EFFECTS OF ADRENOCORTICOTROPHIC AND SOMATOTROPHIC HORMONES OF THE HYPOPHYSIS AND OF CORTISONE ON CERTAIN ASPECTS OF NITROGEN METABOLISM IN EXPERIMENTAL TOXIC HEPATITIS*

S. M. Leites and G. T. Pavlov

From the Department of Pathophysiology (Director – Prof. S. M. Leites)
All-Union Institute of Experimental Endocrinology
(Director – Prof. E. A. Vasiukova), Moscow

(Received January 24, 1958. Presented by V. N. Chernigovskii, Active Member AMN SSSR)

It was found by one of us [3] that in disturbances of protein metabolism involving increased urinary output of nitrogen and intensification of hepatic proteolysis (experimental thyrotoxicosis, febrile states), the administration of adrenocorticotrophic hormone (ACTH), at certain dosage levels, and for certain periods of time, led to a fall in the urinary output of nitrogenous metabolites and in the intensity of the proteolytic process, i.e., it exerted an adaptive, so-called normalizing, effect [2]. For further development of this investigation we thought it necessary to ascertain whether ACTH exerts a similar effect in experimental toxic hepatitis, which is also associated with disturbances in the protein metabolism of the liver, expressed as enhanced breakdown of protein, with reduction of its synthesis. In view, also, of the known activating effect of somatotrophic hormone (STH) on protein synthesis [4], we thought it desirable to investigate its action under conditions of hepatic pathology.

The study of these questions is of some clinical importance, since it may provide data for the formulation of indications or counter-indications for the use of ACTH and somatotrophic hormone in patients suffering from liver disease.

EXPERIMENTAL METHOD

The experimental animals were white rats, weighing from 200 to 250 g. Experimental toxic hepatitis was induced by subcutaneous injection of 0.3 – 0.4 ml of CCl_4 per 100 g body weight (3 injections, given every other day). The animals were killed on the 3rd day after the last injection of CCl_4, i.e., on the 7th day from the 1st injection. Fatty degeneration of the liver was found in all the animals (fat content raised by 70-120% above normal). In the first series of experiments, daily subcutaneous injections of prolonged action ACTH (of Danish manufacture) were given together with the CCl_4, for 6 days, at dosage levels of 2 and 6 units. In the second series cortisone acetate (Roussel, Paris) was given instead of ACTH, in doses of 1 and 3 mg. In the third series of experiments we gave subcutaneous injections of somatotrophic hormone, prepared in the experimental manufacturing laboratory of our Institute, and assayed by the tibial growth test in the Department of Experimental Biology. The somatotrophic hormone was administered for 5 days, in daily doses of 1, 2, and 3 mg. The daily dose was divided into 2 parts, injected morning and evening.

We determined the protein content of the liver (from its nitrogen content), and we followed the incorporation of S-labelled methionine into the tissues and proteins of the liver of normal and CCl_4-poisoned animals, and of the latter during administration of ACTH and cortisone; the same was done for the liver, muscle, and kidney proteins of rats receiving STH. S^{35}-Methionine was injected intraperitoneally 24 hours before killing the animals, at dosage levels of 5000 – 6000 cpm per g body weight. Weighed portions of liver, muscle,

*Read at a meeting of the All-Union Institute of Experimental Endocrinology, March 19, 1957.

or kidney were homogenized, and were spread in a thick layer on aluminum sample pans, for activity measurements by means of an end-window counter. Preliminary experiments designed to construct the self-absorption curve for the tissues showed that in computing the results of measurements of thick samples the basis should be the activity of 12 mg of fresh tissue. Liver proteins were isolated by grinding the tissue with 10% trichloroacetic acid, filtering, washing the precipitate three times with the same solution, and then three times with methanol and ether. The product was then dried in a desiccator, and the activity of 50 mg portions was determined, in which case the thickness of the sample corresponded with 15 mg of protein, and this was taken into account in the calculations. The rate of incorporation of labelled atoms into the protein was expressed as percentages of radioactivity, in cpm in relation to cpm per g body weight, which was taken as 100%. Since the rate of incorporation of S^{35} into the proteins depended both on the rate of its supply to the liver cells and on the intensity of the process of its incorporation, we excluded the effect of the former factor (permeability changes) by calculating the relative activity of the protein from the following formula, proposed by D. E. Grodzenskii:

$$\frac{\text{activity of protein from 1 g of tissue (cpm)}}{\text{activity of 1 g of tissue (cpm)}}$$

The ratio provides a more correct assessment of the actual rate of incoporation of S^{35} into protein.

