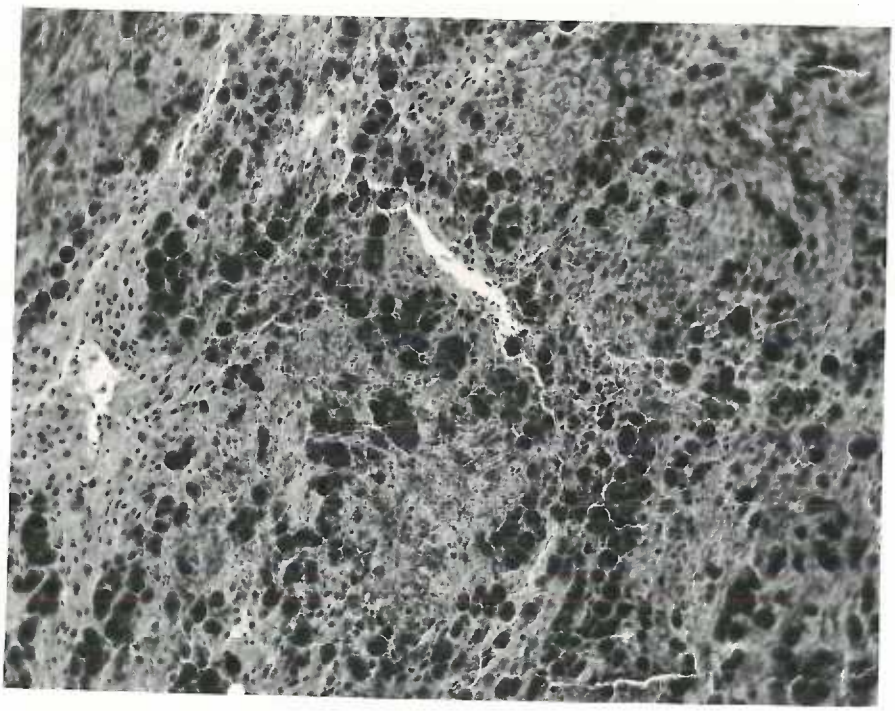
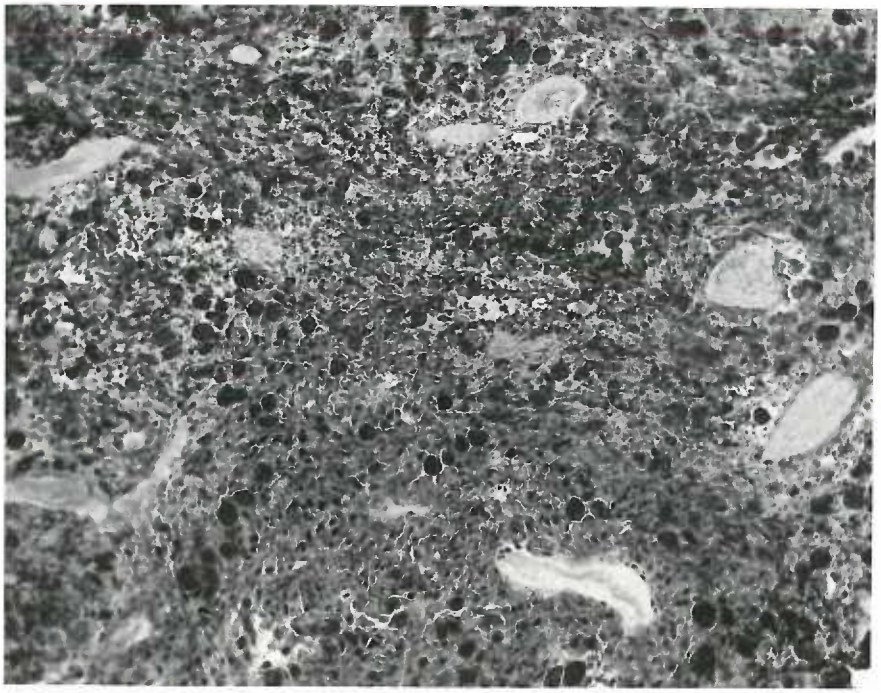


