INCREASED TRANSAMINASE ACTIVITY IN THE LIVER AFTER ADMINISTRATION OF CORTISONE

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It has been shown by several authors that cortisone may act on the transaminase activities of various organs. EISCHEID AND KOCHAKIAN[1] have observed, in the rat, that the subcutaneous implant of cortisone induces, beginning from the seventh day, an increase of glutamic oxalacetic transaminase (GOT) activity in the kidney and heart together with a simultaneous decrease of the same activity in the liver.

The work of RINDI[2] in this field has resulted in somewhat different conclusions: he has been able to show an increased GOT activity in the liver of the rat after prolonged administration of cortisone. This effect, constantly found in the intact animal, was no longer observed if the adrenal glands had been previously removed.

Prednisone, one of the new cortisone derivatives, was employed by FERRARI AND TENCONI[3]: under the action of the hormone the GOT activity of the rat liver did not show any change, while the glutamic pyruvic transaminase activity (GPT) increased significantly.

From these researches, it can be seen that the modifications induced by cortisone on transaminase activities may differ in the various organs: a diminution of these enzymic activities can be found in the liver with a simultaneous increase of kidney and heart activity. In this connection it should be recalled that the liver, heart and kidney are the organs with the highest transaminase activity (AWAPARA AND SEALE[4]; CAMMARATA AND COHEN[5]).

The different behaviour of the various organs under cortisone stimulation suggests that the action of the hormone on the enzymes concerned is a rather complex one; furthermore, it can be argued that a most important role must be played by the metabolic characteristics of the different tissues. On the other hand, it is well known that cortisone, under certain circumstances, may influence the growth of various tissues, both normal (MORSIANI AND LUCCI[6]; ROMANI[7]) and pathological (DUSTIN[8]; HIGGINS AND BENNET[9]; LANNER[10]). A favorable action of the hormone on certain types of human leukemia has also been recently demonstrated (BURCHENAL *et al.*[11]; DAMESHEK[12]).

However, to our knowledge, no study has as yet been published on the action of cortisone upon the transaminase activities of proliferating liver tissue. It appeared to us, therefore, that a study of this kind might be of some interest.

As a model of proliferating tissue, the regenerating rat liver was chosen, this material being particularly rich in transaminase activities.

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METHODS AND MALERIALS

This investigation was carried out on 68 albino Wistar rats, weighing 180–200 g and fed on a free diet. Hepatectomy was performed according to the method of HIGGINS AND ANDERSON[13]. Cortisone was injected intramuscularly two hours after laparotomy, and the injections were repeated daily. Two different dosages of cortisone were used: the first group of 13 rats was given a therapeutic daily dose of 5 mg/kg body weight. Five of these rats were sacrificed on the third day and 8 on the tenth day after administration of the drug. The second group of 11 rats received a toxic* daily dosage of 120 mg/kg body weight; these animals were sacrificed the third day after laparotomy.

As a control group, 24 rats were submitted to partial hepatectomy, but not treated with cortisone.

To a third group of 10 rats, the hormone was given for 3 days either at therapeutic (6 rats) or toxic (4 rats) dosages, without previous hepatectomy. Ten untreated rats were used as control for this last group.

When the technique of HIGGINS AND ANDERSON[13] is used, the amount of liver removed (median and left lateral hepatic lobes) is equal to two thirds of the entire organ; the amount of liver regenerated during the period of the experiment can therefore be calculated as follows: amount of liver at end of experiment minus ½ the amount removed.

The regeneration index can be expressed by the following formula:

$$\% \text{ regeneration} = \frac{\text{regenerated amount}}{\text{removed amount}} \times 100$$

This formula allows a fairly accurate estimate of the degree of regeneration and is not influenced by the possible variations of the weight of the organ in the different animals.

Fragments of the organ, washed and filtered through gauze in order to remove connective and vascular tissue, were transferred in a Potter Elvehjem homogenizer in bidistilled water and then homogenized for 5 min at 1500 r.p.m. A nuclear count was performed on other liver fragments previously homogenized for 3 min at 1500 r.p.m. in 0.77 *M* saccharose solution. A 0.03 % solution of methyl-green was employed for diluting and a Burker chamber was used for counting (ULTMANN *et al.*[14]).

For the determination of GOT activity, the method of KARMEN[15] was adapted to liver tissue. In this method, oxalacetate formed in the reaction

$$\text{aspartic acid} + \alpha\text{-ketoglutaric acid} \rightleftarrows \text{glutamic acid} + \text{oxalacetic acid} \qquad (1)$$

is reduced to malate after addition of excess purified malic dehydrogenase and in the presence of reduced DPN. The oxidation of DPN is followed with the spectrophotometer, in the U.V. range, at a wavelength of 340 mμ. The micromolar extinction coefficient of DPNH being known, it is possible to calculate the number of μmoles of keto acid that have been formed. GOT activity is therefore expressed as μmoles oxalacetate formed/hour/mg fresh liver.

