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THE EFFECT OF ADRENAL STEROIDS AND CORTICOTROPHIN ON THE GROWTH OF A SARCOMA OF HUMAN ORIGIN IN SMALL LABORATORY ANIMALS

BY

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9α Fluorohydrocortisone, prednisolone, and hydrocortisone promoted the growth of the human tumour (H.S.1) in weanling rats, but deoxycortone acetate was ineffective even when injected in very high dosage; Cortrophin ZN, a long-acting corticotrophin preparation, was just as effective as cortisone. This effect of corticotrophin on tumour growth was mediated by the adrenal cortex, and was related to the enhanced secretion of adrenocorticoids; some correlation existed between tumour size and adrenal hypertrophy in the corticotrophin-treated rats. The action of adrenal steroids and of corticotrophin in promoting the growth of the tumour is discussed.

Very few human tumours will proliferate well enough in animal hosts to enable chemotherapeutic and other cancer studies to be performed. However, it has been reported (Toolan, 1951a, 1951b, 1952, 1953a, 1953b, 1954; Toolan and Moore, 1952; Chute, Sommers, and Warren, 1952; Sommers, Chute, and Warren, 1952; Patterson, Chute, and Sommers, 1954; Towbin, 1954; Handler, 1956; Handler, Davis, and Sommers, 1956) that human tissues, both normal and malignant, can be grown in small laboratory animals, providing these hosts are treated by X-irradiation and/or cortisone. Such treatment is essential to reduce the strong natural resistance of the animals to heterografts. Hamsters, however, appear to show much less resistance than any other animal, and tumour growth can occur in untreated newborn hamsters (Chesterman, personal communication).

The tumour H.S.1 was obtained by Toolan in January, 1953, as a soft white mass; a cube of approximately 1 cm. in size, from the calf of the leg of a 43-year-old male, and was one of the few to be successfully passaged in animal hosts. Although fast growing, this tumour has never been grown subcutaneously or intraperitoneally in animals untreated by X-irradiation or by cortisone, except in newborn hamsters (Chesterman, personal communication). Toolan (1954) has confirmed that this tumour contains human antigens when tested by the agar plate method of Ouchterlony (1953).

The present investigation was commenced to extend the studies of Toolan, and in particular to investigate the effect of other adrenocortical steroids, and of corticotrophin on the growth and development of the tumour H.S.1.

METHODS AND MATERIALS

All animals were maintained on a No. 41b cube diet and tap water to which 0.01% oxytetracycline (Terramycin) was added to reduce the incidence of infection. Preliminary investigations showed the importance of using weanling rats; male Wistar rats of the A.R.C. strain of body weights 30 to 35 g. (18 to 21 days old) were found to be the most suitable; if slightly older and larger animals were used the tumours were poor in growth and development or alternatively failed to grow at all. In early experiments, the rats were treated with both X-irradiation and cortisone; they received 150 r total body X-irradiation 1 to 3 days prior to the tumour implant, and 4 subcutaneous injections of cortisone acetate (3 mg./rat). The steroid was injected into the area of the left shoulder, as far away from the site of tumour implant as possible to eliminate any chance of local action of the hormone on tumour growth. In later experiments rats were not X-irradiated but received the cortisone treatment alone. The steroid was injected in 4 subcutaneous doses each of 20 mg./100 g. body weight; the first injection was given at the time of tumour implant, the others at 48 hr. intervals.

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Hamsters received a single subcutaneous injection of cortisone acetate (20 mg./100 g. body weight) at the time of implant, and no further treatment was given.

Weanling albino mice of the A.R.C. strain of body weights 8 to 11 g. were used in a few experiments; these animals received 3 subcutaneous injections of cortisone acetate into the left shoulder area. The first dose (3 mg./mouse) was given at the time of the tumour implant, the remaining injections (2 mg./mouse) were given at 48 hr. intervals.

Tumours were removed aseptically and minced in a sterile tube. A suspension of the tumour tissue was made in a sterile Ringer fluid (NaCl 0.9%, KCl 0.042%, $CaCl_2$ 0.024%, $NaHCO_3$ 0.015%) containing glucose (1.0%), penicillin (200 units/ml.) and streptomycin (200 μg./ml.). Approximately 4 ml. of this solution was added to each gram of tumour tissue. Each rat received 0.5 ml. of tumour suspension injected subcutaneously into the shaved right flank using a No. 16 or 18 needle. Mice received 0.25 ml. of the suspension in a similar site, while hamsters were lightly anaesthetized with sodium pentobarbitone (Nembutal, 9 mg./100 g.) intraperitoneally and were injected with 0.25 ml. of the tumour suspension into one or both of the cheek pouches, or 0.5 ml. into the flank (Handler, 1958). In all hosts, the tumour was harvested on or about the fourteenth day after implantation.

