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Effect of Some Steroids and of Corticotropin (ACTH) on Cellular Activity. (20062)

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The writer pointed out that adrenal cortical extract suppresses the increased capillary permeability induced in inflammatory states by leukotaxine or by the unfractionated crude inflammatory exudate(1). The same type of inhibitory effect was found with Compound E or cortisone(2). Subsequently it was found that the suppressing capacity of cortisone is only effective against an alkaline exudate or leukotaxine, whereas in the case of an acid exudate the hormone was ineffective. Corticotropin (ACTH), was found capable of repressing another factor present in acid exudates also capable of increasing capillary permeability(3,4). In this way two factors were shown to exist in inflammatory exudates capable of increasing permeability, namely an initial substance, leukotaxine, and another factor termed exudin(3-5). Leukotaxine has the additional property of inducing leukocytic migration, but exudin merely causes increased capillary permeability in the later stages of an acute inflammation without essentially displaying any chemotactic effect towards leukocytes. Subsequent studies have indicated that cortisone and ACTH are capable of localizing from the circulating blood into an area of inflammation(6-8).

It is of importance to determine or at least attempt to throw some light on the mechanism of suppression by cortisone and ACTH in inflammation. The present report indicates that cortisone and corticotropin (ACTH), as well as some other hormones derived from the adrenal cortex, suppress cell activity. On the contrary, another steroid which is concerned in protein anabolic processes, testosterone, and a growth-promoting hormone of the pituitary, somatrofin (STH), have no such effect in inhibiting cellular activity. Cellular activity has been measured on Arbacia ova by their ability to segment following fertilization. If the ovum is adversely affected by a given steroid, the incidence of cleavage or developmental capacity is correspondingly reduced.

Method. The first series of experiments on sea urchin ova were performed by the addition of 2.5 mg of commercial cortisone acetate (Merck) suspended in about 0.1 cc of its vehicle in a Syracuse dish containing 10 cc of sea water. A batch of ova in approximately 0.5 cc of sea water was then added. An interval ranging between 15 to 20 minutes or so elapsed before the addition of 5 or 6 drops of sperm suspension. The interval prior to the addition of the sperms thus gave the ova sufficient time to come in contact with the cortisone suspension. For a period of one hour and a half to about 3 hours later, 50 ova taken at random were counted. The eggs which showed some degree of cleavage were recorded, and the eggs which failed to display any segmentation were also noted. In another Syracuse dish containing 10 cc of sea water but no cortisone, the same procedure was followed to control for the results obtained with cortisone. As a *control* for the vehicle in which cortisone is suspended, a few experiments were performed with the filtrate obtained when the cortisone suspension was filtered through ordinary filter paper (Whatman No. 5). Several experiments were also set up in which the ova were exposed to 0.1 cc of the commercial vehicle utilized in the prep-

aration of commercial cortisone. The vehicle was originally obtained through the kindness of the Merck Co. It contains the following ingredients:

.9% benzyl alcohol
.9% sodium chloride
.4% polyoxyethylene sorbitan monooleate
.5% sodium carboxymethyl cellulose (distilled water, q.s. ad 100%)

A *third series* of experiments served to determine the effect of the residue of cortisone acetate following filtration of the suspension on the incidence of cleavage of Arbacia ova. The residue was taken up in approximately 0.2 cc of sea water and added to 10 cc of sea water in a Syracuse dish. The subsequent procedure of adding ova and of fertilizing them was similar to the one described above. Finally, the effect on the *fertilizing capacity of sperms* was observed. Sperms were placed in contact with 2.5 mg or 5 mg of cortisone suspension in 10 cc of sea water for periods ranging from 22 to 47 minutes, and 5 or 6 drops of these cortisone-exposed sperms were then added to untreated ova. The number of ova in cleavage was subsequently counted. To be certain that the ova themselves were not damaged by any cortisone carried over with the drops of treated sperm suspension, eventually untreated normal sperms were also added, and readings on the cleavage of the ova were subsequently recorded. *Compound F* or 17-hydroxycorticosterone was suspended in 0.25 cc propylene glycol and the effect on the development of the ova was also recorded. It is the belief by many that Compound F is considerably more active than cortisone.