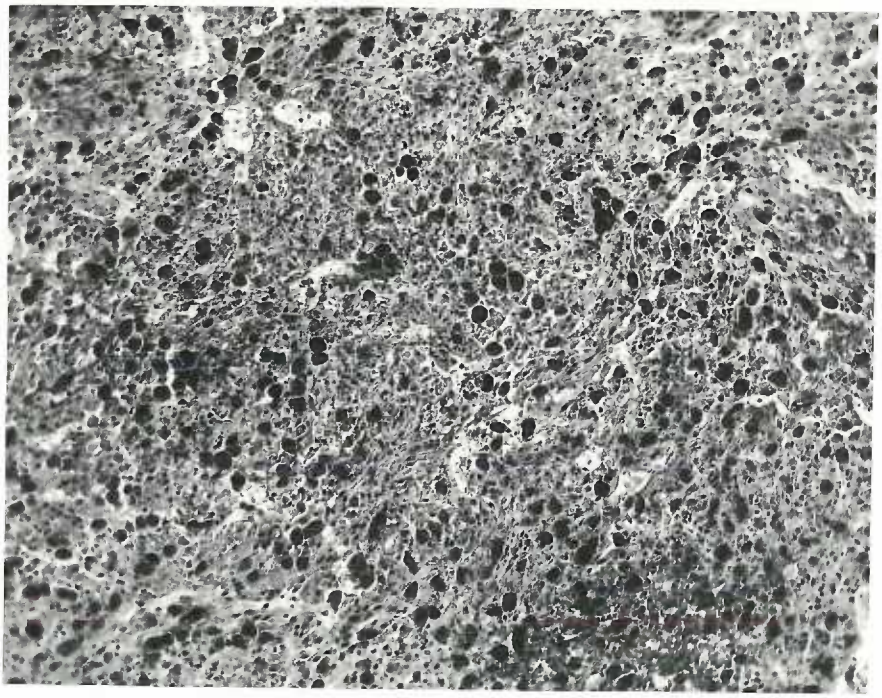
Figure 3:

Low-power photomicrograph of melanoma from Group B animal.

Figure 4:

Low-power photomicrograph of melanoma from Group C animal.





DISCUSSION

In order to understand the rationale behind this experiment it will be necessary to discuss briefly the mechanism of and hormonal effect on melanin formation. According to Fitzpatrick and Lerner[47] melanin is produced in the skin by the polymerization of oxidation products of ortho-dihydroxy phenyl compounds to insoluble substances of high molecular weight. Most of these products in humans are formed from the oxidation of tyrosine by the enzyme tyrosinase, attached to the mitochondria of the melanocytes in human skin, to form dihydroxyphenylalanine (dopa). Dopa can then be readily converted, through several oxidation steps, to melanin by the action of tyrosinase or by other enzymes such as cytochrome oxidase. Tyrosinase, however, is the only enzyme capable of oxidizing tyrosine to dopa. It has been found that an inhibited tyrosinase system is present in unirradiated human skin so that when normal human skin is incubated with tyrosine the melanocytes require an activation of the tyrosinase system by a stimulating factor--for example, UV light--in order to form melanin. On the other hand, a partially inhibited system is present in actively proliferating junctional nevi, while an active system occurs in melanotic and amelanotic melanomas and human skin subjected to UV light[48]. Some of the inhibitors of the tyrosinase system are: (1) sulfur containing compounds such as thiouracil and BAL, which tie up the copper necessary

for tyrosinase activity; (2) parahydroxyphenyl compounds such as hydroquinone and parahydroxypropiophenone; and (3) reducing agents, especially ascorbic acid.

