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ROLE OF SOMATOTROPHIC HORMONE IN THE PRODUCTION OF MALIGNANT NEPHROSCLEROSIS, PERIARTERITIS NODOSA, AND HYPERTENSIVE DISEASE

BY

HANS SELYE, M.D., Ph.D., D.Sc., F.R.S.C.

*Professor and Director of the Institut de Médecine et de Chirurgie Expérimentales.
Université de Montréal, Montreal, Canada*

[WITH SPECIAL PLATE]

During exposure to stress large amounts of corticotrophic and corticoid hormones are discharged into the blood as part of the general-adaptation syndrome (Selye, 1936, 1937, 1950b). Hence the question arose whether, during adaptation to stress, intoxication with endogenous corticoids could predispose the organism to the development of disease.

It was found, by experimentation on animals, that malignant nephrosclerosis, periaarteritis nodosa, as well as morphological changes reminiscent of the hypertensive and rheumatic diseases can be produced by mineralo-corticoid substances such as desoxycorticosterone acetate (D.C.A.) (Selye and Pentz, 1943) or desoxocortisone (Reichstein's "compound S") (Selye, 1950a).

An impure corticotrophic lyophilized anterior pituitary (L.A.P.) preparation also proved to cause hypertension, with lesions in the cardiovascular and connective tissues quite similar to those seen in the so-called "collagen diseases" (Selye, 1944, 1946).

The similarity between the actions of D.C.A. and L.A.P. became even more evident when it was found that both these hormone preparations predispose to the production of experimental arthritis (Selye, 1949a) and to anaphylactoid reactions (Selye, 1949b). It was therefore tentatively assumed that L.A.P. may act by virtue of its corticotrophic effect, perhaps by stimulating the production of mineralo-corticoids.

Subsequent observations revealed that L.A.P. does not elicit the above-mentioned cardiovascular and renal changes in adrenalectomized rats maintained on corticoid extracts. Thus it became extremely probable that the effect of the pituitary preparation is actually mediated through the adrenal cortex (Hall *et al.*, 1946; Ingle, 1950).

However, later, when adequate amounts of highly purified A.C.T.H. became available, it was noted that even the highest tolerable doses of this hormone fail to reproduce the above-mentioned actions of L.A.P. In particular, A.C.T.H. causes no nephrosclerosis; it exerts only irregular effects upon the blood pressure, and, far from eliciting "collagen disease" and predisposing to arthritis or allergy, it actually inhibits their development, both in experimental animals and in man. Crystalline pure gluco-corticoids—for example, cortisone—act essentially like A.C.T.H., not like L.A.P. or D.C.A. Therefore

we had to assume that A.C.T.H. is predominantly a "gluco-corticotrophic" hormone—that is, one which stimulates the adrenal to produce gluco-corticoids. From the above it became obvious that the active principle in L.A.P. could not be A.C.T.H., but since the second corticotrophin has not yet been identified, it was meanwhile merely designated as the "L.A.P. factor," or "X factor," of the pituitary.

If L.A.P. is administered to rats simultaneously with A.C.T.H. the nephrotoxic actions and cardiovascular damage induced by the former are considerably aggravated (Selye, 1950a). This led us to suppose that perhaps the "L.A.P. factor" so conditions the response of the adrenal cortex to A.C.T.H. that the latter becomes "mineralo-corticotrophic" (Selye, 1950b, 1950c).

It will be recalled that L.A.P.—which is merely a dried anterior pituitary powder—contains all the adreno-hypophysial principles and much accompanying non-hormonal protein material. However, the preparation is particularly rich in somatotrophic hormone (S.T.H.), as shown by the fact that it considerably promotes somatic growth, as well as the proliferation of connective and lymphatic tissues. All these actions are shared by electrophoretically pure S.T.H. (Evans *et al.*, 1948; Simpson *et al.*, 1949).

The experiments reported here show that the above-mentioned desoxycorticosterone-like (mineralo-corticoid) actions of the "L.A.P. factor" can be reproduced by electrophoretically pure S.T.H. They also demonstrate that, under suitable experimental conditions, the simultaneous administration of cortisone inhibits these effects of S.T.H., presumably because it depresses A.C.T.H. secretion by the animal's own pituitary.

Materials and Methods

Two series of experiments have been performed in which S.T.H. was administered in conjunction with cortisone. In each instance the changes observed were compared with the norm and with those elicited by cortisone or S.T.H. alone.

As the two experiments were conducted in essentially the same manner they may well be discussed conjointly.

Experimental Animals.—In both experiments female piebald rats weighing 105–140 g. were used exclusively. In

all of them the right kidney was removed on the day preceding the administration of the first injections. The animals received a powdered "Purina Fox Chow" diet and were given 1% NaCl solution instead of drinking-water. The unilateral nephrectomy was done and the NaCl supplement was administered because these factors had previously been shown to increase sensitivity to the nephrosclerotic action of L.A.P. (Selye, 1950b). The first experiment was terminated after 27 days, the second after 17 days. The animals were killed by exsanguination and the organs immediately removed for weighing and histological examination.

Preparation and Administration of the S.T.H. Solution.—In the first experiment two S.T.H. preparations were employed—one supplied by the Armour Company (lot No. R20-5), the other by Professor C. H. Li, of Berkeley, California (lot No. L1643M). Professor Li's preparation proved to be a homogeneous material as judged by electrophoresis studies. Biological assays did not show L.H., F.S.H., lactogenic, or A.C.T.H. contamination, but they revealed a trace of thyrotrophic activity. The Armour preparation has not been subjected to electrophoretic analysis; however, judged by our own studies, it was also essentially free of contaminating pituitary principles. Both S.T.H. preparations were dissolved in 1% NaCl in such a manner that each individual dose was contained in 0.2 ml. of the solvent. Li's preparation was given at the dose of 3 mg., Armour's at the dose of 6 mg. a day, divided into three injections, given once every eight hours throughout the day and night.

