liver and heart mitochondria, on the other hand, progesterone always exerted a inhibitory action.

One may conclude from these experiments that the real influence of progesterone on ATPase activity of mitochondria is an inhibitory one. The fact that acceleration occurs with homogenates from livers stored at – 30°C, as JONES and WADE found and as these researches confirm, may depend upon a damaging of the mitochondria.

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Riassunto

L'autrice ha studiato l'influenza del progesterone sull'attività ATPasica di omogenati e mitocondri di fegato e cuore di ratto. Sui mitocondri di cuore il progesterone ha sempre azione inibente sia *in vivo* che *in vitro*. Sui mitocondri di fegato ha azione leggermente accelerante. Sugli omogenati di fegato preparati da tessuti freschi ha azione inibente. Se si conservano i tessuti a – 30°C si ha invece un aumento dell'ATPasi *in vitro*.

The Buffering Effect of Adrenal Tissue During Corticoid Overdosage

(Further studies on the physiological antagonism between gluco- and mineralo-corticoids)

The theory of stress and the diseases of adaptation is largely based upon the following assumptions: (1) that the adrenals can produce glucocorticoids (which, as far they have been examined uptodate, proved to be also antiphlogistic, lympholytic and catabolic) and mineralocorticoids (which, as far as they have been examined up todate, proved to be also prophlogistic, antilympholytic and anticatabolic) independently of each other, (2) that adjustments of corticoid secretion during stress is an important factor in regulating resistance, and (3) that derangements not only in the total amount, but also in the balance between these two types of corticoids is an important factor in the pathogenesis of various diseases[1].

The recent demonstration that cortisol[2] and aldosterone can be detected not only in adrenal tissue but also in the circulating blood has definitely rendered the "unitarian theory" of adrenocortical function untenable[3] yet, during the last four years, several investigators expressed doubts concerning the importance, or even the existence, of any true antagonism between mineralo- and gluco-corticoids. We have therefore devoted a great deal of attention to means of perfecting experimental techniques for the demonstration of such antagonisms, which to our mind are extremely important both in physiology and in pathology.

Experimental Procedure. Eighty Sprague-Dawley rats, having an average body-weight of 131 g (range 125–140 g) were subdivided into eight equal groups and treated as outlined in the Table.

Bilateral adrenalectomies were performed in Groups V–VIII, through the lumbar route and treatment with the steroids was begun on the same day. Microcrystal suspensions of cortisol acetate (250 µg/day) and DCA (1 mg/day) were administered in 0·2 ml of water, both steroids being injected subcutaneously in the ventral region. In the rats of all groups, granuloma-pouches were prepared two days later, under the shaved skin of the back, in the usual manner[1]. Under ether anesthesia, 25 ml of air was first injected, this being immediately followed by the introduction of 0·5 ml of an 0·5 % croton-oil solution (in corn-oil). The animals were maintained exclusively on "Purina Fox Chow" and tap water.

The formation of exudate was followed daily by trans-illumination with an electric flashlight and all surviving animals were killed 12 days after preparing the granuloma-pouch. Immediately after autopsy, the exudate in the granuloma-pouches was accurately measured by aspiration into a graduated syringe. At the same time, the adrenals, thymuses and spleens of all animals were dissected and fixed in Susa solution for subsequent weighing and histological study.

Experimental Observations. Our principal observations are summarized in the Table, so that it will suffice merely to mention the most salient features here.

It should be said at the outset that many preliminary experiments showed the impossibility of keeping alive, for 12 days, more than a few exceptional adrenalectomized rats with granuloma-pouches, if they received neither hormone treatment nor salt supplements. Hence, in the present experiment, only two rats of Group V survived so that here the means listed in the Table could not be accompanied by the standard errors (as was the case in the other seven groups in which all animals survived until the end of the experiment).

The *adrenals* in the intact-untreated rats (Group I) were of normal size, while those of the rats treated with cortisol, DCA or a combination of the two hormones showed varying degrees of the well known phenomenon of "compensatory atrophy". Thus, in this respect, the two types of steroids are not antagonistic and, indeed–as we have shown elsewhere[2]–at higher dose-levels, they are even synergistic.

In the intact rats, the formation of *exudate* under the influence of local croton-oil irritation, was only insignificantly affected by cortisol, DCA or the combination of the two agents, at the dose-levels used. Conversely, after adrenalectomy, cortisol alone proved to be clearly antiphlogistic (Group VI) and DCA prophlogistic (Group VII), while combined treatment with both hormones resulted in a mutual antagonism between them (Group VIII).