EXPERIMENTAL RESULTS

As is evident from the data presented in Table 1, administration of CCl_4, in doses of 0.3 ml per 100 g body weight given 3 times, every other day, leads to a fall in the protein content of the liver and in the rate of incorporation of methionine-S^{35} into its proteins. When 2 units daily of prolonged action ACTH were administered at the same time as the CCl_4 the protein content of the liver rose, as did the rate of incorporation of methionine-S^{35}. This effect was also seen with doses of 10 units of ACTH, although at this dosage level the protein content of the liver was not raised. Hence ACTH, at a certain dosage level (2 units), inhibits proteolytic processes proceeding in toxic hepatitis. At higher dosage levels (10 units), ACTH does not affect the protein content, although it still activates incorporation of methionine-S^{35} into hepatic proteins. It appears that large doses of ACTH stimulate both the processes of synthesis and of breakdown of protein, so that the resultant protein content is unchanged.

In the second group of experiments (Table 1, Group 6) we administered ACTH for 5 days, beginning with the day on which CCl_4 injection was discontinued, i.e., during the period of regeneration. The protein content of the liver of the control group (Group 5) remained low over this period, as did the relative activity of the protein, pointing to continuing inhibition of protein synthesis.* Under these experimental conditions, ACTH causes a rise in the relative activity of the protein, and slightly increases its content in the liver. In normal animals administration of 10 units of ACTH for 6 days causes a fall in the percentage incorporation of methionine-S^{35} into hepatic proteins, and in their relative activity.

Thus, at a given dosage level, ACTH retarded incorporation of methionine into the liver proteins of normal animals, but had the opposite effect in animals suffering from disturbances of assimilatory processes, as in toxic hepatitis. As in our previous work on thyrotoxicosis and febrile states, ACTH (at a certain dosage level) exerts an adaptive, "normalizing" effect in animals in which catabolic processes prevail in the protein metabolism of the liver, such as in CCl_4 poisoning.

When smaller doses of CCl_4 are administered (0.2 ml; 3 doses at 48 hr-intervals) the rate of incorporation of labelled methionine falls within the same limits as for normal animals, and in such animals the rate of incorporation into hepatic proteins is lowered by ACTH.

We next examined the effect of adrenal glucocorticoids, in order to ascertain to what extent the effect of ACTH on incorporation of methionine into liver proteins is due to stimulation of the adrenal cortex. We first administered cortisone to normal animals.

*Increase in percentage incorporation of S^{35} into the proteins, with a fall in their relative activity, shows that the former effect is associated with intensification of entry of the labelled atoms into the tissue, but not with increase in the rate of their incorporation into the proteins.

TABLE 1

Effect of ACTH on the Protein Content of Liver and on Incorporation into the Proteins of Methionine-S^{35} in Normal Rats and in Experimental Toxic Hepatitis

Group	Nature of treatment	Protein content %	Percentage incorporation of S^{35} into proteins	Relative activity	No. of animals
1	Control, normal	21,0	8,95 (8,0—9,4)	0,54 (0,43—0,62)	12
2	CCl_4; 3 doses of 0.3 ml CCl_4 every other day	18,9	6,3 (5,3—6,7)	0,36 (0,29—0,46)	14
3	CCl_4, 0.3 ml every second day + ACTH, 2 units daily	20,0	7,07 (4,3—9,4)	0,49 (0,40—0,60)	10
4	CCl_4, 0.3 ml every second day (3 doses) + ACTH, 10 units daily	18,8	7,74 (5,9—9,0)	0,47 (0,29—0,60)	14
5	CCl_4, 0.3 ml, as before (3 doses); rats killed 5 days after the last injection	16,9	8,3 (7,3—9,9)	0,35 (0,34—0,38)	7
6	The same, + ACTH, 10 units for 5 days after the last injection of CCl_4	17,3	8,1 (6,9—7,7)	0,44 (0,36—0,52)	8
7	ACTH, 10 units daily for 6 days	21,2	7,65 (7,1—8,2)	0,39 (0,37—0,42)	6
8	CCl_4, 0.2 ml, 3 doses, given (every second day)	21,0	7,3 (5,3—9,1)	0,55 (0,52—0,58)	9
9	CCl_4, 3 doses of 0.2 ml, given every second day + ACTH, 10 units daily	20,5	5,6 (4,6—6,6)	0,46 (0,43—0,48)	9

Note: Mean values, range shown in parentheses.