Preparation and Administration of the Cortisone Solution.—In both experiments cortisone was used in the form of cortisone acetate micro-crystals, as prepared by Merck and Co. (25 mg. per ml.), suspended in saline solution with added suspending agents. In the first experiment 5 mg. was injected subcutaneously once daily during the first seven days and 2.5 mg. daily during the rest of the experiment. In the second experiment 5 mg. a day was given during the first seven days and 10 mg. a day during the rest of the period of observation.

Measurement of Water Turnover.—In both experimental series only the water intake was measured daily, as the accurate collection of urine is technically difficult in the rat. In the tables the corresponding figures represent the mean values during the 10 last days of observation.

Measurement of the Blood Pressure.—The blood pressures were determined by the tail-cuff method, under light ether anaesthesia, once a week. The values so obtained are intermediate between systolic and diastolic pressures, but closer to the former. The figures given in the tables represent the means of the last blood-pressure measurements in all instances.

Production of Experimental Arthritis.—In both series an experimental arthritis was produced on the eighth day of the experiment by a modification of the "topical irritation arthritis" test. While in earlier studies a formalin solution was employed to cause local irritation (Selye, 1949c), we have now employed a 10% suspension of mustard powder, of which 0.1 ml. is injected under the plantar aponeurosis in the metatarsal region of the left hind leg. The degree of swelling and inflammatory granuloma formation is expressed in a scale from 0 to +++, and the mean values are listed in the tables. The severity of the lesion was estimated three days after the injection of the mustard powder.

Results of First Experimental Series

Changes in Body and Organ Weights

The distribution of the animals into the six groups of this experimental arrangement, as well as the body and organ weights, is summarized in Table I, together with the standard errors for each figure. In order to facilitate the evaluation of the changes observed, the weights of all organs have been expressed both in mg. (with the standard error) and in mg./100 g. of body weight. This was deemed advisable because the final body weight of the rats in the various groups of this experiment were very different, owing to the anabolic effect of S.T.H. and the catabolic action of cortisone.

In accord with expectations, S.T.H. (groups II and V) greatly increased *somatic growth*, while cortisone actually diminished body weight below the initial value (group III). It is also evident that the catabolic effect of cortisone has been abolished by simultaneous treatment with either of the two S.T.H. preparations (groups IV and VI). Indeed, the animals receiving S.T.H. plus cortisone had a higher final body weight than the untreated controls, although they were not as heavy as the rats receiving S.T.H. alone. We may conclude that there is a direct antagonism between the anabolic effect of S.T.H. and the catabolic action of cortisone. These observations suggest that the individual variability in the requirements for cortisone in patients, in whom the hormone is given for therapeutic reasons, may likewise be largely conditioned by the availability of S.T.H. within the organism.

The weight of the *heart* (expressed either in absolute terms or as a percentage of the body weight) was greatest in the animals receiving S.T.H. (Li) plus cortisone. There was also some cardiac enlargement in the rats receiving

TABLE I.—Summary of Organ Weights in the First Experiment*

Group:	I Normal Controls	II S.T.H. (Li)	III Cortisone	IV S.T.H. (Li) + Cortisone	V S.T.H. (Armour)	VI S.T.H. (Armour) + Cortisone
No. of rats per group	6	3	6	3†	3	3
Initial body weight in g.	113	116	114	113	113	113
Final body weight in g.	147 ± 5.8	175 ± 20	100 ± 6	153	181 ± 25	166 ± 6.3
Heart	532 ± 23	864 ± 49	512 ± 32	1,018	734 ± 146	667 ± 46
in mg./100 g.	362	494	512	665	405	402
Kidney	794 ± 123	1,460 ± 68	673 ± 117	2,076	1,187 ± 283	983 ± 73
in mg./100 g.	540	834	673	1,357	656	592
Liver	6,332 ± 131	10,879 ± 36	4,596 ± 507	11,282	8,633 ± 509	7,634 ± 116
in mg./100 g.	4,307	6,216	4,596	7,374	4,770	4,599
Adrenals	37 ± 1.8	57 ± 0.07	11 ± 1.6	50	49 ± 8.2	20 ± 0.09
in mg./100 g.	25	33	11	33	27	12
Thymus	192 ± 18	81 ± 61	33 ± 3.4	18	163 ± 60	59 ± 20
in mg./100 g.	131	46	33	12	90	35
Spleen	745 ± 47	1,090 ± 60	433 ± 42	1,154	879 ± 146	810 ± 94
in mg./100 g.	507	623	433	754	486	488

* In the text we did not evaluate the statistical significance of each figure, especially since the interested reader can readily do so with the aid of the standard errors given here. It should be emphasized, however, that many of the inferences drawn in the text are based on a conjoint consideration of the data given in the four tables and of histological observations which do not lend themselves to statistical evaluation.

† Two of the rats in this group succumbed several days before the termination of the experiment. Hence, their organ weights were not pooled with the rest and thus standard errors cannot be given in this column. These animals died with severe nephrosclerosis and myocarditis, without signs of adrenal atrophy. This fact speaks (even more definitely than organ weights would) in favour of our conclusion that under these conditions when cortisone fails to cause adrenal involution it aggravates the nephrotoxic and cardiotoxic actions of S.T.H.

S.T.H. (Li) or S.T.H. (Armour) alone. However, the cardiac enlargement caused by S.T.H. (Armour) was not further increased by simultaneous cortisone administration. Indeed, the animals given S.T.H. (Armour) plus cortisone had a slightly smaller heart weight than those receiving S.T.H. (Armour) alone. An attempt at a theoretical evaluation of these differences in the actions of the two S.T.H. preparations is made later, in the section entitled "Discussion." However, for the sake of clarity, it may be mentioned here that in this (and in all other pertinent experiments performed in our Institute) cortisone tended to aggravate the cardiac, vascular, and renal damage caused by S.T.H. overdosage whenever it failed to cause marked adrenocortical involution; conversely, cortisone actually inhibited these S.T.H. effects under experimental circumstances which permitted it to induce the usual "compensatory atrophy" of the adrenal cortex.