Recent studies on the hormonal aspects of this problem have brought to light some interesting facts. Studies by Lerner, Shizume and Fitzpatrick[49] have shown that there is a pituitary hormone which has a definite influence on the amount of melanin formation in any individual. More recent investigations by Lerner, Shizume and Bunding[50] have brought a greater understanding of these facts. This hormone, formerly called intermedin, melanophore hormone, melanophore dilating principle, etc., is now known as the melanocyte stimulating hormone (MSH) and has recently been prepared in a more purified form than was formerly possible[51]. By clinical studies these workers were able to determine that MSH secretion can be regulated to a great extent by the administration of cortisone and/or hydrocortisone. In other words, these adrenal hormones inhibit the release of MSH from the pituitary gland in a manner similar to their regulation of ACTH excretion.

As was shown by Sugiura in 1934[22], intermedin, which was a weak and impure preparation of MSH, definitely stimulated the growth of Harding-Passey melanomas. This was the only hormone that had been found to have any effect upon this type of tumor. In the present

experiment, with this fact in mind and also with the knowledge that adrenalectomy should remove inhibition by the adrenal steroids of MSH secretion, it was postulated that melanomas might grow more rapidly under the influence of increased MSH. It is seen in Tables I and III, and in the statistical evaluation that there is no significant difference between Group C (control) and Group A (adrenalectomized mice). The reason for this is not clear and cannot be explained at present, but it is possible that the tumor growth is not under the control of MSH.

On the other hand, the marked inhibition of tumor growth on high doses of hydrocortisone correlates well with what is known about the action of this hormone on the pituitary secretion of MSH. That this fact is the reason for growth inhibition cannot be positively stated, but it may certainly be a possibility. The premature demise of most of the animals in this group can probably be ascribed to the tendency of steroids to prevent localization of infection, along with their ability to mask any systemic symptoms of bacterial invasion. Whether this intercurrent infection had any effect upon the rate of tumor growth cannot be stated. It has been observed in previous experiments with melanomas in this laboratory that infection has often been present terminally, but not in such marked degree as occurred in this group.

The use of antibiotics in these animals might have prolonged their survival time and should have been considered. Another possible cause of early mortality corroborated by the pulmonary edema present in all the animals, is that of salt and water retention under the influence of hydrocortisone.

As one would anticipate, the completion of an experiment of this type presents some unique and difficult problems. Three previous similar experiments had to be discontinued because of various reasons, the chief ones being high surgical mortality and increased susceptibility of adrenalectomized animals to infection, as well as temperature changes.

In general, the microscopic appearance of these tumors are the same as that described originally by Harding and Passey[1] with the exception that the present melanomas contain fewer melanin-filled macrophages. The melanomas described by Sugiura[22] are almost identical in appearance as those seen here, i. e., scattered pigment-containing phagocytes, fusiform epithelial-like cells composing the tumor parenchyma with vesicular nuclei of various sizes and numerous mitotic figures, and fibroblasts characterized by various shapes, little or no pigment and inconspicuous nuclei (Figures 2, 3 and 4).

The relationship of Harding-Passey melanomas to their human

counterparts has not yet been delineated. Although both have similar cell types, their behavior and growth are quite different. The mouse melanoma rarely metastasizes while the human type is notorious for its early spread.

In considering future research in this field, it might prove interesting to determine what effect the administration of MSH to adrenalectomized animals with melanomas has on the growth of these tumors. Another possibility which might be considered is the effect of epinephrine upon the growth of these tumors in previously adrenalectomized animals. It would also be of interest to vary the dosage of hydrocortisone in tumor bearing mice to determine the optimal dosage of this steroid. Hypophysectomy has never been done in mice with melanomas and might provide some information on the hormonal aspects of this problem. There are many facets of the problem which have not as yet been explored, some of which may prove to be of great interest and value.

CONCLUSIONS

1. Bilaterally adrenalectomized mice showed no alteration in the rate of growth of Harding-Passey melanomas as compared to the normal controls.

2. Bilaterally adrenalectomized mice given one milligram of hydrocortisone daily showed definite inhibition of the growth rate of Harding-Passey melanomas as compared to the control animals.

3. Hydrocortisone was considered as the probable cause of the early demise of some of the animals from pulmonary edema and bronchopneumonia.

4. Considerations for future investigation in this field using the present data as a starting point were discussed.

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