For the determination of GPT activity, the method of HENLEY *et al.*[16] was used. This method is based upon the interaction of pyruvate formed in the transamination reaction

$$\text{alanine} + \alpha\text{-ketoglutaric acid} \rightleftarrows \text{glutamic acid} + \text{pyruvic acid} \qquad (2)$$

with an excess of lactic dehydrogenase and in presence of DPNH. To determine GOT activity, the μmoles of pyruvate formed per hour and per mg of fresh liver can be easily calculated.

RESULTS

As illustrated in Tables I and II and in Figs. 1 and 2, cortisone constantly induces an increase of transaminase activities, both in normal and regenerating liver. This increase is significantly higher when toxic doses of the hormone are used.

Furthermore, in every group of rats, the percentage increase of GPT activity is larger than for GOT activity; therefore, following treatment with cortisone, the ratio between these two enzymic activities is shifted in favour of the first one.

Owing to the simultaneous diminution of cellularity in the treated animals, the increase of both transaminase activities is even more marked when referred to the

* Although usually considered as "toxic" similar dosages have been recently employed for the treatment of human leukemias.

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number of cells; this is particularly evident in the case of GPT activity the values for which may be found to be about 100% higher than in the controls.

TABLE I

EFFECT OF CORTISONE ON TRANSAMINASE ACTIVITIES OF NORMAL AND REGENERATING RAT LIVER

(Daily dose: 3 mg/kg body wt)

Days after hepatectomy	Control group				Treated group			
	GOT		GPT		GOT		GPT	
	μmoles oxalacetate /hour/mg fresh liver ± σ	10^{-6} μmoles oxalacetate /hour/cell ± σ	μmoles pyruvate /hour/mg fresh liver ± σ	10^{-6} μmoles pyruvate /hour/cell ± σ	μmoles oxalacetate /hour/mg fresh liver ± σ	10^{-6} μmoles oxalacetate /hour/cell ± σ	μmoles pyruvate /hour/mg fresh liver ± σ	10^{-6} μmoles pyruvate /hour/cell ± σ
0 *	3.46 ±0.59	17.3 ±4	0.38 ±0.06	1.9 ±0.16	3.97 ±0.35	20.7 ±2.15	0.50 ±0.14	2.61 ±0.22
3	4.08 ±0.85	27.2 ±6.06	0.30 ±0.04	2 ±0.18	4.61 ±1.2	34.10 ±9	0.43 ±0.09	3.18 ±0.81
10	3.47 ±0.54	15.7 ±2.46	0.37 ±0.1	1.7 ±0.21	4.52 ±0.49	23.9 ±4.3	0.56 ±0.1	2.96 ±0.53

* *i.e.* normal liver.

TABLE II

EFFECT OF CORTISONE ON TRANSAMINASE ACTIVITIES OF NORMAL AND REGENERATING RAT LIVER

(Daily dose: 120 mg/kg body wt)

Days after hepatectomy	Control group				Treated group			
	GOT		GPT		GOT		GPT	
	μmoles oxalacetate /hour/mg fresh liver ± σ	10^{-6} μmoles oxalacetate /hour/cell ± σ	μmoles pyruvate /hour/mg fresh liver ± σ	10^{-6} μmoles pyruvate /hour/cell ± σ	μmoles oxalacetate /hour/mg fresh liver ± σ	10^{-6} μmoles oxalacetate /hour/cell ± σ	μmoles pyruvate /hour/mg fresh liver ± σ	10^{-6} μmoles pyruvate /hour/cell ± σ
0 *	3.46 ±0.59	17.3 ±2.55	0.38 ±0.06	1.9 ±0.10	5.78 ±0.42	31.24 ±5.2	0.69 ±0.19	3.73 ±0.26
3	3.52 ±0.69	23.4 ±4.9	0.32 ±0.02	2.12 ±0.15	5.34 ±0.83	43 ±5.5	0.51 ±0.09	4 ±0.93

* *i.e.* normal liver.

DISCUSSION

Cortisone administered at therapeutic or toxic dosages does not change substantially the rate of hepatic regeneration as measured by the HIGGINS-ANDERSON method. On the contrary, a diminution of cellularity can be observed after administration of the hormone; this is in keeping with the histological finding of an increase of the cellular volume and of the number of polyploid cells, previously observed by other authors (EINHORN *et al.*[17]; DUNN *et al.*[18]) and confirmed by us on histological liver sections.

After administration of the hormone, transaminase activities of the liver increase

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markedly; this increase is even more conspicuous when referred to the number of cells.