The weight of the *kidney* (both in absolute terms and when expressed as a percentage of body weight) was largest in the animals receiving cortisone and S.T.H. (Li) simultaneously. Both S.T.H. preparations, when given alone, also caused a marked, absolute, and relative renal enlargement. However, the increase in kidney weight induced by S.T.H. (Armour) was actually diminished by simultaneous cortisone treatment.

Both S.T.H. preparations caused a pronounced increase in the weight of the *liver*. This was further accentuated by simultaneous cortisone administration in the case of S.T.H. (Li), but was diminished by this same steroid in the animals receiving S.T.H. (Armour). The fact that cortisone augmented the hepatic weight increment caused by S.T.H. (Li) is all the more significant since in these same animals the somatic weight increase elicited by S.T.H. (Li) was actually diminished by cortisone. Apparently, under these conditions, there is a dissociation between the general anti-anabolic effect of cortisone (as revealed by the somatic weight) and its action upon the growth of renal and hepatic tissue.

It is relevant to mention that in certain species, such as the rabbit, cortisone itself causes a pronounced increase both in the absolute and in the relative weight of the liver (Timiras, unpublished). The reason for this species difference in the responsiveness of the liver to cortisone has not yet been elucidated.

The *adrenals* underwent pronounced compensatory atrophy in the rats treated either with cortisone alone or with cortisone plus S.T.H. (Armour). Conversely, in the rats receiving S.T.H. (Li) in conjunction with cortisone the adrenal weight was far above that of the uninjected controls. When expressed as a percentage of the body weight the adrenal weight in the animals receiving S.T.H. (Li) plus cortisone was almost exactly the same as in those receiving this S.T.H. preparation alone. This implies that, under the conditions of this experiment, cortisone failed to inhibit the adrenal-stimulating effect of S.T.H. In sharp contrast to the above-mentioned findings, the rats treated with S.T.H. (Armour) showed a much less pronounced adrenal enlargement; indeed, this was almost negligible when the adrenal weight was expressed as a percentage of the body weight. Furthermore, in the animals treated with S.T.H. (Armour) simultaneous injection of cortisone caused considerable adrenal atrophy both in absolute and in relative terms.

The *thymus* of all four groups receiving cortisone, alone or in combination with one of the S.T.H. preparations, was almost maximally involuted; hence the appar-

ent differences between these groups do not lend themselves well to an analysis of the underlying mechanism. Yet it is noteworthy that simultaneous treatment with S.T.H. (Li) tended to accentuate, and S.T.H. (Armour) to counteract, the thymus involution normally caused by cortisone alone. Although the figures leave much doubt about the significance of this antagonism they clearly show at least the absence of a synergism of cortisone with S.T.H. (Armour) such as is noted with S.T.H. (Li). This (like the adrenal changes) suggests that some A.C.T.H.-like effect accompanied the administration of S.T.H. (Li) under these conditions.

The *spleen* was smallest in the animals treated with cortisone alone, while it was much enlarged after S.T.H. (Li). S.T.H. (Armour) caused a lesser splenic enlargement, which failed to be manifest if the organ weight was expressed per 100 g. of body weight. It is particularly noteworthy that, although the somatic weight increase caused by either S.T.H. preparation was so considerably inhibited by simultaneous cortisone treatment, the splenic enlargement was not significantly affected by this steroid. This fact (as well as the above-mentioned observations concerning cardiac, renal, and hepatic weights) further illustrates the lack of proportionality between somatic anabolism in general and the growth of certain organs in rats treated with S.T.H. plus cortisone. Apparently the cardirotrophic, renotrophic, hepatotrophic, and splenotrophic actions of S.T.H. are much less readily counteracted (and may even be increased) by cortisone than is its general somatotrophic action.

Clinical and Histological Observations

Only some of the clinical and histological observations can be summarized in the form of a table (see Table II). These, as well as other findings, are discussed in more detail below.

TABLE II.—Summary of Some Clinical and Histological Observations in the First Experiment

Group:	I Normal Controls	II S.T.H. (Li)	III Cortisone	IV S.T.H. (Li) + Cortisone	V S.T.H. (Armour)	VI S.T.H. (Armour) + Cortisone
Myocarditis	0	++	0	++	+	0
Nephritis + nephro- sclerosis	0	++	0	+++	+	0
Pancreatic periarteritis	0	Trace	0	++	Trace	0
Water intake in ml. . .	56 ± 2	196 ± 8.2	39 ± 0.36	166 ± 23	104 ± 7.8	64 ± 2
Blood pres- sure in mm. Hg	128 ± 7.6	177 ± 53	153 ± 11.5	142	153 ± 14	149 ± 14
Arthritis test	++	+++	0	+	+++	+

It may be stated at the onset that the changes observed in the heart, kidney, and the pancreatic vessels were essentially similar to those previously observed after treatment with D.C.A. or L.A.P. (Selye, 1950b). In the table these are listed under the headings "myocarditis," "nephritis plus nephrosclerosis," and "pancreatic periarteritis" respectively. Their severity is expressed in a scale of 0 to + + +, which represents the mean change in each group. The designation "trace" is employed when the mean reading falls below one +.

In the *hearts* of the rats treated with S.T.H. (Li) we found the usual granulomatous nodules, situated either between the muscle fibres or underneath the endocardium, rarely in the subepicardial layer. These nodules consist of proliferating connective tissue cells, many of which are in various phases of mitotic division.