Results

Choice of Host Animal.—The tumour was successfully grown in cortisone treated rats, hamsters, and mice; the rats were the animals of choice for general investigations. In all the experiments reported here, the animals were treated with cortisone or other adrenal steroid alone; X-irradiation in combination with cortisone was only used in the initial experiments on the passage of the tumour. Since there appeared to be no difference between the rate of growth of the tumour in rats receiving cortisone alone and in those treated with a combination of X-irradiation and cortisone, the former treatment was selected. Fig. 1 shows the appearance of the tumour in a rat 14 days after implantation, the implant having been made subcutaneously into the right flank.

Toolan (1954) reported that with the strain of rats used in her experiments, the tumour killed the host within 12 to 15 days from implantation, the animal dying in a cachectic and emaciated condition. In the present series of experiments this mortality was seen in some of the animals, especially in those bearing large tumours which sometimes grew to 25% of their body weight; in other rats there was a gradual necrosis and regression of the tumour with no apparent ill effect on the animal host. This regression of the tumour was also seen when rats were treated with corticotrophin instead of cortisone.

Hamsters were used solely to provide a reserve supply of tumour tissue for routine passage; mice were used in a few experiments, but they had no distinct advantage over rats; moreover, in many instances mice showed a marked toxic reaction to the cortisone treatment (D'Arcy and Howard, 1958).

The tumour was successfully passaged within and between the three species of host, namely from rat to rat, from rat to hamster cheek pouch, and from rat to mouse and *vice versa*. Some difficulty was occasionally experienced in mouse to mouse passage; the reason for this was not fully evident, but it is believed to be due, at least in part, to the poor condition of the mice receiving large doses of cortical steroids.

The Effects of Some Adrenal Steroids on Tumour Growth.—The effects of some of the more recent analogues of cortisone and of hydrocortisone on the growth and development of the sarcoma in the rat were investigated. The results of experiments with cortisone acetate, hydrocortisone acetate, prednisolone, 9α fluorohydrocortisone acetate, and deoxycortone acetate are summarized in Table I. The doses of steroid were injected subcutaneously in saline suspension immediately after tumour implantation and were repeated three times at 48 hr. intervals.

TABLE I

THE EFFECT OF SOME ADRENAL STEROIDS ON THE GROWTH OF SARCOMA H.S.1 IN WEANLING RATS

The steroids were administered four times subcutaneously on alternate days.

Steroid (mg./100 g. Body Wt.)	Results
Cortisone acetate:	
80	Toxic
40	"
20	Growth
10	"
5	Slight growth
Hydrocortisone acetate:	
20	Growth
10	"
Prednisolone (Δ^1 hydrocortisone):	
5	Growth
2·5	No growth
9α Fluorohydrocortisone acetate:	
4	Toxic
2	Growth
1	"
0·5	Slight growth
Deoxycortone acetate:	
200	No growth
100	" "
20	" "

