

Effect of Cortisone upon Growth *in vitro* of Femur of the Chick Embryo.* (21896)

W. BUÑO AND H. GOYENA.

From the Department of Histology and Embryology, Faculty of Medicine, Montevideo, Uruguay.

In adequate doses, cortisone produces a distinct and rapid inhibition of growth in the rat(1), with marked abnormalities in the newly formed cartilage and bone(2). Suspension of treatment allows of a rapid recovery in weight(3). In other animal species (mice, guinea-pigs, rabbits) the inhibitory action of cortisone was also evident(4). ACTH also shows a marked inhibitory action upon growth of rats(5,6). An inhibitory effect upon growth has also been shown in the chick embryo(7,8).

It has not been shown whether such effects are the result of a direct action of the hormone upon bone growth or of an action indirectly through some other gland and/or the general metabolism, especially in view of the catabolic effect on proteins possessed by this corticoid.

Cortisone acts directly upon cells in cultures(9-13), on the segmentation of the egg of *Arbacia*(14); and on mastocytes *in vivo* (15) and *in vitro*(16).

In our studies, cortisone acted directly on bone *in vitro*, thus precluding all possibility of intermediate factors. This same technic has

been employed to study the effect on bone growth of thyroxin, vit. A, growth promoting hormone and estradiol(17-20).

Material and technic. Femurs were used of Rhode Island chick embryos in their 7th day of incubation, and cultivated according to the technic of Fell, upon a clot in a moist chamber(18). The embryo was dissected and both femurs removed; one femur was cultivated in the medium containing hormone, the other femur being cultivated as a control in a medium without hormone. Hormone was added to the medium, producing a very fine suspension in the Tyrode at a concentration of 0.1 mg per ml. Cortisone acetate and hydrocortisone acetate were used in the final medium in a concentration of 0.025 mg per ml. Every 2 or 3 days the femurs were removed to a fresh clot, identical in composition to that used in the first explantation, and were measured with a micrometric eyepiece, the lengths being expressed in arbitrary units. In 2 series of experiments, the first and second explantations were made in a medium containing cortisone, but subsequently the successive transplantations were made without this

TABLE I. Effect of Cortisone on Increase in Length of Chick Embryo Femur, *In Vitro*.

Days after explantation	Series	Control		+ Cortisone		Difference	P
		No. of cultures	M $\pm$ E*	No. of cultures	M $\pm$ E*		
2	a	11	3.86 $\pm$.25	11	2.41 $\pm$.28	1.45	.001
	b	14	4.93 $\pm$.33	14	2.92 $\pm$.24	2.01	.001
4	a	11	6.07 $\pm$.29	11	4.16 $\pm$.25	1.91	.001
	b	13	6.96 $\pm$.36	13	4.21 $\pm$.24	2.75	.001
6	b	13	9.03 $\pm$.37	13	5.94 $\pm$.31	3.09	.001
7	a	11	8.77 $\pm$.35	11	5.61 $\pm$.27	3.16	.001
8	b	7	10.03 $\pm$.14	7	6.78 $\pm$.43	3.25	.001
9	a	11	9.50 $\pm$.34	11	6.43 $\pm$.27	3.07	.001
10	b	4	10.12 $\pm$.38	4	7.06 $\pm$.95	3.06	.001
12	a	10	10.17 $\pm$.4	10	6.92 $\pm$.26	3.25	.001
15	a	7	11.50 $\pm$.45	7	7.89 $\pm$.33	3.61	.001
17	a	7	11.67 $\pm$.52	7	8.03 $\pm$.25	3.64	.001
19	a	3	11.66 $\pm$ 1.20	3	8.16 $\pm$.33	3.50	.001

* M = arithmetical average.

E = standard error.

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EFFECT OF CORTISONE ON BONE GROWTH "IN VITRO"