The question arose as to the effect of another adrenal cortical hormone, namely desoxycorticosterone. Both the glucoside and the acetate in concentrations ranging from 2.0 to 2.5 mg were assayed on the ova. The desoxycorticosterone glucoside was a Ciba preparation whereas the acetate was a Schering preparation. Upon addition of 2.5 mg of desoxycorticosterone acetate in 0.5 cc of propylene glycol to 10 cc of sea water, a white precipitate formed which was probably calcium or magnesium or perhaps both. The effect of propylene glycol was also determined in order to have a control for the vehicle utilized with desoxycorticosterone acetate and Compound F (Table II). It became of interest to determine whether the effects of the steroids of the adrenal cortex were specific or whether any other steroids would have the same effect on the cleavage of fertilized Arbacia ova. For this reason, experiments were set up to determine the effect of 2.5 mg of testosterone propionate (Ciba) on the cleavage of fertilized Arbacia ova. Has ACTH only an effect on the adrenal cortex, or has it also a direct effect? The evidence previously obtained(4,5,8) and unpublished studies on adrenalectomized rats, indicate that some ACTH (corticotropin) can apparently have a direct effect. In an endeavor to find out whether this fact could be demonstrated with isolated cells, studies on the segmentation of Arbacia ova were performed. Two preparations of ACTH were utilized: The preparation made by the Armour Co., and the preparation made by the Organon Co. The Armour ACTH was taken in sea water in doses ranging from 2 mg to 5 mg. In the case of Organon preparation, the ACTH was dissolved in the vehicle supplied along with the hormone. The solvent is water containing 2.0% glycerine, and 0.5% phenol as a preservative, adjusted to approximately pH 3.0. This vehicle alone was added to the control samples. The doses of Organon ACTH were approximately of the same magnitude as those used with the Armour preparation.

To ascertain that the effects obtained with ACTH were quite specific, another anterior pituitary hormone was assayed. This was somatrofin (STH). Concentrations of 2.5 mg STH were utilized in the experiments undertaken. The addition of this hormone to sea water induced the formation of a large precipitate. In this way it could be seen whether the effect of the solubility or insolubility of a substance was of importance in the final observations. In the foregoing experiments ACTH and somatrofin had been utilized. Therefore, to rule out the effect of proteins or protein derivatives on fertilized ova, a series of experiments were accordingly set up as follows: In 4 experiments, besides 10 cc of sea water, each contained 2.5 mg of the pseudoglobulin-albumin fraction of acid inflammatory exudates. This fraction contains crude exudin(3,4,5); in 2 experiments, 2.5 mg

TABLE I. Effect on Cleavage Development of Adding 2.5 mg of Cortisone Acetate in .1 cc of Vehicle to Suspension of Fertilized Arbacia Ova in 10 cc of Sea Water.

Exp. No.	Exposure Hr	Exposure Min.	No. of ova in cleavage	No. of ova not in cleavage
2 Exp.	1	37	0	50
Control	1	32	49	1
4 Exp.	2	51	15	35
Control	2	51	49	1
5 Exp.	2	25	4	46
Control	2	35	48	2
6 Exp.	2	05	8	42
Control	2	12	41	9
7 Exp.	1	40	14	36
Control	1	45	49	1
8 Exp.	1	50	11	39
Control	1	55	48	2

of the leukocytosis-promoting factor of inflammatory exudates (LPF) was used; in 4 experiments, 0.5 cc of the aqueous diffusate obtained after dialysis of either canine or rabbit inflammatory exudate was utilized; and finally in 2 experiments, 0.5 cc of the diffusate of canine blood serum was employed. These particular diffusate fractions were all Biuret positive and the diffusate fractions from inflammatory exudates were in each case also Ninhydrin positive. This indicates that these fractions were protein or at least protein derivatives.

Results. The addition of cortisone acetate to sea water, and the subsequent exposure of Arbacia ova to this hormonic preparation results in a suppression or an appreciable reduction in the number of ova capable of segmentation following their fertilization. These effects are conspicuous when the incidence of cellular division is compared to that observed in the controls. The data of 8 such experiments are assembled in Table I.