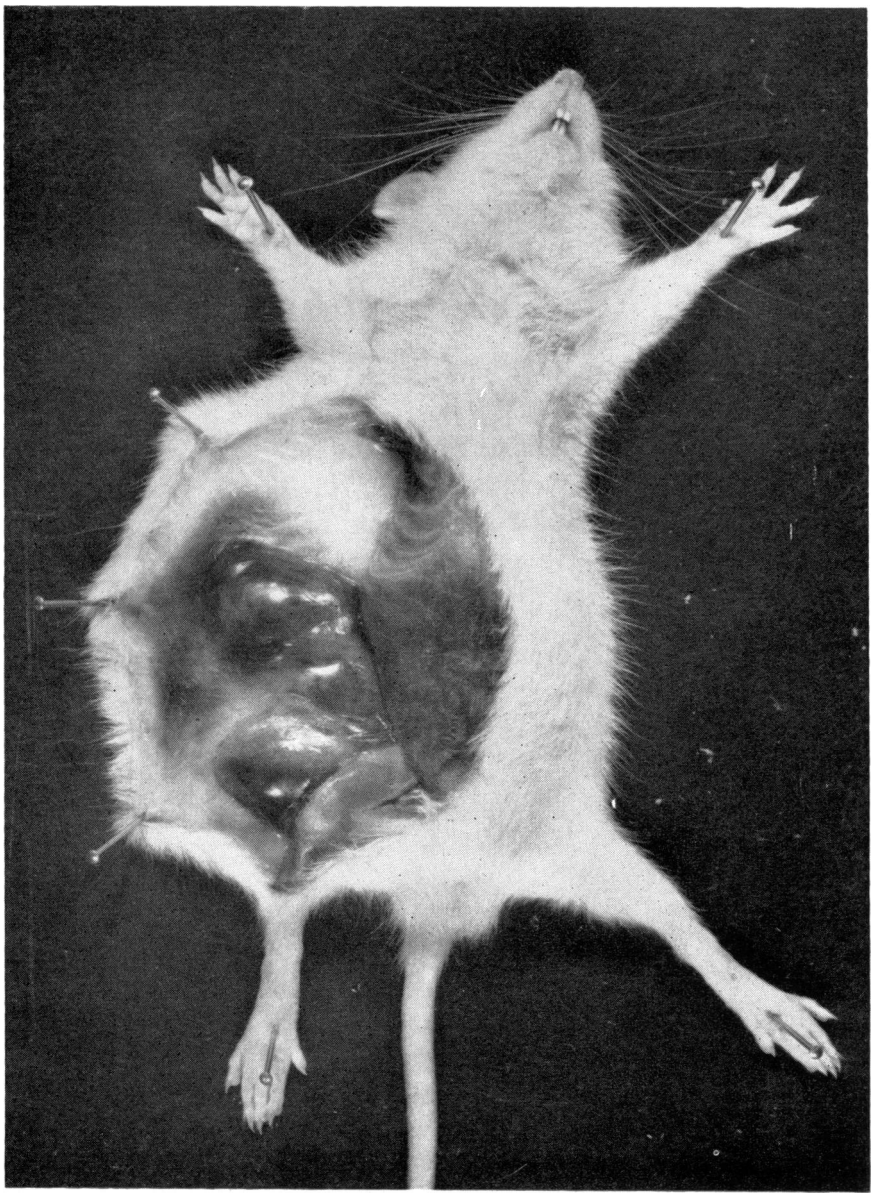

FIG. 1.—Sarcoma H.S.1, 14 days after implantation in the right flank of a weanling rat. The animal was given 20 mg. of cortisone acetate/100 g. body weight subcutaneously at the time of implantation of the tumour and 3 further doses at 48 hr. intervals.

