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THE INFLUENCE OF ADRENALECTOMY AND OF CORTISONE TREATMENT ON ARGINASE AND ESTERASE ACTIVITIES IN LIVER TISSUE

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INTRODUCTION

The effect of hormones on enzymic reactions within the cell represents one of the burning problems of modern biochemistry. While hitherto it was possible in only a few cases to establish an effect of a hormone, added *in vitro* to enzyme preparations, an increasing number of cases has been reported in recent years in which changes in the activity of extracted enzyme systems have been observed after the animals had received hormone treatment; in particular, treatment with adrenal corticoids has been found to be effective in this respect. In slice experiments, the glucose-6-phosphatase level in the liver of animals treated with 17-hydroxycorticosterone was seen to be increased (ASHMORE, HASTINGS, NESBETT AND RENOLD[1]). In other experiments the treatment of rats with cortisone had a considerable effect on the enzymes concerned with the metabolism of tryptophan (BROWN AND BERG[2], KNOX AND AUERBACH[3]). Conversely, the effect of an enzyme (phosphorylase) on the synthesis and the release of corticosteroids has also been claimed (HAYNES AND BERTHET[4]). Adrenalectomy decreases the activities of many, but not of all, enzymes; the levels of succinic dehydrogenase and of various nucleases, for instance, tend to rise after adrenalectomy (STEVENS AND REID[5]), while that of catalase is unaffected (TROOP AND STANLEY[6]).

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All workers in the field agree that the activity of arginase in the organs of adrenalectomized animals is markedly depressed, and this has recently been confirmed by THOMSON AND MOSS[7]. There are, however, discrepancies in the reports on the effect on the level of hepatic arginase following administration of adrenal corticoids to normal and to adrenalectomized animals. The early work of FRAENKEL-CONRAT, SIMPSON AND EVANS[8], who reported a marked activating effect of small doses of cortisone administered to fasting and growing but otherwise normal rats on the hepatic arginase level, stimulated further research under more easily reproducible experimental conditions. On the other hand, injections of cortical extracts had no such effect on the liver arginase under the experimental conditions of KOCHAKIAN AND BARTLETT[9]. As to the ability of adrenal hormones to restore the level of arginase, initially depressed by adrenalectomy, the issue is also controversial (KOCHAKIAN AND VAIL[10]). FOLLEY AND GREENBAUM[11] reported a considerable restoring effect on the hepatic and mammary arginase levels after administration of various adrenal corticoids to adrenalectomized, lactating rats. 11-Desoxycorticosterone, given in large doses, was particularly effective (in contrast to previous findings by other workers[8]); 11-dehydrocorticosterone (compound A) and cortisone were also active. In the opinion of the authors, the degree of efficiency in restoring the arginase level depended on the dietary protein level, a view which is in accordance with earlier claims of LIGHTBODY AND KLEINMAN[12]. FOLLEY AND GREENBAUM emphasise that there may be a link between arginase and the fact that the mammary gland is "notable as being the site of rapid and continuous synthetic processes". It has also been suggested that arginase may play a notable role in the metabolism of fast growing and of malignant tissue (BACH AND LASNITZKI[13], BACH AND SIMON-REUSS[14]) and that the metabolism of arginine appears to be considerably enhanced during the growth of malignant tissue (BACH AND MAW[15]). It is this aspect of the problem which induced the writers to reinvestigate, under easily reproducible conditions, the effect of adrenalectomy and of cortisone on arginase activity. It was also thought desirable that investigations should be carried out on another hydrolytic enzyme, and liver esterase was chosen for the purpose. An attempt was made in this work to reduce as much as possible the scatter of results normally associated with biological experiments. For this purpose, the changes in the arginase and esterase levels in the liver were observed by means of biopsies carried out at intervals, and the results observed by this method showed a considerable degree of consistency.

METHODS

Experimental animals

The experimental animals used were adult (10–20 weeks old) male albino rats of 200–250 g weight. They were fed throughout on standard laboratory diet consisting of rat cake supplied by Aberdeen Flour Millers and provided with ample drinking water (0.9% saline in the case of the adrenalectomised animals); the rats maintained effectively constant body weights throughout the experiments.

Operative techniques

All operations were carried out under ether anaesthesia. For liver biopsy, an incision of about 0.5 cm was made in the abdominal wall, and 30–40 mg of tissue were cut from the edge of the left lateral lobe of the liver. This liver tissue was kept on ice during the short intervening period between removal and homogenisation. For adrenalectomy, the adrenal glands were removed using the standard technique.

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Cortisone treatment

Cortisone injections were made subcutaneously using an aqueous suspension containing 10 mg cortisone acetate/ml. Each injection was adjusted to contain a dose of 2 mg cortisone acetate/100 g body weight. The cortisone acetate was supplied by Organon Laboratories, London.