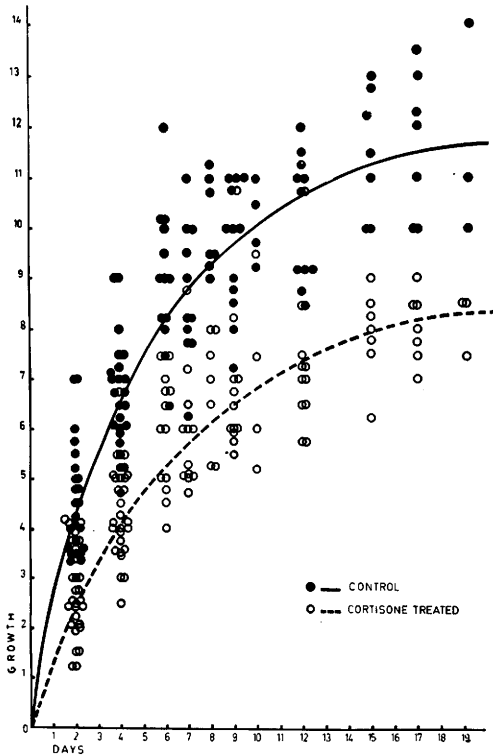


FIG. 1. Graph showing effect of cortisone on growth of chick embryo femur *in vitro*. Lines indicate average growth of control (solid line) and cortisone treated (dotted line). No matter which initial length of the bone was at the beginning of the experiment it has been taken as zero, and therefore the lines show daily increase of length.

hormone. All experiments were rejected in which deformities or curvatures appeared which could affect the results. In all, 59 experiments were conducted. At varying times after the first explantation, the material was fixed in formalin-calcium solution (Baker) and histological study made. Thus, in one series of experiments (a) the skeletal anlagen were kept in a hormone-containing medium throughout, but with a varying duration for each individual experiment. In another series (b) the skeletal anlagen were cultivated in a hormone-containing medium for only 48 hours, and then the culture was continued without hormone. The longest experimental series lasted 19 days, entailing 7 transplantations, the first 3 at 2-day intervals, the rest every 3 days.

Results. The results obtained in a total of

28 experiments, in which the anlage was kept in a medium with hormone throughout, are shown in the table and in Figs. 1 and 2. The data show that all the anlagen of bones cultivated in a medium containing cortisone or hydrocortisone were inhibited in their growth. This effect was already evident at the moment of the first transplantation, *i.e.*, 48 hours after cultivation was begun. At this time, control bones had grown an average of 3.86 ± 0.25 , while those treated had grown 2.41 ± 0.28 . This difference increases until the 15th day after the beginning of the experiment, after which the difference reached does not vary materially. All the experiments carried out showed the same type of abnormality, although to a varying degree. This

EFFECT OF CORTISONE ON CHICK EMBRYO FEMUR "IN VITRO"

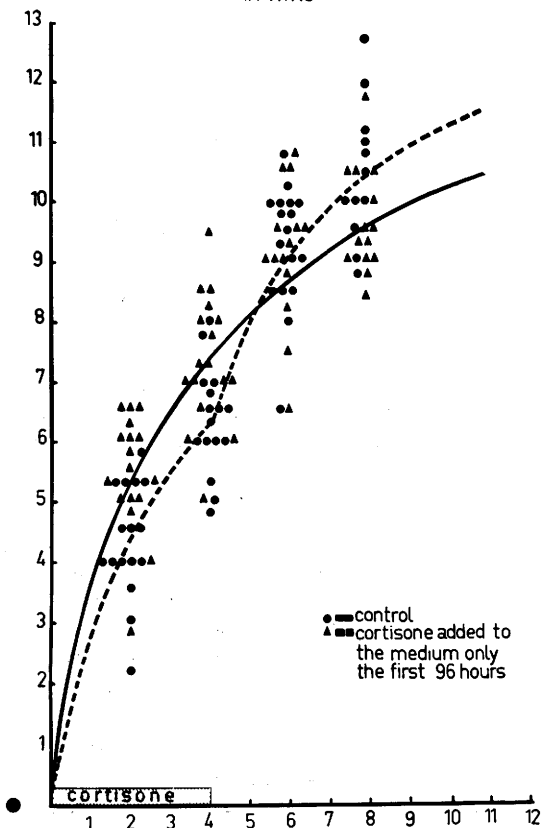


FIG. 2. Same as Fig. 1 but in this case cortisone was added to medium only in 1st and 2nd transplantation. After the 2nd transplantation (96 hr) both, control and treated, were transplanted to normal medium without cortisone.

fact, together with the actual values of the differences, makes the results highly significant. There was no appreciable difference between the effects of cortisone and hydrocortisone.

Quite an unexpected result was obtained in the experiments in which the cortisone or hydrocortisone was present in the explantation and was absent at the first or the second transplantation. Forty-eight or 96 hours after explantation, the anlagen suffered the usual arrest in growth we have described above. On the other hand, when these treated femurs were transplanted to a fresh medium without hormone, growth began again at a very high rate, the final dimensions being, in some cases, greater than those of the controls (Fig. 2).