The results with cortisone are not referable to the vehicle in which the commercial cortisone acetate is suspended. The absence of any effect on the cleavage pattern can be observed with either the filtrate of the cortisone preparation utilized or with the commercial vehicle employed. The averages of the data are presented in Table II.

The cortisone residue obtained as a result of filtration, however, tends to suppress the incidence of cleavage. Such residue should not be suspended in distilled water, for in the latter medium the cortisone residue tends to sink to the bottom of the dish. Even though the distribution of the hormone is not homogeneous, sea water is found a more effective medium for the cortisone residue than is distilled water. The average data on these experiments are shown on Table II.

Are the sperms likewise affected by cortisone acetate or can the effect be only detected in the fertilized ova? When Arbacia sperms are in contact with cortisone for a variable amount of time ranging between 22 to 47 minutes at concentrations of either 2.5 mg or 5 mg of the hormone suspension per 10 cc of sea water, it is found that on transferring such sperms to a dish containing normal ova in 10 cc of sea water, the fertilizing capacity of such sperms is wholly absent. None of the ova show any subsequent segmentation. The average data and the number of experiments appear in Table II. The ova have not been affected by transferring 5 to 6 drops of sperms from the cortisone-containing dish, for if now one adds normal untreated sperm, the ova proceed to be fertilized and to segment in the usual fashion. It would seem that cortisone *per se* affects cellular activity irrespective of whether one is dealing with ova or with sperms.

Compound F or 17-hydroxycorticosterone suspended in propylene glycol likewise has the same suppressing tendency on the incidence of normal cell division in fertilized ova.* The concentration of this hormone was 5 mg in 10 cc of sea water and 0.25 cc of propylene glycol was employed in each case. In all these experiments whether with cortisone or Compound F or with other hormones of the adrenal cortex when cleavage did occur, even the cell division pattern was often pathological in nature. Abnormal or atypical cleavage with unequal division was of frequent occurrence. The average data on Compound F are summarized in Table II.

Desoxycorticosterone seems to have a similar suppressing tendency on the incidence of

* Recent unpublished studies indicate that Compound F acts on capillary permeability in inflammation just like cortisone. This hormone represses leukotaxine, but not exudin, which in turn is repressed by ACTH. Compound F was obtained through courtesy of Merck Co.

TABLE II. Composite Data on Effect of Various Fractions of Cortisone Acetate and of Other Substances on Cleavage Development of Fertilized Arbacia Ova.

Type and amt of material employed	No. of exp.	Avg No., of 50 exp. ova counted, found in cleavage	Avg No., of 50 control ova counted, found in cleavage
.20-.5 cc filtrate of cortisone acetate	3	48	49.3
.1 cc vehicle in which cortisone acetate is suspended	3	47	48
.1 cc suspension of residual fraction of cortisone acetate following filtration of 25 mg of steroid preparation	4	36	48.25
2.5-5 mg cortisone on fertilizing capacity of sperms	3	0	41.3
5 mg compound F in propylene glycol	4	23.5	46.2
.25 cc propylene glycol *per se*	2	43.5	44
2-2.5 mg desoxycorticosterone glucoside	6	27.4	46.9
2.5 mg desoxycorticosterone acetate	2	.5	46
2.5 mg testosterone propionate	5	45.4	46.4
2-5 mg ACTH (Armour preparation)	6	29.5	47.6
2.5 mg ACTH (Organon preparation)	6	21.5	41.7
2.5 mg somatrofin (STH)	4	43.6	45
2.5 mg exudin (impure)	4	41.6	43.1
2.5 mg leukocytosis-promoting factor (LPF) of canine exudate	2	38	36
.5 cc of canine or rabbit diffusate from inflammatory exudates	4	42.7	45.3
.5 cc of diffusate of canine blood serum	2	45	44

cleavage in fertilized ova. When only 2 mg of desoxycorticosterone glucoside per 10 cc of sea water was utilized this suppressing tendency seemed to be transitory; but when a slightly higher concentration of 2.5 mg was employed the suppressing effect seemed irreversible. The toxic effect was even more pronounced in the case of desoxycorticosterone acetate. One wonders whether this is wholly due to the effect of the hormonic preparation or whether the formation in the sea water of a white precipitate, which is possibly calcium, upon addition of this acetate hormone preparation, may also not have had some effect on the ultimate results. The averages of the data are accordingly summarized in Table II.