It is known that the main goal of transamination processes consists in the direct conversion of an α-amino acid into the corresponding keto acid without production of ammonia. The resulting keto acid can subsequently be transformed into carbohydrates (gluconeogenesis), into acetate and fat, or it can enter the oxidative cycle. As has been clearly shown in the past years, cortisone increases gluconeogenesis and imposes a negative nitrogen balance upon the organism. Our data suggest that cortisone might produce these effects by enhancing the transamination processes. Furthermore, the finding that GPT activity increases more than GOT activity, may furnish the explanation for the rise in the pyruvic acid level in the blood observed by GITELSON[19] after administration of cortisone.

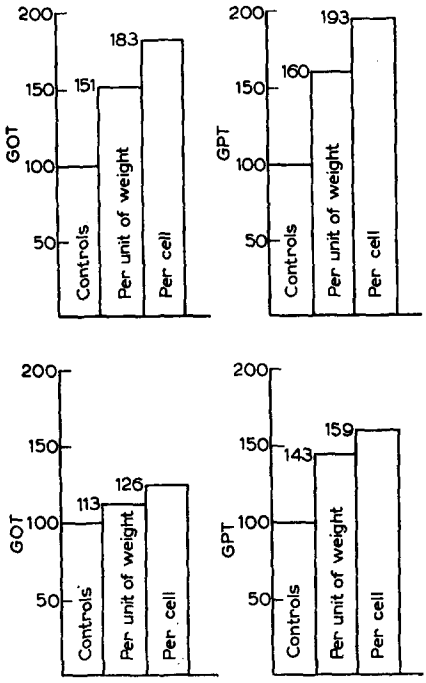

Fig. 1. Percentual changes of GOT activity (left) and GPT activity (right) of regenerating rat liver induced by toxic (top) and therapeutic (bottom) doses of cortisone.

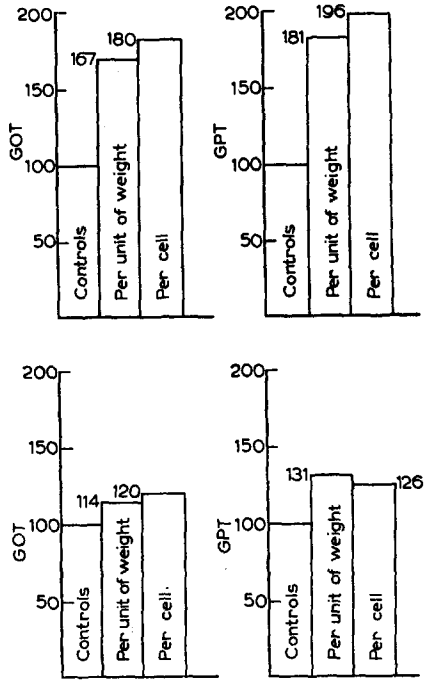

Fig. 2. Percentual changes of GOT activity (left) and GPT activity (right) of normal rat liver induced by toxic (top) and therapeutic (bottom) doses of cortisone.

The increase of transaminase activities is clearly evident both in resting and regenerating liver. It seems likely that the stimulus to regeneration does not modify the cortisone reactivity of the hepatic tissue, at least as far as this section of protein metabolism is concerned: on the other hand, as indicated above, even toxic doses of cortisone fail to influence the rate of regeneration of the hepatic tissue.

SUMMARY

The action of therapeutic and toxic doses of cortisone on the normal and regenerating liver has been studied.

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The drug does not seem to influence the rate of hepatic regeneration, but it does induce, in both types of liver, a significant increase of GOT and GPT activities, and particularly of the latter.

It is suggested that the hormone may enhance gluconeogenesis by activating the transamination processes; in this connection, regenerating liver does not behave differently from normal liver.

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AN HYDRAULIC HOMOGENIZER* FOR THE CONTROLLED RELEASE OF CELLULAR COMPONENTS FROM VARIOUS TISSUES**

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Procedures now available for dispersing tissues yield, as a rule, not only mixtures of cells, nuclei, and mitochondria, but also products of their destruction. An adequate and simple means of controlling the products obtained by tissue homogenization has, so far, not been devised. Ideally, such a procedure should produce only *whole* cells, the *intact* products of whole cells (*i.e.*, nuclei, mitochondria, and microsomes), or the dispersed contents of nuclei plus the other cellular inclusions. In addition, homogenization should be accomplished rapidly, at low temperatures, and should yield metabolically active products. From the standpoint of these requirements, the instrument described here has proved greatly superior to glass homogenizers used in this laboratory.

* Commercially available from Microchemical Specialties Co., 1834 University Avenue, Berkeley 3, California.

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