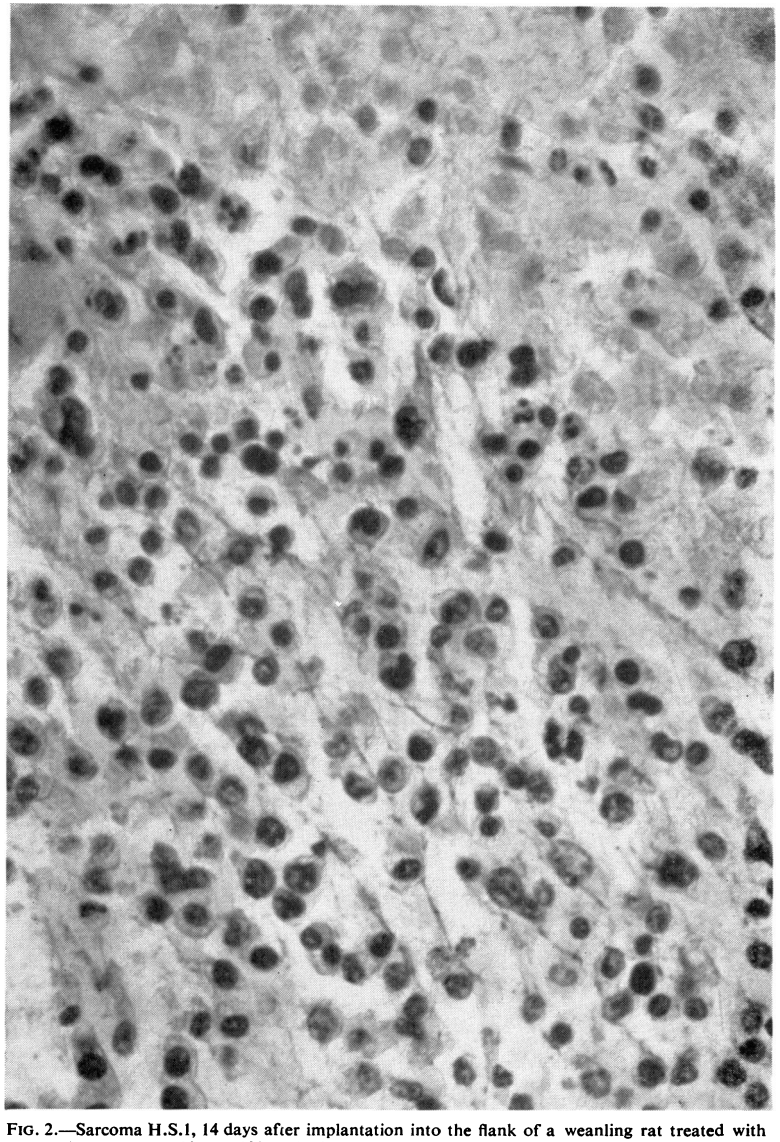

FIG. 2.—Sarcoma H.S.1, 14 days after implantation into the flank of a weanling rat treated with cortisone acetate, 20 mg./100 g. body weight subcutaneously at the time of implant and 3 further doses at 48 hr. intervals. A multinucleated round cell sarcoma with extensive fibrous elements. Magnification, × 350.

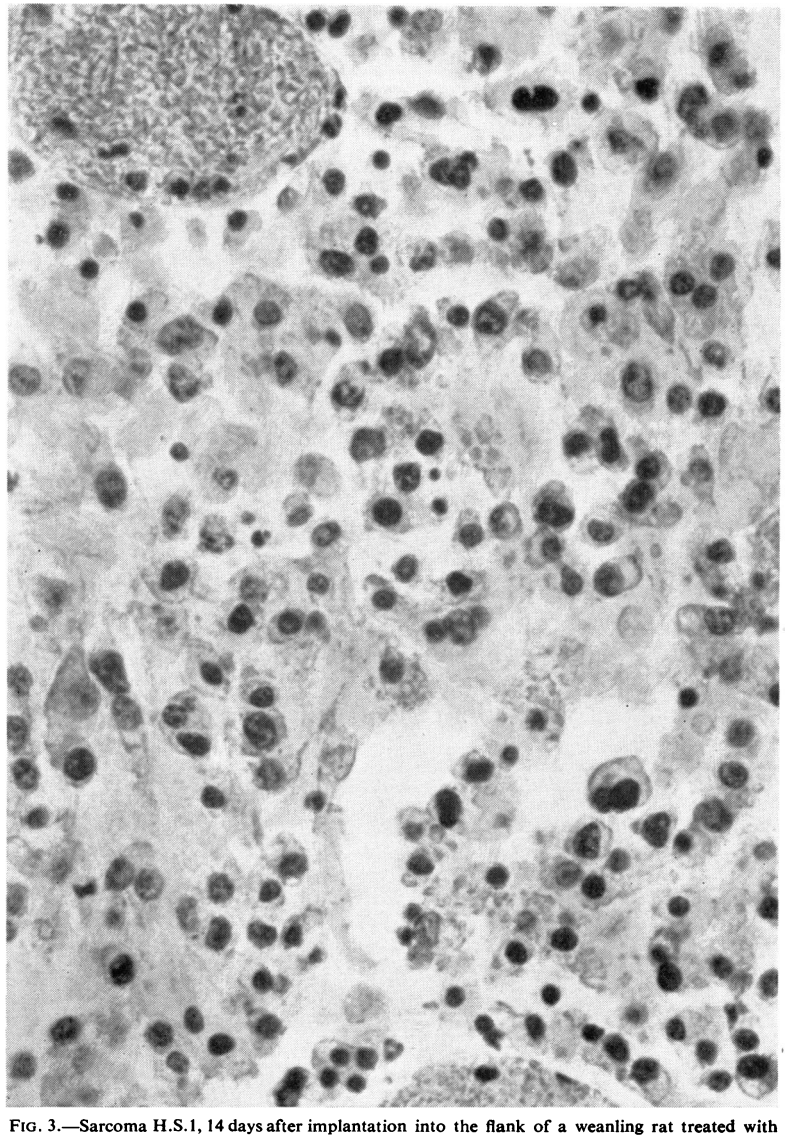

FIG. 3.—Sarcoma H.S.1, 14 days after implantation into the flank of a weanling rat treated with Cortrophin ZN, 5 units daily/rat intramuscularly for 14 days. Note the similarity of the tumour to that from the cortisone treated animal (Fig. 2). Magnification, × 350.