Does any steroid have a similar toxic effect on the incidence of the cleavage pattern in fertilized Arbacia ova as the hormone of the adrenal cortex? To test this point, a series of experiments were undertaken by exposing ova to 2.5 mg of testosterone propionate in 10 cc of sea water. A summary of the average data appears in Table II. It is quite clear that this steroid, which is also important in protein anabolic processes, has no effect in restraining normal segmentation in fertilized sea urchin ova.

Recent studies(7,8) have indicated that corticotropin (ACTH) penetrates from the circulating blood directly into an acutely inflamed area and once there it exerts a suppressing effect on the inflammatory reaction. As pointed out at the time(7,8), one does not deny the well known physiological mechanism in the mammalian organism of an action of this pituitary hormone on the adrenal cortex; but as employed in a pathological process, such as inflammation, where there is a marked local increased capillary permeability, some and only some ACTH from the circulation seems to seep through the capillary wall and there appears to have a direct effect on the inflammatory reaction (7,8). This viewpoint, reached as a result of numerous experiments on rabbits and dogs(7, 8), and in adrenalectomized rats, was studied again with Arbacia ova. In this set up, there is a system of isolated cells. Has ACTH any direct effect on the incidence of the cleavage pattern in a way similar to cortisone or Compound F? The procedure adopted has been

described in the section on *Method*. Generally speaking, as the average data on Table II indicate, ACTH in concentrations varying from 2 mg to 6 mg seems likewise to have a suppressing effect on the incidence of cleavage of fertilized Arbacia ova. Here also, whenever cleavage did occur, this was frequently characterized by unequal or an atypical cleavage pattern. In several instances the effect of corticotropin (ACTH) was sometimes transient and after several hours most of the ova were in some state of cellular division. In other experiments the damaging effect on the incidence of cleavage seemed irreversible. It would seem that ACTH has essentially the same direct damaging effect on the fertilized Arbacia ova as the hormones of the adrenal cortex described above. Two experiments were performed with 5 mg of Armour ACTH preparations, which also indicated that this hormone in 50% sea water likewise tends to injure Arbacia sperms, so that their capacity to fertilize normal ova with the subsequent usual cleavage pattern was appreciably diminished.

Is the effect of corticotropin (ACTH) quite specific or would another anterior pituitary hormone behave in a similar manner? The somatrofic growth hormone, somatrofin (STH), was obtained through the courtesy of Frank W. Horner, Limited, of Montreal. 2.5 mg of STH was added to 10 cc of sea water. A white suspended precipitate resulted. The fertilized ova in contact with this suspended hormone were not suppressed in their segmentation (Table II). Consequently, it is clear that a protein pituitary growth hormone has no apparent effect on the division of the ova. The effect of ACTH seems therefore, to be a direct one and it seems to be quite specific. Furthermore, the insolubility resulting in a suspended STH precipitate has no significant effect on the incidence of the normal cleavage pattern. This is important in connection with the powerful effect of the insoluble cortisone suspension on the frequency of the absence of cleavage in sea urchin ova (Tables I and II).

To rule out any protein effect or effects caused by derivatives of proteins, the ova were exposed to several fractions derived from inflammatory exudates. The data are shown in Table II. The pseudoglobulin-albumin fraction of acid exudates which contains exudin, the substance that induces increased capillary permeability in the later stages of an acute inflammation(3), was incapable of altering the usual cell division of ova. Concentrations of 2.5 mg of that fraction in 0.25% sea water placed in 10 cc of sea water failed to suppress cell division. The leukocytosis-promoting factor (LPF) which is an alpha globulin recovered from inflammatory exudates(9) likewise in 2.5 mg concentration failed to alter the cleavage pattern. Finally, the diffusate fractions of exudative material or of blood serum were ineffective in suppressing the incidence of the cleavage of ova. These diffusate fractions, as pointed out above, contained probably protein derivatives alongside with inorganic elements.