The two *lymphatic organs*, the thymus and the spleen, may be discussed conjointly, as they reacted essentially in the same manner to the two types of steroids. The lympholytic effect of cortisol was comparatively slight in intact animals, and correspondingly, the anti-lympholytic effect of DCA could likewise not become very obvious. Conversely, after adrenalectomy cortisol produced a marked thymolysis and splenic involution (Group IV), DCA had an inverse effect (Group VII) and, even more significantly, the effect of cortisol upon these lymphatic organs was very effectively counteracted by simultaneous treatment with DCA (Group VIII).

[1] H. SELYE, *Stress, The Physiology and Pathology of Exposure to Systemic Stress* (Acta Inc., Med. Publ., Montreal, 1950); *The Story of the Adaptation Syndrome* (Acta Inc., Med. Publ., Montreal, 1952).

[2] In agreement with the suggestion made by SHOPPEE [C. W. SHOPPEE, in: Ann. Rev. Biochem. *22*, 261 (1953)], the term cortisol is used here in preference to "hydrocortisone" since it eliminates confusion with 4,5-dihydrocortisone and obviates the possible implication that "hydrocortisone" is to be regarded primarily as a mere derivative of cortisone.

[3] S. A. SIMPSON and J. F. TAIT, in: Fourth Annual Report on Stress by H. SELYE and G. HEUSER (Acta Inc., Med. Publ., Montreal, 1954).

[1] H. SELYE, J. Amer. Med. Ass. *152*, 1207 (1953).

[2] H. SELYE, *Stress, The Physiology and Pathology of Exposure to Systemic Stress* (Acta Inc., Med. Publ., Montreal, 1950).

The buffering effect of adrenal tissue during corticoid overdosage

Group	Treatment	Adrenal Wt. in mg	Exudate in ml	Thymus in mg	Spleen in mg	Body Wt gain in g
I	Intact no Treatment	53·6 ± 3·6	13 ± 2·5	304 ± 25	801 ± 67	27 ± 2·2
II	Intact cortisol	46·3 ± 1·4	11 ± 1·8	218 ± 11	713 ± 61	16 ± 3·9
III	Intact DCA	44·4 ± 4·3	9·7 ± 2·5	308 ± 23	824 ± 84	27 ± 2·8
IV	Intact cortisol + DCA	43·8 ± 2·3	13 ± 1·8	278 ± 27	687 ± 40	18 ± 4
V	Adrenalectomized no Treatment	–	12·5	428	587	– 4
VI	Adrenalectomized cortisol	–	7·2 ± 1·8	195 ± 24	471 ± 53	– 14 ± 1·9
VII	Adrenalectomized DCA	–	17 ± 2·4	472 ± 29	1045 ± 86	32 ± 5
VIII	Adrenalectomized cortisol + DCA	–	13 ± 2·4	295 ± 20	914 ± 74	18 ± 2·6

Finally, in intact animals, the gain in *body-weight* was likewise not very markedly influenced by the two types of hormones, at this dose-level; yet, in the adrenalectomized rats, cortisol (Group VI) produced a particulary marked loss and DCA-treatment (Group VII) counteracted the growth-inhibitory effect induced by adrenalectomy in itself (Group V). Here again, the most significant finding is the total inhibition of the cortisol-induced catabolism by simultaneously administered DCA (Group VIII).

It should perhaps be especially mentioned that all these actions of combined cortisol and DCA treatment cannot be interpreted as merely due to a generally better maintenance of the deficient animals. Numerous preliminary experiments had shown that if cortisol is given at a daily dose higher than 250 µg (without dietary NaCl supplements) exudate-formation, as well as growth of the lymphatic organs and of the body as a whole, are even more markedly suppressed than in Group VI of the present series.

Discussion. It is particularly noteworthy that, in all these respects, the actions of cortisol are inhibited by the presence of living adrenal tissue (Group II), approximately in the same manner as–in adrenalectomized animals–by the injection of DCA (Group V). It will be recalled that, at the time when DCA was still in scarce supply, it was found necessary to remove the adrenals in order to obtain clearcut prophlogistic effects (myocarditis, periarteritis nodosa, arthritis) with the minimal amounts of this steroid which were then available for the demonstration of some interrelation between the adrenal cortex and connective-tissue diseases[1]. Subsequently, several investigators reported observations, both in animals and in Addisonian patients, indicating that sensitivity to injected corticoids is greatly enhanced by removing the adrenals. (For review of pertinent literature cf.[2].)

In assessing the significance of the experiments reported in this paper, it is of particular interest that, according to INGLE[3], various metabolic alterations induced by cortisone overdosage are also enhanced by adrenalectomy. The impression was gained–in comparing groups of intact and adrenalectomized cortisone-treated rats–that some of the organ-changes produced by cortisone may have been accentuated in the absence of the adrenals.