Some of these elements develop several irregular processes and thus lose their normal spindle shape. Their cytoplasm often exhibits a diffuse basophilia. The endothelial cells which line the capillaries and small arterioles tend to become hypertrophic and in many places protrude deeply into the lumina of the vessels, thus resembling a cuboidal or cylindrical epithelium. These granulomas are frequently invaded by lymphocytes, plasma cells, and macrophages, and less commonly by a few polymorphonuclear leucocytes and eosinophils. They often also contain polymorphonuclear giant cells, which appear to form either by confluence of endothelial cells in the capillaries or by division of the nuclei (without concomitant division of the cytoplasm) in proliferating connective-tissue cells. Between the cells are masses of more or less homogeneous eosinophilic "hyalin" material. Not infrequently these nodules develop around small arterioles which undergo changes typical of periarteritis nodosa. When such nodules develop underneath the mural endocardium or on the valves they may ulcerate, and then fibrin is deposited on the denuded surface. This fibrin gradually becomes organized by invading connective-tissue strands and vessels. There is a striking similarity between these myocardial changes and those observed in acute rheumatic fever, lupus erythematosus, and Libman-Sacks disease (Plate, Fig. 7).

The above-mentioned cardiac changes were particularly severe in the rats receiving S.T.H. (Li) plus cortisone, but almost as marked in those treated with S.T.H. (Li) alone. They were less pronounced in the animals given S.T.H. (Armour) alone, and here they were completely prevented by concurrent treatment with cortisone.

The kidneys of the rats treated with S.T.H. (Li) exhibited a typical malignant nephrosclerosis with periarteritis-nodosa-like changes of the small renal arterioles and glomerular hyalinization. All these changes had been previously noted in D.C.A.-treated animals. In nephrons with early stages of the lesion, the glomerulus is merely enlarged and apparently permeable to plasma proteins, since coagulated proteinaceous material is found in the spaces of Bowman's capsules as well as in the convoluted tubules. These coagula form hyaline casts which may completely obliterate the tubular lumina so that above their level the nephrons are greatly distended with urine. At a later stage the glomerulus becomes highly cellular; its endothelium and the visceral lamina of Bowman's capsule undergo marked hypertrophy and hyperplasia, often accompanied by invasion with polymorphonuclear leucocytes. At this time erythrocytes may appear in the lumina of Bowman's capsules and of the tubules. In some kidneys the hyalinization of vessels and glomeruli, in others the proteinuria, haematuria, and inflammatory glomerular changes predominate. Thus we note various intermediate stages between typical malignant nephrosclerosis and a haemorrhagic type of glomerular nephritis. Lymphoid infiltrates are occasionally seen between the tubules.

The connective tissue around the renal pelvis is always very oedematous and infiltrated by inflammatory cells. In some instances a typical pyelonephritis ensued.

All these changes were more pronounced in the rats treated with S.T.H. (Li) than in those given S.T.H. (Armour); cortisone tended to aggravate them in the former and prevented them in the latter case.

The only respect in which the kidneys of S.T.H.-treated rats appeared to differ from those of D.C.A.-intoxicated animals is that the former usually show very pronounced regenerative changes in the tubules and consequently a

much greater weight increase than has been noted after D.C.A. This "renotrophic effect" may well be specific of S.T.H. and quite distinct from its nephrotoxic action, as will be seen in the discussion of these findings below.

The rats treated with cortisone alone showed only marked glomerular hyperaemia with the extreme distension of the glomerular capillaries which is quite characteristic and constant in rats treated with this hormone (Selye, 1950b). (Figs. 1-6. All the photomicrographs in the Plate illustrate organ changes observed in the first experiment.)

In the pancreas, obvious changes developed only in the vessels, while the acini and the Langerhans islets remained essentially intact. Pronounced periarteritis nodosa was observed in all the animals treated with S.T.H. (Li) plus cortisone, while S.T.H. (Li) alone caused only minor lesions in one of the animals. Thus, in this respect again, cortisone aggravated the tendency of S.T.H. (Li) to produce "collagen disease." On the other hand, S.T.H. (Armour), which also produced periarteritis nodosa in one of the animals, did not exert such an action in any of the rats treated simultaneously with cortisone (Fig. 8). Histological signs of oedema in the pancreatic stroma ran approximately parallel with the degree of vascular lesion in all groups.

It should be mentioned here that macroscopically visible reddish nodules of *periarteritis nodosa in the mesentery* are almost invariably observed when renal damage is produced by D.C.A. or by the "endocrine kidney technique." Such lesions are quite exceptional, however, in rats in which similar renal damage is elicited by L.A.P. The mesenteric periarteritis nodosa caused by D.C.A. can be inhibited by simultaneous treatment with cortisone, even in animals in which the renal damage is actually aggravated by the latter hormone (Selye, 1950b). In the present experimental series, mesenteric periarteritis nodosa outside the pancreas was not observed in any of the animals—an observation which is compatible with the view that S.T.H. is the nephrotoxic factor responsible for the L.A.P. effect.

The liver revealed marked parenchymatous hyperplasia and hypertrophy in all S.T.H.-treated animals, whether or not they were also given cortisone. The hepatic cells were enlarged, some of them showed mitotic figures, and in general the cytoplasm exhibited a more or less pronounced basophilia. The hepatic stroma occasionally contained infiltrations with lymphoid and myeloid elements.

The adrenals of the rats receiving cortisone alone underwent the usual cortical atrophy, while the medulla remained essentially intact. The involution affected all three layers of the cortex, although the glomerulosa was least atrophic. This may be due to the fact that even normally the glomerulosa cells are poorly developed. The reticulo-endothelial phagocytes of the sinusoids were often laden with brownish blood-pigment derivatives.

Under the conditions of this experiment, S.T.H. (Li) caused a pronounced hypertrophy and hyperplasia of the adrenal cortex, often accompanied by lymphatic and myeloid infiltration islets. These same changes had previously been noted in animals treated with L.A.P. (Selye, 1950b). In rats concurrently given S.T.H. (Li) and cortisone the lymphatic infiltration was significantly less pronounced than in those receiving S.T.H. (Li) alone, presumably because of the well-known antilymphatic effect of gluco-corticoids.