TABLE II

COMPARISON OF THE EFFECTS OF CORTISONE AND CORTROPHIN ZN ON THE GROWTH OF SARCOMA H.S.1 IN WEANLING RATS

See text for method of scoring tumour size.

Expt.	Treatment	No. of Rats	Size of Tumour 14 Days after Implantation								Total Score/Group
A	Cortisone acetate 20 mg./100 g. subcutaneously at time of implant plus 3 further injections at 48 hr. intervals	8	+ + + +	+ + +	+ +	+ +	+ +	+ +	+	+	17
	Cortrophin ZN 5 units daily/rat intramuscularly for 14 days	8	+ + +	+ + +	+ +	+ +	+ +	+	+	+	15
B	Cortisone acetate 20 mg./100 g. subcutaneously at time of implant plus 3 further injections at 48 hr. intervals	8	+ + +	+ +	+ +	+ +	+ +	+ +	+	+	15
	Cortrophin ZN 5 units daily/rat intramuscularly for 14 days	8	+ + +	+ +	+ +	+ +	+	+	+	+	13

With the exception of deoxycortone acetate all the steroids promoted the growth of the sarcoma; from a comparison of the effective doses it was apparent that 9α fluorohydrocortisone was the most active steroid followed in order by prednisolone, then by hydrocortisone and cortisone which were equally active. However, apart from the smaller dosage necessary these steroids had no better effect than cortisone on the growth of the tumour. With large doses of the steroids, the rats lost body weight due to the catabolic effect of the hormones; some died probably because of a combination of this effect with the sudden flare-up of a dormant infection.

The Effect of Corticotrophin on Tumour Growth.—Experiments were also performed to determine the effect of corticotrophin. The first experiments with soluble corticotrophin failed to induce tumour growth, and a long-acting preparation, namely corticotrophin precipitated with zinc hydroxide (Cortrophin ZN, Organon), was used in order to ensure that the blood concentrations of the hormone were maintained. The preparation was administered daily by intramuscular injection for 14 days; the first dose was injected into the hind limb immediately after the tumour implantation. The results of two separate experiments are summarized in Table II, in which tumour growth in rats treated with Cortrophin ZN (5 units/rat daily) was compared with the growth of the tumour in rats receiving the standard cortisone treatment. Tumour sizes have been assessed by visual observation on a scale extending from 1 plus (+) to 4 plusses (++++), and a numerical score has been allotted to the results by summing the plusses. In early experiments, the tumours were weighed as well as being assessed visually, and it was found that there was good correlation between the two estimates of tumour size. It is apparent from these results that Cortrophin ZN, when injected daily, will promote tumour growth to the same degree as the cortisone treatment.

If the dosage of Cortrophin ZN was reduced to 2 units/rat/day tumour growth occurred, while with a dose of 1 unit daily the tumour sometimes commenced to develop but regressed while still quite small, usually about the ninth day after implantation. When the dose of the preparation was increased to 4 or 8 units/rat daily, tumour growth was only slightly better than that produced by the 2 unit dosage. These results are summarized in Table III; it is also evident that Cortrophin ZN caused a decrease in the body weight and an increase in the adrenal weights of the treated rats; both these effects were related to the dose of the preparation. It was also found that the administration of corticotrophin need not necessarily start immediately after implantation of the tumour; if the first dose of Cortrophin ZN

TABLE III

THE EFFECT OF VARIOUS DOSES OF CORTROPHIN ZN ON THE GROWTH OF SARCOMA H.S.1 IN WEANLING RATS

Cortrophin ZN was given intramuscularly daily for 14 days. The asterisk indicates that some of these tumours grew slightly for 7 days and then regressed.