Nevertheless, INGLE concluded that, because of "some difficulty in appraising these changes quantitatively and objectively, no claim is made that we have proved a true difference in the incidence and extent of pathology between these two groups".

INGLE also raised the fundamental question: "Does it (the adrenal) have the capacity to inactivate some exogenous cortisone or does it continue to secrete substances which either balance or inhibit the effects of cortisone?" Naturally, this point is of the greatest interest, in connection with our theory of stress and the diseases of adaptation, which postulates the importance of an antagonistic balance between pro- and antiphlogistic corticoids in health and disease.

Although adrenal-inactivation of corticoids may also occur, the observations reported in this paper clearly indicate that we need not postulate it to explain the observed facts; even after adrenalectomy, the various actions of cortisol are antagonized by simultaneous DCA treatment. On the other hand, as shown by the phenomenon of "compensatory adrenal atrophy", it is also clear that these hormones are not necessarily antagonistic in every respect; their ability to synergize each other as regards life maintenance of adrenalectomized animals further proves this point.

Addendum in proof. Since this manuscript was submitted, it has been possible to demonstrate that as little as 100 µg/day of DCA suffices fully to antagonize the actions of 400 µg/day of cortisol in such tests. Partial inhibition of this same dose of cortisol may even be obtained with as little as 30 µg/day of DCA, while aldosterone (kindly supplied by Doctor A. WETTSTEIN, of Ciba Limited, Basle) exhibited little, if any anti-cortisol effect at this same doselevel. These experiments have, in the meantime, been described at length elsewhere (H. SELYE and P. BOIS, in: Fourth Annual Report on Stress by H. SELYE and G. HEUSER, Acta Inc., Med. Publ., Montreal, 1954); they are mentioned here only because they suggest the possibility of some dissociation between mineralo-corticoid and prophlogistic corticoid activity.

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Zusammenfassung

Experimente an Ratten zeigten, dass sowohl die antiphlogistischen, lympholytischen und katabolischen Wirkungen von Cortisol als auch die entgegengesetzten

[1] H. SELYE, O. SYLVESTER, C. E. HALL, and C. P. LEBLOND, J. Amer. Med. Ass. *124*, 201 (1944).

[2] H. SELYE, *Stress. The Physiology and Pathology of Exposure to Systemic Stress* (Acta Inc., Med. Publ., Monetral, 1950); First Annual Report on Stress (Acta Inc., Med. Publ., Montreal, 1951); in: Fourth Annual Report on Stress by H. SELYE and G. HEUSER (Acta Inc., Med. Publ., Montreal, 1954). – H. SELYE and A. HORAVA, Second Annual Report on Stress (Acta Inc., Med. Publ., Montreal, 1952); Third Annual Report on Stress (Acta Inc., Med. Publ., Montreal, 1953).

[3] D. J. INGLE, Proc. Soc. Exper. Biol. Med. *79*, 184 (1952).

Wirkungen von DCA viel evidenter in adrenalektomierten als in intakten Tieren sind. – Durch gleichzeitige DCA-Behandlung konnte sogar nach kompletter Adrenalektomie die Cortisol-Pufferwirkung der lebenden Nebenniere nachgeahmt werden. Anscheinend sind die Wirkungen dieser beiden Typen von Nebennierenhormonen weitgehend maskiert, wenn Nebennierenrindengewebe eine einseitige Überdosierung durch die Sekretion von antagonistischen Corticoiden hemmen kann.

Spontaneous Peristaltic Activity of Veins of Chick Embryos and Newly Hatched Chickens Explanted *in vitro*

In a previous study by ATTARDI, GANDINI, and MARCON[1] it has been shown that the chick embryo arteries cultivated *in vitro* exhibit spontaneous contractions of a peristaltic type, with a frequency up to 20 per minute, which last for 2 or 3 days: these results have been recently confirmed by TONI[2]. A similar contractile activity has been observed also in the rabbit embryo arteries cultivated *in vitro* (ATTARDI[3]).

We have undertaken the present investigations, of which the first results are reported here, with the aim of establishing whether a contractile activity can be demonstrated also in venous segments of the circulatory tree, especially in those regions where, on account of the particular conditions of the venous return, the participation of the vein walls in the blood propulsion is most probable. We should mention here the existence, already demonstrated in some species of Vertebrates, of auxiliary hearts, which help the venous circulation in particular regions, as the portal heart of Bdellostoma (CARLSON[4]) and the pulsatile veins of the bat wings (discovered by WHARTON JONES[5] and recently studied *in vitro* by MISLIN[6]).