The sinusoids of the adrenal cortex were replete with lymphoid cells, especially in the rats receiving S.T.H.

(Li) alone. In some fields the cortical sinusoids contained almost only lymphoid elements so as to give the impression of a lymphatic leukaemia. However, in other organs we did not observe any comparable increase in the intracapillary lymphocyte number.

In the rat, scattered islands of cortical cells are normally found within the substance of the medulla. They resemble the cells of the reticularis. In our S.T.H.-treated rats these "medullary cortical cells" were particularly prominent and enlarged. All these cortical changes were much more pronounced in the rats treated with S.T.H. (Li) than in those given S.T.H. (Armour). Furthermore, simultaneous administration of cortisone failed to prevent the adrenal hypertrophy caused by S.T.H. (Li), while it almost completely abolished that elicited by S.T.H. (Armour) (Figs. 9-11).

The *thymus* showed obvious histological signs of involution, with disintegration of the thymocytes and proliferation of the reticulum in all cortisone-treated animals, but this was least pronounced in those also receiving S.T.H. (Armour).

The *spleen* exhibited a pronounced atrophy of the lymphoid elements in all the animals treated with cortisone alone. This was accompanied by an involution of the splenic megakaryocytes, which were scarce, and even the few that could be found were subnormal in size. Conversely, the rats treated with S.T.H. (Li)—and to a lesser extent those receiving S.T.H. (Armour)—revealed a marked hyperplasia of lymphoid and myeloid elements, especially in the marginal zone of the Malpighian corpuscles. This was accompanied by a pronounced increase in the number of eosinophilic leucocytes within the splenic parenchyma. Normally, sessile eosinophils are extremely rare in the rat spleen. An extraordinarily large number of hyperplastic megakaryocytes was striking in both S.T.H.-treated groups. Simultaneous treatment with cortisone did not significantly alter the response to either of the S.T.H. preparations. However, in all the animals simultaneously treated with cortisone and either of the S.T.H. preparations there was an obvious increase in the storage of brownish blood-pigment derivatives within the splenic macrophages (Fig. 12).

The *water intake* was greatly increased in all the animals treated with S.T.H. (Li) and, to a less extent, in those given S.T.H. (Armour). Simultaneous treatment with cortisone did not significantly alter the increased water turnover of the rats given S.T.H. (Li), but it almost completely abolished the polydipsia and polyuria of those receiving S.T.H. (Armour). Cortisone in itself caused only a slight inhibition of water turnover.

The mean *blood pressure* was highest in the group receiving S.T.H. (Li). Although cortisone in itself also caused some hypertension, the rise in blood pressure was less pronounced in the rats given S.T.H. (Li) plus cortisone. This is noteworthy since the renal changes were more severe in the latter group. The interpretation of this finding must await further observations, especially since the pressor effect of S.T.H. (Armour) did not appear to be significantly influenced by concurrent cortisone treatment.

The *topical irritation arthritis* test showed that both S.T.H. preparations increase the ability of the rat to produce an inflammatory granuloma at the site of irritation, while cortisone prevents it. This agrees with our earlier observations, which revealed that L.A.P. aggravates, while cortisone inhibits, this type of arthritis (Selye, 1949c). The above observation is also compatible with

the view that S.T.H. exerts D.C.A.-like actions, since the latter hormone likewise augments the inflammatory response to local irritation in the joint regions. It is especially noteworthy, however, that both S.T.H. preparations greatly diminished the anti-arthritis effect of cortisone. This agrees with our view that the cortisone requirements of patients are also dependent upon endogenous S.T.H. production.

Results of Second Experimental Series

Perhaps the most clear-cut outcome of our first experiment was that, under our experimental conditions, cortisone influenced the effect of S.T.H. (Li) and of S.T.H. (Armour) in a different manner. It must be kept in mind, however, that S.T.H. (Li) was administered at the rate of 3 mg., and S.T.H. (Armour) at the rate of 6 mg. a day. The reason for this difference in dosage was merely that we did not have enough of the former preparation.

Our results made it imperative, however, to determine whether cortisone would inhibit the cardiovascular and renal toxicity of S.T.H. (Li) if the latter were given at a higher dose level. Endocrinology furnishes many examples showing that the action of hormones at low dose levels may be diametrically opposite to that at high dose levels. Thus small amounts of testosterone cause testicular atrophy, large amounts hypertrophy; thyroxine stimulates somatic growth at low and inhibits it at high dose levels, etc.

We also had to consider the possibility that the handling of our experimental animals (blood-pressure measurements, topical irritation arthritis test, repeated injections, etc.) stimulated endogenous A.C.T.H. production, which may have overcompensated for the effects of small doses of S.T.H. This would of course likewise interfere with the ability of cortisone to produce compensatory atrophy of the adrenals. Several (unpublished) preliminary experiments had revealed that cortisone prevents the cardiovascular and renal toxicity of S.T.H. only under conditions which permit it to cause adrenocortical involution. For these reasons we decided to refrain from the publication of the first experimental series until we were able to obtain an additional supply of S.T.H. (Li). Recently, in a second experiment, we increased the daily dose both of S.T.H. (Li) and of cortisone. We hoped that under these conditions any incidental endogenous A.C.T.H. secretion would be comparatively less important in proportion to the high S.T.H. and cortisone dosages injected. Unfortunately, we had only enough electrophoretically pure S.T.H. (Li) to continue this experiment for 17 days, but, even so, it became obvious that under these experimental conditions S.T.H. (Li) behaved in the same manner as the S.T.H. (Armour) had in the first series. In other words, under favourable experimental circumstances the toxicity of either of the two S.T.H. preparations is counteracted by cortisone. Hence, the apparent difference between the two somatotrophic extracts must not be ascribed to inherent dissimilarities of their actions, but to the dose level at which they were given.