Discussion. The foregoing experiments indicate that various steroids from the adrenal cortex, such as cortisone, Compound F, and desoxycorticosterone directly affect the fertilized ova of *Arbacia punctulata* so that the incidence of normal cleavage is considerably reduced. The same direct effect is obtained with corticotropin (ACTH). With this pituitary hormone the incidence of normal cleavage is either retarded or suppressed. In many instances with either hormones of the adrenal cortex or with corticotropin (ACTH) even when cleavage occurs, the cleavage pattern tends to be abnormal in the form of unequal or partial cleavage. All these substances are considered to be protein catabolic agents. On the other hand another steroid, testosterone propionate, or the anterior pituitary growth hormone, somatrofin (STH), fails to alter the normal cleavage pattern of fertilized Arbacia ova. These latter substances are considered to be promoters of protein anabolism. The cellular division of the fertilized ova can be considered to be an index of normal cellular activity. It, therefore, would seem reasonable to assume that cortisone, and ACTH which penetrate into an acutely inflamed area from the circulation owing to an increase in local capillary permeability(6-8) exert their suppressing effect on inflammation by directly reducing cellular activity. At present investigation are underway in an endeavor to deter-

mine whether cortisone and ACTH suppress some or any of the biochemical factors formed during the course of development of an acute inflammation, and which in turn are responsible for some of the biological manifestations of inflammation(9). Studies to be reported *in extenso* elsewhere indicate that the direct injection of Compound F into an inflamed area either suppresses the formation of leukotaxine or at least tends to inactivate this substance, and also the presence of this steroid in the area of inflammation inhibits apparently the formation of the LPF (Leukocytosis promoting factor). Both of these important biological substances in inflammation are presumably formed by the injured cell(9). It would thus seem as if the mechanism of suppression by the anti-inflammatory substances is at a cellular level.

Previous studies have pointed out that an extract of the adrenal cortex would retard growth of the chick embryo(10). Karnofsky and his collaborators(11) have shown the retarding influence of cortisone and of Compound F on the growth of the chick embryo. The view that the presence of cortisone and ACTH in an inflamed area may suppress cellular activity in that area is therefore a distinct possibility, and the present studies substantiate this view.

Summary. 1. Cortisone acetate (Merck) (2.5 mg in 10 cc of sea water) tends to suppress the incidence of normal cleavage in fertilized *Arbacia punctulata* ova. This is not referable to the vehicle in which the steroid is suspended. The vehicle does not alter the cleavage pattern. Filtering the suspension of cortisone acetate yields a cortisone residue capable of suppressing the incidence of normal cleavage, whereas the filtrate which is essentially the suspending vehicle has also no such inhibitory effect. The sperms are likewise inactivated by cortisone acetate (2.5 mg or 5 mg in 10 cc of sea water). Compound F (5 mg in 10 cc of sea water) suspended in propylene glycol suppresses the incidence of normal cleavage of the fertilized ova. 2. Desoxycorticosterone acetate and the glucoside tend to have the same inhibitory effect on the cleavage of ova. 3. Two to 6 mg of ACTH (corticotropin) (Armour and Organon products) directly affects the cleavage pattern of Arbacia ova. There is also a greater incidence of suppression or at least retardation of segmentation of the ova. This indicates that this pituitary hormone can likewise have a direct toxic effect on the ova without the mediation of the adrenal cortex. 4. In contrast, a protein anabolic steroid, testosterone propionate (Ciba), in 2.5 mg concentration, has no effect on the incidence of normal cleavage in Arbacia ova. 2.5 mg of Somatrofin (STH), (a Horner product), a growth hormone from the anterior pituitary has likewise no effect on the cleavage pattern. 5. The suppressing action of ACTH is not a protein effect and it is not due to any differential solubility of the hormone. 6. The specific results are discussed from the standpoint that the suppression of cellular activity, as measured by cell division, by cortisone, Compound F, and ACTH may throw light on the mechanism of suppression at a cellular level by some of these hormones in inflammation as reported in 1940 and 1942 by the writer.

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