It appears from the data of Table 2 that administration of cortisone to normal rats, at a rate of 1 mg daily for 6 days, leads in most cases to lowering of the protein content of the liver, and of its relative activity, while percentage inclusion of labelled methionine remains at the normal level. These effects can be interpreted as giving evidence of an increase in the rate of entry of methionine into the hepatic tissues. As has been shown above, the actual rate of incorporation of labelled methionine into liver proteins is given by the relative activity. Daily injection of 3 mg of cortisone acetate for 6 days lowers both the relative activity of the liver proteins and the percentage incorporation of labelled methionine into the proteins.

M. B. Lebedeva [1] administered cortisone in dosages of 5-7.5 mg for 2 days, and found that the rate of incorporation of glycine-1-C^{14} into liver proteins was raised for 1-6 hours after the injections. When cortisone was given in daily doses of 2.5 mg for 6 days, and of 5 mg on the 7th day, and the animals were killed 6 hours after administration of glycine, she found that the rate of incorporation of glycine into liver proteins was lowered, as was the total nitrogen content of the liver; these results are similar to those found by us in the study of the effect of injecting cortisone acetate, at dosage levels of 3 mg per diem daily for 6 days, on the rate of incorporation of methionine-S^{35} into liver proteins, 24 hours after its intraperitoneal injection.

Injection of cortisone (1 mg daily for 6 days) simultaneously with injection of CCl_4 raised the rate of incorporation of methionine-S^{35} into liver proteins (Table 2), i.e., it had the same effect as ACTH. There was, however, no increase in the protein content of the liver, showing that protein anabolic processes were balanced by catabolic ones. At higher dosage rates of cortisone (3 mg daily) we found a fall in the protein content of liver, and of percentage incorporation into the proteins of methionine; the relative activity did not rise. It thus appears that relatively high doses of cortisone (in contrast to small ones) did not stimulate protein anabolic processes in toxic hepatitis.

Injection of somatotrophic hormone at the same time as CCl_4, in doses of 1, 2, and 3 mg daily for 5 days (Table 3), prevents the halt in increase of body weight found in the control animals. The protein content of the liver rose slightly at STH dosages of 1 and 2 mg, but did not change at dosage levels of 3 mg. Percentage incorporation of methionine-S^{35} into liver proteins, and their relative activity, rose slightly after administration of STH at a dosage level of 2 mg*, but showed no change at dosage levels of 1 and 3 mg. We could find no changes in the protein content of muscle, percentage incorporation therein of methionine, or in the relative activity of the proteins, after administration of STH. The protein content of kidney tissue, the percentage incorporation therein of labelled methionine, and the relative activity of the proteins rose at dosage levels of 1 mg of STH; at a dosage level of 2 mg the protein content rose somewhat, while percentage incorporation of methionine and the relative activity of the proteins remained unchanged. At a dosage level of 3 mg of STH both the protein content of the kidneys, and the relative activity of the proteins fell.

TABLE 2

Effect of Cortisone on the Protein Content of Liver, and on the Incorporation into Liver Proteins of Methionine-S^{35} in Normal Rats and in Experimental Toxic Hepatitis

Group	Nature of treatment	No. of animals	Body weight (g)		Protein content (%)	Percentage incorporation of S^{35} into proteins	Relative activity
			initial	final			
1	Control, normal	12	—	—	21,0	8,95 (8,0—9,4)	0,54 (0,43—0,62)
2	Cortisone, 1 mg daily for 6 days	10	234	237	19,0	8,70 (6,5—10,8)	0,44 (0,34—0,54)
3	Cortisone, 3 mg daily for 6 days	12	221	215	20,0	6,5 (5,0—7,7)	0,47 (0,34—0,54)
4	CCl_4, 0.3 ml, 3 doses given every other day	14	225	214	18,9	6,3 (5,3—6,7)	0,36 (0,29—0,46)
5	The same, + cortisone, 1 mg daily for 6 days	8	237	224	19,0	8,2 (6,3—10,4)	0,44 (0,40—0,49)
6	CCl_4, 0.3 ml (3 doses, given every other day) + cortisone, 3 mg daily for 6 days	12	220	202	17,8	4,6 (3,1—6,4)	0,34 (0,28—0,41)

Note: Mean values; range shown in parentheses.