The exact experimental procedure has already been outlined above in the section "Materials and Methods." The results as regards body weights, organ weights, and clinical and histological changes were so similar to those reported above for S.T.H. (Armour), alone and in combination with cortisone, that it would be redundant to discuss these here in detail. Suffice it to refer to Tables

III and IV, which are constructed in the same manner as the corresponding Tables I and II of the first experiment.

TABLE III.—*Summary of Organ Weights in the Second Experiment*

Group:	I Normal Controls	II S.T.H. (Li)	III Cortisone	IV S.T.H. (Li) + Cortisone
No. of rats per group	6	3	3	6
Initial body weight in g.	125	130	125	130
Final body weight in g.	143 ± 3.8	215 ± 11	103 ± 6.7	167 ± 2.3
Heart	521 ± 13	763 ± 6.5	486 ± 26	636 ± 32
in mg.	364	355	472	381
Kidney	715 ± 8.9	1,501 ± 259	658 ± 58	1,028 ± 36
in mg./100 g.	500	698	639	615
Liver	5,661 ± 392	11,756 ± 409	5,114 ± 771	9,226 ± 224
in mg.	3,955	5,468	4,965	5,524
Adrenals	38 ± 0.39	66 ± 6.5	12 ± 0.48	22 ± 2.6
in mg./100 g.	26.4	30.8	12.2	13.2
Thymus	136 ± 27	171 ± 54	29 ± 3.7	34 ± 4.6
in mg.	95	79	28	20
Spleen	780 ± 50	1,286 ± 655	373 ± 54	1,146 ± 409
in mg./100 g.	545	598	362	686
Prepu- tials	47 ± 2.8	101 ± 48	55 ± 10	174 ± 13
in mg./100 g	33	70	53	104

TABLE IV.—*Summary of Some Clinical and Histological Observations in the Second Experiment*

Group:	I Normal Controls	II S.T.H. (Li)	III Cortisone	IV S.T.H. (Li) + Cortisone
Myocarditis ..	0	++	0	0
Nephritis + nephro- sclerosis ..	0	++	0	0
Pancreatic periari- teritis ..	0	+	0	0
Water intake in ml.	43 ± 2	191 ± 21	73 ± 4.5	62 ± 3.9
Blood pressure in mm. Hg ..	136 ± 3	155 ± 5	154 ± 7	175 ± 19
Arthritis test ..	++	++	0	++

It will be seen that under these experimental conditions S.T.H. (Li) produced marked cardiac and renal enlargement as well as diuresis, all of which were inhibited by simultaneous cortisone treatment. Significantly, at the dose level at which it was employed here, cortisone caused marked adrenal atrophy despite S.T.H. treatment; it is tempting to ascribe the inhibition of the cardiovascular and renal effects to this cortical involution. Thus there is no reason to assume any important qualitative difference in the actions of the two S.T.H. preparations which we employed.

It will be noted that in this second experiment we also examined the *preputial glands*. These were only moderately influenced by cortisone or S.T.H. alone, but underwent a most pronounced enlargement in the rats treated with both these hormones simultaneously. The observation recalls some of our earlier experiments which showed that the atrophy of the preputial glands produced by hypophysectomy can be prevented by an impure anterior pituitary extract. The latter even causes preputial-gland enlargement both in intact and in hypophysectomized rats, this effect being considerably augmented by testosterone, androsterone, progesterone, and Δ^5 -pregnenolone, but not by D.C.A. At that time pure gluco-corticoids (such as cortisone) were not yet available to us. From these observations we concluded "that the preputial gland stimulating effect (just as the mammogenic action) of steroids is at least partly dependent upon the simultaneous action of hypophysial hormones" (Selye and Clarke, 1943).

The present observations suggest that S.T.H. is the factor which was responsible for the stimulating effect of our impure anterior pituitary extract on the preputial gland. Experiments now under way in this Institute are designed to examine the specificity of this effect and its possible suitability for the bio-assay of S.T.H. Pre-

liminary investigations have shown that, under suitable experimental conditions, S.T.H. causes marked preputial-gland enlargement within 48 hours. Perhaps the sensitivity of this test might be further augmented by topical application of S.T.H. We mention these preliminary findings only because of the urgent need for a clinically applicable sensitive bio-assay method for the estimation of S.T.H. in biological fluids. Perhaps they will stimulate others to explore such possibilities.

Discussion

The principal outcome of these experiments was to show that the mineralo-corticoid-like actions of L.A.P. can be reproduced by electrophoretically pure S.T.H. They also demonstrate that under suitable experimental conditions the concurrent administration of cortisone inhibits these effects of S.T.H., perhaps because it depresses A.C.T.H. secretion by the animal's own pituitary.

It had long been known that certain impure pituitary extracts contain a principle, "the L.A.P.-factor" or the "X-factor," which exerts mineralo-corticoid actions, presumably because it induces the adrenal cortex to secrete D.C.A.-like steroids. It was obvious that this factor is not the commonly known corticotrophic hormone A.C.T.H., because the latter produces gluco-corticoid actions by inducing the adrenal to secrete predominantly cortisone-like (or "compound F"-like) substances. The experiments reported here suggest that this X-factor is identical with S.T.H. In a certain sense, therefore, one might consider S.T.H. to be "mineralo-corticotropic," just as A.C.T.H. is "gluco-corticotropic." Yet there seems to be an essential difference between the mechanism of action of S.T.H. and A.C.T.H., in that the latter maintains adrenal weight even in the hypophysectomized animals, while S.T.H. fails to do so (Simpson *et al.*, 1949).

It will be recalled, however, that, on the basis of indirect evidence, we predicted that the "X-factor" in L.A.P. could exert its mineralo-corticotropic actions only in the simultaneous presence of A.C.T.H. (Selye, 1950b). Thus, A.C.T.H. would be required not only for the secretion of gluco-corticoids but also for the maintenance of the adrenal cortex in a condition of responsiveness to S.T.H. In this event, when A.C.T.H. is eliminated, either by hypophysectomy or by the administration of adequate amounts of cortisone, then S.T.H. of course could not exert its mineralo-corticotropic effects. The above interpretation is all the more probable since some of our unpublished data showed that A.C.T.H. injections increase the ability of S.T.H. to produce cardiovascular and renal damage with severe polyuria. (Incidentally, this is one of the few respects in which A.C.T.H. and cortisone exhibit diametrically opposite effects.)