Cortrophin ZN (Units)	No. of Rats	Tumour Growth	Mean Wt. of Rat (g. ± S.E.)	Mean Paired Adrenal Wt. (mg. ± S.E.)
8	8	Growth	40 ± 1·2	67·4 ± 6·8
4	8	,,	41 ± 1·7	58·2 ± 1·6
2	8	Slight growth	49 ± 2·0	31·2 ± 1·8
1	8	*No growth	78 ± 4·4	23·8 ± 1·0
Zinc suspension control	8	No growth	85 ± 3·8	12·4 ± 0·8

was delayed for a period of 1 day, or, on one occasion, 2 days after implantation, the tumour grew; if the delay between implantation and treatment was extended beyond 2 days then the tumour did not develop.

The histological appearance of the tumour removed from cortisone treated rats did not differ in any way from that removed from rats treated with Cortrophin ZN. In both cases (Figs. 2 and 3) microscopical examination of the tumour tissue revealed a multinucleated round cell sarcoma with extensive fibrous elements. This appearance was in accord with the findings of Toolan (1954).

Mode of Action of Corticotrophin on Tumour Growth.—A further series of experiments were carried out to investigate the mode of action of corticotrophin (Table IV). The tumour failed to develop in untreated adrenalectomized or hypophysectomized animals, but grew well if these animals were treated with cortisone. Corticotrophin was without effect in adrenalectomized animals, but promoted tumour growth after hypophysectomy. It appeared that the action of corticotrophin on tumour growth was by way of the adrenal cortex and was related to the enhanced adrenocortical secretion which it produced.

TABLE IV

THE EFFECT OF REMOVAL OF ENDOCRINE GLANDS AND REPLACEMENT THERAPY ON THE GROWTH OF SARCOMA H.S.1 IN WEANLING RATS

An asterisk indicates no evidence of tumour growth.

Procedure	Tumour Growth
Cortisone acetate 20 mg./100 g. body wt. × 4, subcutaneously	Growth
Cortrophin ZN 5 units daily/rat, intramuscularly for 14 days	,,
Adrenalectomy	No growth
,, +cortisone acetate	Growth
,, +Cortrophin ZN	No growth
Hypophysectomy	*Rats died
,, +cortisone acetate	Growth
,, +Cortrophin ZN	,,

In a number of experiments, the adrenal glands were removed from corticotrophin treated rats exhibiting tumours; doses of this hormone ranged from 1 unit to 20 units/rat daily for 14 days. As might be expected, the adrenal glands showed varying degrees of hypertrophy depending on the dose of corticotrophin administered. The glands were preserved in formol saline and subsequently weighed. In the same animals the size of the tumour was assessed and graded: no growth (−), partial growth (±) or positive growth (+ to ++++ and greater than ++++). An attempt to relate adrenal size with tumour growth was made. Fig. 4 shows that the tumour failed to grow in corticotrophin-treated rats when the combined weight of both adrenal glands was less than about 30 mg.; partial tumour growth was associated with glands slightly larger than this, while positive tumour growth occurred in animals with paired adrenals larger than about 50 mg. The paired adrenal glands from untreated rats weighed about 12 mg. These results indicate that there is a degree of correlation between adrenal enlargement and the growth of the tumour.

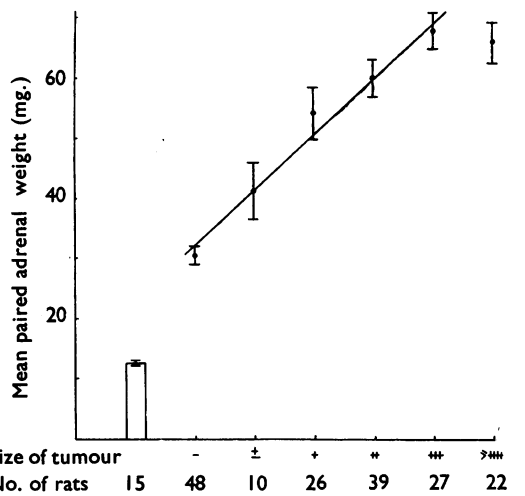

FIG. 4.—Relation between adrenal weight and tumour size in Cortrophin ZN treated rats. The regression line ($y=9.24\ x+22.97$) has an index of precision (λ)$=0.082$; the slope is very highly significant ($P<0.001$). Each point represents the mean paired adrenal weights of a group of animals, the vertical lines indicate the standard errors. Size of tumour is graded: no growth (−); partial growth (±); and positive growth (+ to > ++++). The histogram represents the mean paired adrenal weights of a group of untreated control rats.