It thus appears that in experimental toxic hepatitis the effects produced by our STH preparation on protein anabolism vary, according to the dose given and to the organ examined. At a dosage level of 2 mg STH exerts an activating effect on protein synthesis in the liver; this effect is seen with doses of 1 mg in the kidneys. A dosage level of 3 mg even lowers the protein content of the kidney. This effect might possibly be due to the presence of thyrotrophic hormone in our preparation, which could have an appreciable action at the higher dosage levels taken by us. The STH preparations used by us had no effect on protein synthesis in muscle. It should be noted that in those cases in which STH administration had no effect on the percentage content of protein in the liver, muscles, and kidneys, its absolute amount was increased, owing to increase in weight of the organs and tissues under examination.

* At this dosage level of STH we have observed some activation of protein synthesis in the liver of normal animals.

TABLE 3

Effect of Pituitary Somatotrophic Hormone (STH) on the Protein Content of the Liver, Muscles, and Kidneys, and on Incorporation of Methionine-S^{35} into the Proteins (mean values)

Nature of treatment	No. of animals	Body weight (g)			Liver			Muscles			Kidneys		
		initial	final	difference	proteins (%)	% incorporation of S^{35} into proteins	relative activity	proteins (%)	% incorporation of S^{35} into proteins	relative activity	proteins (%)	% incorporation of S^{35} into proteins	relative activity
CCl_4, 3 doses of 0.3 ml, every other day	14	219	215	—4	17,9	6,42	0,31	19,3	1,80	0,25	15,9	12,0	0,32
CCl_4, 3 doses of 0.3 ml every other day + STH, 1 mg daily for 5 days	14	216	228	+12	19,4	6,35	0,32	20,0	1,73	0,26	16,8	16,0	0,44
CCl_4, 3 doses of 0.4 ml every other day	13	210	198	—12	18,9	6,13	0,26 $m \pm 0,015$	20,2	1,74	0,30	16,7	13,1	0,35
CCl_4, 3 doses of 0.4 ml every other day + STH, 2 mg daily for 5 days	13	212	216	+4	19,9	7,0	0,33 $m \pm 0,016$ $t=3,7$	20,7	1,78	0,30	17,8	13,8	0,36
CCl_4, 3 doses of 0.4 ml every other day	8	205	203	—2	17,4	4,91	0,24	18,0	1,47	0,20-	15,7	12,3	0,32
CCl_4, 3 doses of 0.4 ml every other day + STH, 2 mg daily for 5 days	7	205	213	+8	17,7	5,10	0,26	18,7	1,40	0,24	13,9	12,2	0,26

SUMMARY

In experimental toxic hepatitis of rats caused by administration of CCl_4 a fall is found in the protein content of the liver, and in the rate of incorporation of methionine-S^{35} into these proteins. When prolonged action ACTH (2-10 minutes) or cortisone (1 mg) are injected at the same time as the CCl_4 the rate of incorporation of methionine-S^{35} is raised. Higher doses (3 mg) of cortisone had the opposite effect. Administration of somatotrophic hormone prevents loss of weight of CCl_4-poisoned rats. Only a small rise in the protein content of the liver, and in the rate of incorporation of methionine-S^{35} into the proteins were found at a dosage level of 2 mg of STH. The protein content of the kidneys, and the rate of incorporation of methionine-S^{35} are increased with dosage levels of 1 mg of STH. At a dosage level of 3 mg a fall in the protein content of the kidneys was found. Administration of STH did not affect the protein content of muscles, or the rate of incorporation of methionine-S^{35} into the muscle proteins.

LITERATURE CITED

[1] M. B. Lebedeva, Voprosy Med. Khim. No. 4, 278-286 (1956).

[2] S. M. Leites, Arkhiv Patol. No. 6, 45-52 (1956).

[3] G. T. Pavlov, Proceedings of the Combined Session of the All-Union and Ukrainian Institutes of Experimental Endocrinology, pp. 75-76 (Moscow, 1954); Proceedings of the Scientific Meeting of the All-Union Institute of Exp. Endocrinol. devoted to Discussion of Hypophyseal – Corticoadrenal Problems, pp. 39-41 (Moscow, 1956).

[4] B. Ketterer, P. J. Randll and F. G. Young, Ergebnisse der Physiologie, Berlin, 1957, Bd. 49, S. 127-211.