Histological study of the bone anlagen carried out at variable times during cultivation, showed a normal, healthy appearance of the cells until the 10th or 12th day; after this, degenerative changes were seen both in the treated and the control femurs, their tissues being practically alike. Study of the lipids showed, within the cells, droplets made evident by the use of Sudan black, and which increased with the duration of the experiment. Employing the periodic acid-sulfofuchsin method, a weakly positive reaction appeared in the osteoid zone. Comparison of the histological appearance of the treated and the control anlagen did not lead to any conclusions. Inhibition of growth does not appear to be due to a decrease in the size of the cells or in the amount of fundamental substance, but rather to a diminished proliferation of the former.

Discussion. The arrest of skeletal growth observed in experimentation *in vitro*, is in agreement with the results already mentioned, obtained *in vivo*. In the first case, it is evident that the cortisone acts directly upon the cartilage. We believe that the same conclusion can be applied to the action of the hormone in the intact animal, its action not being through the glands, such as the hypophysis, or through a general metabolic action. When the hormone is withdrawn, after a period of treatment, the rapid recuperation of growth is also similar *in vitro* and *in vivo* (3), although

greater in the former case, wherein the dimensions of the treated anlage surpass those of the untreated control. The action appears to be a direct inhibition of cell proliferation, inasmuch as we have not seen cellular atrophy or reduction in the fundamental intercellular substance of the cartilage.

This marked action of cortisone upon the growth of the skeletal anlage, contrasts with the negative results obtained with the same technic, but using growth hormone (19); on the other hand, thyroxin and vitamin A are evidently active (17,18).

Summary. Cortisone and hydrocortisone act *in vitro* inhibiting the growth of explanted chick embryo femur anlagen. This effect is evident 24 hours after explantation, and increases until the 15th day after the beginning of the experiment. When the hormone is withdrawn at the first transplantation, the anlage recovers active growth and in some instances surpasses the dimensions of the control.

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Comparative Effects of Muscle Work and Insulin upon Plasma Amino Acids in Eviscerated Rats.* (21897)

DWIGHT J. INGLE, GLORIA TORRALBA, AND VIOLETA FLORES.

From the Ben May Laboratory for Cancer Research, The University of Chicago, Chicago, Ill.

Insulin will suppress the rise in plasma amino acids and enhance glucose tolerance in the eviscerate animal. Stimulation of muscle has an insulin-like effect upon the glucose tolerance of normal, diabetic and eviscerate animals(1). Under the conditions of the present experiments muscle work failed to simulate insulin in suppressing the rise in plasma amino acids following evisceration of the rat.

Methods. Male rats of the Sprague-Dawley strain were fed Archer Dog Pellets. At a weight of 250 ± 2 g non-fasted rats were anesthetized with cyclopal sodium and functionally eviscerated. The stimulation of muscle to contract 5 times per second was by the method of Ingle(2). The left hind leg was weighted with 100 g. The stimulus passed from the right hind foot to the left hind foot, thereby activating all of the musculature of both hind legs. A solution of glucose, with and without insulin, was infused into the jugular vein by a continuous injection apparatus which delivered fluid from each of 6 syringes at the rate of 20 cc in 24 hours. The experiment lasted 3 hours, whereupon the blood was collected by cannula from the abdominal aorta. Blood glucose was determined by the method of Miller and Van Slyke(3) and plasma amino acids by the method of Hamilton and Van Slyke(4).

Experiments and results. The conditions of each experiment are given in Table I. The data of Groups 1, 2 and 3 illustrate the char-

acteristic rise in plasma amino acids following evisceration and its suppression by insulin. In Group 4, the stimulation of muscle greatly increased glucose tolerance but failed to suppress the rise in plasma amino acids. When insulin was administered with glucose there was partial suppression of the rise in plasma amino acids. The average value for plasma amino acids was lower in the insulin-treated resting rats than in the insulin-treated working rats; the difference between the means (2.20 ± 0.533) meets the usual requirements for statistical significance. The combination of insulin and muscle work (Group 5) had a greater effect upon glucose tolerance than either one alone.

Discussion. The relationship between the effects of insulin upon the utilization of carbohydrate and upon the metabolism of amino acids and proteins is not understood although it is commonly assumed that the effects of insulin upon protein metabolism are secondary to some primary action upon the utilization of carbohydrate. Both insulin and muscle work increase the transfer of glucose and other sugars into cells(5). It has been suggested (6) that the increase in the rate at which glucose enters the cell represents the primary action of insulin to which all other consequences of its action are secondary. Since muscle work simulates the action of insulin upon glucose utilization and glucose space, its failure to have an insulin-like action upon the rise of plasma amino acids following evisceration of the rat requires further explanation.

In confirmation of earlier studies(1) the data of Group 5 (Table I) show that a com-

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