DISCUSSION

The soft tissue sarcoma H.S.1 was taken from a patient about five years ago and has since been transplanted over 150 times in cortisone-treated hamsters, rats, and mice. The growth and development of the tumour were also promoted by treating the animals with adrenal steroids other than cortisone; 9α fluorohydrocortisone was effective in this respect at a dosage about one-tenth that of cortisone, while prednisolone was active at half the cortisone dosage. Hydrocortisone appeared to have similar activity to cortisone, but deoxycortone acetate did not induce tumour growth even when injected in very high dosage.

Soluble corticotrophin was not effective in promoting tumour growth; however, a long-acting preparation, Cortrophin ZN, was just as effective as cortisone at a dose of 2 units or more/rat/day.

The tumour failed to develop in untreated adrenalectomized or hypophysectomized rats but grew well if they were treated with cortisone; corticotrophin was without effect in adrenalectomized animals, but promoted tumour growth after hypophysectomy; thus it was apparent that the effect of Cortrophin ZN on tumour growth was mediated by the adrenal cortex and was related to the enhanced secretion of adrenocorticoids. The marked hypertrophy of the adrenal glands removed from Cortrophin ZN treated rats with tumours provided further evidence to support this view. In spite of the considerable variation in adrenal size, it was evident that up to a certain stage of adrenal enlargement there was a relation between tumour development and adrenal hypertrophy. Furthermore, rats which showed a positive tumour growth when treated either by cortisone or by corticotrophin all showed greatly diminished body growth. If the dose of Cortrophin ZN was too small to promote tumour growth, then body weight of the animal was not greatly diminished. This reduced body weight in the cortisone-treated animals is believed to be due to the catabolic action of the steroid (D'Arcy and Howard, 1958); in the case of the Cortrophin ZN treated rats it is likely to be due to a similar action of the enhanced adrenal output.

How exogenous or endogenous adrenocorticoids are able to break down the resistance of a host animal to the growth of malignant heterologous tissue is uncertain. Toolan (1955) suggested that cortisone may reduce the natural immunity of the host by reducing antibody function. The results obtained in this present work can add little to this discussion of the mechanism involved; however, two facts are clear. Firstly, age is a factor in the development of the resistance to the growth of heterologous tissue, and in older rats it is impossible to break down this resistance by the administration of adrenal steroids. Secondly, stimulation of the rat adrenal cortex by corticotrophin produces a sufficiently high blood titre of endogenous adrenocorticoids to enable the tumour to develop; this suggests that physiological adrenocortical stimulation may be a factor in the growth of the tumour.

There is a possible analogy between the regression of the H.S.1 tumour in rats when Cortrophin ZN treatment is stopped and the regression of some metastatic breast and prostate tumours after adrenalectomy. Although in the latter condition the patients normally receive cortisone replacement therapy, it is possible that the doses administered may be much less than the quantities of equivalent corticosteroid formerly secreted by the patients' own adrenal glands. It may be that the breast and prostate tumours which respond to adrenalectomy are those which are capable of producing an antibody or other defence mechanism on the part of the patient, and that this antibody formation is suppressed by the presence of the endogenous corticoids; such a mechanism by its emergence after adrenalectomy may be able to bring about a temporary cessation in the growth of the metastasizing tumour. On the other hand, in these cases it has been held that because the adrenal gland is an extraneous source of sex hormones, stimulating the growth of normal or malignant breast tissue, its removal may produce improvement because the output of such sex hormones is diminished. It is even possible that both of these mechanisms may have some bearing on the regression of the tumour.

The ability of corticotrophin to induce tumour growth, as shown in the present studies, may be correlated with a further clinical finding; namely in patients from whom the primary growth has been removed, there is a tendency for metastases to appear after subjection to stress, in particular to the surgical stress of a further operation. It seems likely that the stress-induced hypersecretion of corticotrophin, producing enhanced adrenocortical output, may be the contributory cause of this syndrome.

In view of the points of possible analogy between the clinical findings in the treatment of some cases of mammary cancer, and the present study of a human sarcoma growing in rats, it would seem to be profitable to investigate the extent to which any hyperactivity of a patient's own pituitary-adrenal system might influence the course of tumour development.

While this communication was awaiting publication, Palm, Teller, Mercker, and Woolley (1958) have shown that hydrocortisone had a similar effect to that of cortisone on the growth of the tumour H.S.1.

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