Another fundamental difference between the actions of the two pituitary principles is that A.C.T.H. does not appear to produce any direct actions; hence it is ineffective in the absence of the adrenals. On the other hand, S.T.H. stimulates somatic growth even after adrenalectomy (Emery and Gottsch, 1941). It may be added that in the experiments reported here we often noted the development of fibroma-like connective-tissue proliferations at the site of S.T.H. injections. Preliminary observations (unpublished) showed, furthermore, that the injection of S.T.H. into the joint region (using our technique for the production of topical irritation arthritis) causes very extensive granuloma formation in the peri-

articular structures. Here we are obviously also dealing with a direct effect of S.T.H. Perhaps the development of experimental replicas of the so-called "collagen diseases" under the influence of S.T.H. overdosage is dependent upon a dual mechanism—namely, the production of mineralo-corticoids by the adrenals and the direct stimulation of connective-tissue proliferation by the S.T.H. itself.

It is probably because of this dual action that S.T.H. overdosage does not merely copy the manifestations of D.C.A. intoxication. Large doses of S.T.H. reproduce the effects of excessive D.C.A. administration in the cardiovascular system and the kidney, but the syndrome produced by S.T.H. intoxication is invariably also accompanied by the specific actions of the latter hormone upon somatic growth in general, the spleen, the liver, etc. These latter changes cannot be duplicated by D.C.A. Presumably, S.T.H. stimulates the proliferation of connective tissue and its ability to respond with granuloma formation upon local injury, both by the production of mineralo-corticoids and by some of its direct peripheral effects. Through this peripheral action S.T.H. presumably sensitizes the connective tissues to the actions of the mineralo-corticoids discharged by the adrenal cortex under the influence of this same pituitary hormone.

We have seen that the cardiovascular and renal damage caused by S.T.H. intoxication is prevented by cortisone only if the latter hormone succeeds in producing a pronounced adrenocortical atrophy. This agrees with the view that the above-mentioned toxic actions of S.T.H. are mediated through the adrenal cortex. Conversely, under experimental conditions which prevent the production of adrenocortical atrophy by cortisone, the toxicity of S.T.H. is further aggravated by this steroid. This agrees with our previous finding that the nephrotoxic effects of mineralo-corticoids (e.g., D.C.A.) are likewise enhanced by cortisone.

In other respects the interaction is less predictable between exogenous (e.g., injected D.C.A. or desoxo-cortisone) and endogenous mineralo-corticoids (e.g., those produced under the influence of S.T.H.) on the one hand, and exogenous (e.g., injected cortisone) or endogenous gluco-corticoids (e.g., those produced under the influence of A.C.T.H.) on the other hand. The production of mesenteric periarteritis nodosa by mineralo-corticoids is readily inhibited by gluco-corticoids, but the accompanying cardiac and pancreatic periarteritis nodosa may be prevented or aggravated by them, depending upon conditions (Selye, 1950b). L.A.P. (which contains both S.T.H. and A.C.T.H.) regularly causes marked renal damage, while cardiac lesions and pancreatic periarteritis nodosa are less common and mesenteric periarteritis nodosa is hardly ever observed. The resulting syndrome corresponds closely to that obtained by concurrent treatment with D.C.A. and cortisone.

In animals simultaneously injected with L.A.P. and cortisone the above-mentioned lesions are particularly severe, presumably because the A.C.T.H. contained in the L.A.P. prevents the compensatory adrenal atrophy normally elicited by cortisone; hence only the detrimental extra-adrenal synergism between the two preparations can manifest itself (Selye, 1951a). A similar aggravation of the L.A.P. effect is obtained by simultaneous treatment with pure A.C.T.H. (Selye, 1951b), probably for similar reasons. In both these cases the adrenal cortex is well developed and presumably maintains its responsiveness to S.T.H.

Electrophoretically pure S.T.H. is not yet abundantly available. However, it has been possible to show in the rat that the malignant nephrosclerosis and myocarditis normally produced by conjoint treatment with L.A.P. and cortisone can be completely prevented by adrenalectomy. As cortisone does not produce such renal and cardiac lesions either in intact or in adrenalectomized animals, it is probable that the active principle of L.A.P. (presumably S.T.H.) exerts its cardiotoxic and nephrotoxic effects through (or at least under the influence of) the adrenals.

It is particularly significant that adrenalectomized animals maintained on cortisone are highly resistant to normally lethal doses of L.A.P. In one such experiment two groups of rats were treated with cortisone and L.A.P. simultaneously; one group was adrenalectomized, the other not. The rats in possession of their adrenals died from nephrosclerosis and myocarditis when their adrenalectomized partners still survived in good condition. Here we are faced with a rather unusual situation in that adrenalectomy preserves life (Selye, 1951c, in press). This experiment will be repeated, using pure S.T.H. instead of L.A.P., in order to make certain that, in this respect also, the former is the active principle of the latter. It is already obvious, however, that some factor present in our anterior pituitary preparation exerts its fatal effects only in the presence of the adrenal cortex, presumably through the liberation or activation of D.C.A.-like compounds.

One additional pertinent fact must be considered. In a hitherto unpublished experimental series, pure S.T.H. and D.C.A. were administered, either singly or in combination, to rats sensitized to mineralo-corticoid actions (by unilateral nephrectomy and a high NaCl intake). Here concurrent administration of S.T.H. and D.C.A. produced fatal nephrosclerosis and myocarditis much more rapidly than either of the hormones given alone. Thus there is an obvious synergism between S.T.H. and the mineralo-corticoid substance D.C.A.