We have first taken into consideration the veins of the portal system of chick embryos and newly hatched chickens (omphalomesenteric vein, mesenteric vein, right portal vein[7], left portal vein[8]), where the blood is almost lacking in *vis a tergo*, owing to the fact that it has flowed through the capillary network of the yolk-sac and of the alimentary canal, and it probably needs help in order to overcome the resistance of the hepatic capillary bed. Reference should be made, in this connection, to the above mentioned portal heart of Bdellostoma and to the observations by RONCATO[9], concerning the occurrence of rhythmical movements of relatively high frequency (up to 15–20 per minute) and long duration (up to 6 h) in segments of the portal and superior mesenteric veins of adult dogs, suspended in autologous blood serum and submitted to a certain stretch.

We have examined also the left umbilical vein, for which it can likewise be assumed that the *vis a tergo* is not sufficient by itself to ensure the blood flow towards the heart, as it tends to exhaust itself through the considerable length of the vessel.

For these investigations chick embryos from 6 days of incubation up to the hatching, and newly hatched chickens up to 5 days of age have been employed. The explants have been prepared on coverslips by the usual hanging-drop method in a medium composed of homologous plasma and 10-day-old chick embryo extract in equal parts. Usually fragments of vein 2 to 3 mm in length have been explanted; whenever possible more than one fragment of each vein have been tested. We have tried to place the fragments in the medium as far as possible in an extended state, as this condition is favourable to the contractile activity. – The results of our observations are presented in the Table.

The contractile activity started generally a few minutes after the explantation or anyhow within the first hour and became exhausted after a few hours (from 2 to 15 h), only slight undulations of the walls persisting after that time. The spontaneous contractions occurred at periods alternating with periods of rest of different duration. The contractions, more or less powerful, were generally of a peristaltic type, and propagated along the whole segment or only one part of it; one could observe rather often peristaltic waves alternating with antiperistaltic ones. In some cases the rhythmical contraction, instead of peristaltic, was longitudinal or annular in a more or less limited part of the fragment, without any sign of propagation.

The fact should be noted that the veins examined have shown a contractile activity *in vitro* in a higher percentage of explants (about 45%) than the embryonic arteries placed under the same experimental conditions. This may be due to the fact that the state of extension of the walls which is favourable to the contraction is more easily produced, by the plasma clot retraction and the action of the temperature, in the veins than in the arteries, owing to the histological features of the former (smaller content of elastic fibres and muscle cells, greater looseness of the structural components, etc.). The contractile activity which is exhibited by the veins of chick embryos and newly hatched chickens differs from that of the embryonic arteries for the lower power, the early beginning and the relatively short duration of the contractions.

In the explants of the umbilical vein derived from the region of the umbilical cord of chick embryos in the first half of incubation (from the sixth day), we have observed very often a rather powerful contractile activity, which appeared to be localized outside the venous walls, in muscle cells which had become differentiated within the mesenchyme of the umbilical cord[1]: a certain amount of this tissue was in fact always explanted together with the venous segments, on account of the difficulty of isolating completely the vein in this region. In the second half of incubation a contractile activity proper to the walls of the umbilical vein could on the contrary be demonstrated also in this portion of the vessel. It is however worth noticing the fact that even when the contractions appeared to occur in the tissue adherent to the vein, they had a clear influence on the thin walls of the vessel, producing rhythmical movements of them. It is therefore likely that the contractile activity of the muscular tissue which develops in the umbilical cord has an adjuvant effect on the circulation through the umbilical vein in the first periods of incubation, when this vessel does not yet present its own contractile activity. A detailed study

[1] G. ATTARDI, E. GANDINI, and L. MARCON, Boll. Soc. ital. Biol. sper. *24*, 1333 (1948).

[2] G. TONI, Boll. Soc. ital. Biol. sper. *29*, 6 (1953).

[3] G. ATTARDI, Boll. Soc. ital. Biol. sper. *25*, 1057 (1949).

[4] A. J. CARLSON, Z. allg. Physiol. *4*, 264 (1904).

[5] T. WHARTON JONES, Philos. trans. roy. Soc. London, 1852.

[6] H. MISLIN, Helv. physiol. Acta *5*, C 3 (1947); *5*, C 18 (1947); *7*, C 15 (1949); Exper. *9*, 425 (1953).

[7] Trunk formed by union of the omphalomesenteric and mesenteric veins.

[8] Independent vessel which collects part of the blood from the gizzard and proventriculus and enters the left lobe of the liver.

[9] A. RONCATO, Arch. Fisiologia *20*, 159 (1922).

[1] Probably these muscle cells belong to that part of the amnion which contributes to form the umbilical cord.