Determination of enzyme activities

The 30–40 mg portion of liver tissue was carefully blotted to remove surface fluid and weighed on a torsion balance. The tissue was then homogenised in a Potter homogeniser with 2–3 ml aqueous solution containing manganese sulphate ($4 \cdot 10^{-2} M$) and sodium maleate ($1 \cdot 10^{-2} M$) (referred to below as manganese–maleate solution). The homogenate was quantitatively transferred to a 5-ml measuring cylinder and the volume made up with manganese–maleate solution. The homogenate was then centrifuged at 1° C, and the supernatant used for the enzyme estimations.

Estimations of arginase. The arginase unit was defined as that amount of enzyme which in 10 minutes at pH 9.0 and 30° will liberate 1 μl urea from a 50 mM L-arginine hydrochloride solution. 0.2 ml homogenate was incubated with 1.8 ml 0.1 M glycine buffer pH 9.0 for 15 min, after which 0.5 ml 0.25 M L-arginine hydrochloride were added and the mixture incubated for a further 10 min. The enzyme was then inactivated by adding 1.0 ml 3 M acetate buffer pH 4.6, and the urea estimated manometrically according to the method of KREBS AND HENSELEIT[16]. The reagent blank was negligible when fresh arginine solutions were used. The amount of preformed urea in the homogenate was also negligible. Under the conditions described, the reaction is of zero order.

Estimation of esterase. The esterase unit was defined as that amount of enzyme which in 5 min at 30° under the conditions described below will liberate 1 μl CO_2. To 1.0 ml manganese–maleate solution in a Warburg flask were added 0.3 ml 0.09 M sodium bicarbonate and 2.0 ml 0.4% ethyl butyrate. The side-arm contained 0.7 ml of the homogenate. After the mixture had been equilibrated for 20 min at 30°, the homogenate was mixed with the contents of the main compartment, and the amount of CO_2 evolved between 5 and 10 min after mixing was taken as the "5 min period". In each case a blank estimation, replacing the homogenate with manganese–maleate solution, was carried out and found to be negligible. Under these conditions, which appear to be critical, the quantity of CO_2 evolved was shown to be directly proportional to the amount of enzyme present, up to the diffusion limit of the apparatus.

All arginase and esterase estimations were carried out in duplicate, and the average of each pair expressed as units/mg wet weight of liver. Unless stated otherwise, each value shown on the Figures and in Table I represents the average of groups of rats comprising 8–20 animals. Standard errors are given in each case. The "p" values given in the text refer to FISHER'S "t" Test[17].

RESULTS

Effect of liver biopsy on the hepatic arginase level (Fig. 1)

The usefulness of the biopsy technique depended on the degree to which the surgical treatment as such, involved in this method, affected the hepatic arginase level. When the intervals between the biopsies were not less than 3 days, it was found that the changes in the arginase level were comparatively small. Fig. 1 shows the average arginase level of 91 normal rats, unaffected by any previous biopsy, to be 113.0 units/mg wet wt. with a standard error of $\pm$ 1.5. Fig. 1 also shows an experiment with 56 rats which were subjected to biopsies at 3 day intervals, but which were otherwise untreated. The average fall of the arginase level, presumably due to the cumulative effect of the biopsies, was approximately 18% after 12 days, with small standard errors throughout. When the interval between the biopsies was increased to 28 days, a tendency to recovery to normal arginase values was observed.

Effect of adrenalectomy and of cortisone treatment on the hepatic arginase level

In comparison with the above-mentioned slight changes in the arginase levels of the control experiments, a very considerable fall in the arginase level was observed 7 days after adrenalectomy (Fig. 1). The fall of the enzyme level appeared to be

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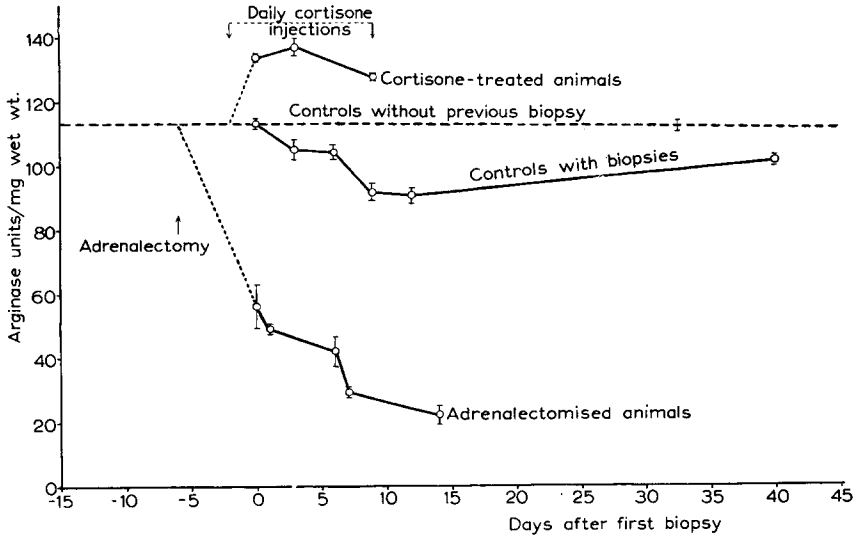