Conclusions

Under suitable experimental conditions, electrophoretically pure somatotrophic hormone (S.T.H.) produces cardiovascular and renal changes quite similar to those elicited by toxic doses of mineralo-corticoids.

Cortisone in doses capable of producing adrenocortical atrophy, or surgical removal of the adrenal glands, prevents the above-mentioned lesions, which are normally elicited by pure S.T.H. or by S.T.H.-containing anterior pituitary preparations. On the other hand, both A.C.T.H. and D.C.A. aggravate these S.T.H. actions.

As regards the mechanism through which S.T.H. could exert its toxic effects upon the cardiovascular system and the kidney, the following possibilities come to mind:

1. A.C.T.H. is a gluco-corticotrophic hormone—that is, one which stimulates the adrenal to produce gluco-corticoids—and S.T.H. possesses mineralo-corticotrophic effects. However, while A.C.T.H. can maintain the integrity of the adrenal cortex—for example, even after hypophysectomy—S.T.H. fails to do so. Hence the latter cannot elicit its potential mineralo-corticotrophic actions after the adrenal cortex has become atrophic owing either to cortisone overdosage or to hypophysectomy.

2. The secretion of mineralo-corticoids by the adrenal cortex is independent of hypophysial regulation. However, owing to a peripheral synergism, the mineralo-corticoids (such as D.C.A.) sensitize, while the gluco-corticoids—for

Further experiments are now in progress to verify the correctness of these three possibilities, but at the present time the third hypothesis seems to be most readily compatible with all the known facts. It is illustrated by the accompanying diagram.

Schematic Diagram Illustrating the Principal Interrelations Between the Hypophysis, the Adrenal Cortex, and the Peripitellar Target Organs During the General-adaptation Syndrome.—The *stressor* (trauma, infection, burns, etc.), acting upon the cells, evokes a stimulus which induces the anterior pituitary to produce A.C.T.H.; in certain circumstances it may also cause a discharge of S.T.H. The nature of this first mediator between the directly injured organ and the anterior pituitary is not yet known (humoral, nervous?). Hence here it is indicated merely by an interrupted line, labelled with a question mark. A.C.T.H. induces the adrenal cortex to produce predominantly *gluco-corticoid* compounds, whose effect upon the response of the various target organs is generally inhibitory—for example, catabolism, diminution of granuloma formation and of allergic responses. Conversely, S.T.H. enhances a variety of defensive reactions in the target organs—for example, anabolism, augmentation of granuloma formation and of allergic responses—primarily by stimulating the connective tissue. Part of this action is undoubtedly not mediated through the adrenal cortex, but this direct effect sensitizes the connective-tissue elements to the—essentially

It is assumed that S.T.H. is the active principle responsible for the toxic actions of L.A.P. upon the kidney and the

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When they met last September, the Expert Committee on Tuberculosis of the World Health Organization drew attention to the value of routine chest examinations in general hospitals as a way of detecting unsuspected cases of tuberculosis. They quoted experience in the United States, where 247 general hospitals are now taking x-ray pictures of all out-patients, admissions, and hospital staff. In one hospital in New York State, for example, where a total of 7,187 patients (74%) were radiographed, 290 (4%) showed signs of tuberculosis. At the Chicago University dispensaries 15,000 people have been examined in this way since the start of the scheme in 1942, and again over 4% of them showed tuberculous lesions. From this and other studies in Chicago—for example, a survey of 25,000 negroes—it has been suggested that there are in the U.S. something like 600,000 patients undergoing medical treatment for other complaints while they have active undiagnosed tuberculosis. The Committee recommends that general hospitals should take up this challenge.

HANS SELYE: ROLE OF SOMATOTROPHIC HORMONE

FIG. 1.—Kidney of control rat (group I). Note normal appearance of glomeruli and tubules.

FIG. 2.—Kidney of S.T.H. (Li)-treated rat (group II). Intense hyalinization of some glomeruli; distension of Bowman's capsules and some tubules. Arrow indicates markedly hyalinized and almost occluded afferent glomerular arteriole.

FIG. 3.—Kidney of cortisone-treated rat (group III). Extraordinary hyperaemia of glomeruli, with dilatation of glomerular capillaries. Arterial system and tubules not affected by cortisone.

FIG. 4.—Kidney of rat treated with S.T.H. (Li) and cortisone (group IV). Glomerular and tubular changes even more pronounced here than in animal illustrated in Fig. 2.

FIG. 5.—Kidney of S.T.H. (Armour)-treated rat (group V). Changes are essentially similar to those produced with S.T.H. (Li), though their frequency in this group was considerably less.

FIG. 6.—Kidney of rat treated with S.T.H. (Armour) and cortisone (group VI). Nephrosclerotic and nephritic changes completely suppressed, though glomerular hyperaemia characteristic of cortisone is detectable.

FIG. 7.—Insertion of mitral valve in rat treated with S.T.H. (Li) and cortisone (group IV). Infiltration of valve by inflammatory cells and deposition of fibrinous material at site of an endothelial defect.

FIG. 8.—Arteriole from pancreas of rat treated with S.T.H. (Li) (group II). Hyaline necrosis of the vessel wall with proliferation of typical granuloma tissue.

FIG. 9.—Adrenal of rat treated with S.T.H. (Li) (group II). Extraordinary predominance of small lymphoid elements within the cortical sinusoids.

FIG. 10.—Another region from the adrenal shown in Fig. 9. Note appearance of lymphoid and myeloid elements between the cortical cells.

FIG. 11.—Adrenal medulla from a rat treated with S.T.H. (Li) (group II). Hypertrophy of several typical cortical cells (indicated by arrows) interspaced between the medullary elements.

FIG. 12.—Many large megakaryocytes in spleen of rat treated with S.T.H. (Li) (group II). There were also many eosinophilic granulocytes in the spleen, but they are not readily distinguishable here.