Fig. 1. The effect of adrenalectomy, and cortisone treatment on the level of hepatic arginase. (O = Biopsy; vertical lines indicate standard errors.)

progressive and a comparison of the slope of the control curve with that of the experimental curve indicated that the further decline in the arginase activity after the first biopsy was to some extent due to the adrenalectomy and did not merely reflect the changes in arginase due to repeated biopsies. On the other hand, it was seen that cortisone treatment of normal rats caused a distinct rise in the arginase activity (Fig. 1). The cortisone effect was much more marked when the hormone treatment was applied to adrenalectomised rats as seen from Fig. 2, where in two parallel experiments cortisone treatment was given before and after adrenalectomy. In confirmation of the results of Fig. 1, Fig. 2 also shows that cortisone treatment increased

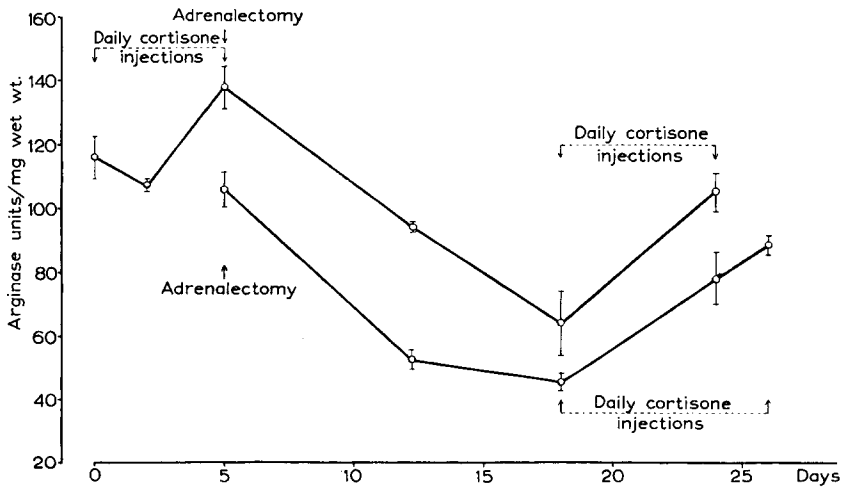

Fig. 2. The effect of a combination of adrenalectomy and cortisone treatment on the level of hepatic arginase. (O = Biopsy; vertical lines indicate standard errors.)

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arginase activity in normal animals, and that adrenalectomy caused a distinct fall. This fall was to a great extent reversed by subsequent cortisone injections.

Effect of adrenalectomy and cortisone treatment on the level of liver esterase

A few similar experiments were carried out with liver esterase (Fig. 3). After five daily cortisone injections during which period the liver esterase levels rose to 123% of the normal level, cortisone treatment ceased and the animals were adrenalectomised. After a further 7 days the average esterase level had fallen to 42% of the normal value. Then cortisone treatment was renewed and, after 7 daily injections, the esterase level rose slightly, which suggested that the rapid decrease following adrenalectomy had been arrested if not actually reversed. The average level of the esterase in the control experiments with, except for biopsy, untreated animals showed little change during the experimental period. While the scatter of values was comparatively small in the cortisone and adrenalectomy experiments, the control experiments showed considerable individual variations.

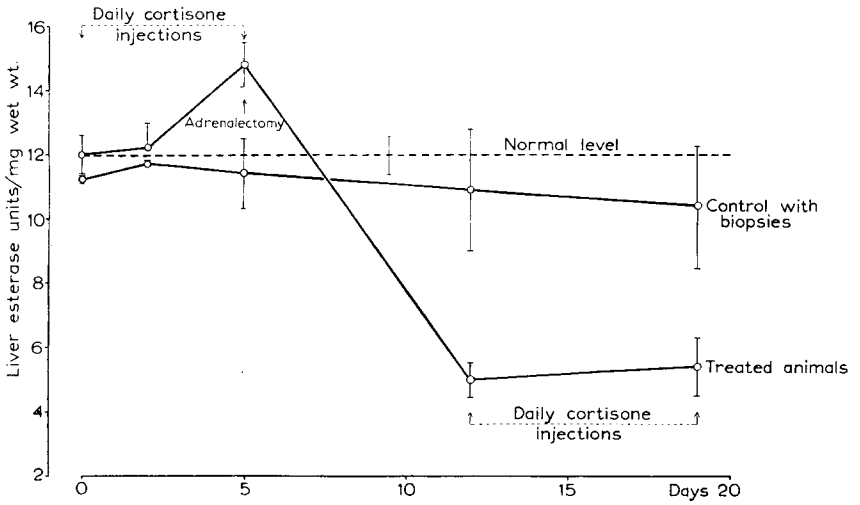

Fig. 3. The effect of a combination of adrenalectomy and cortisone treatment on the level of liver esterase. (O = Biopsy; vertical lines indicate standard errors.)

The effect of surgical injury on arginase

A few experiments were devoted to the question whether the specific liver injury or the general operational shock was principally responsible for the above-mentioned slight fall in the arginase level following biopsy. In the experiments shown in Table I biopsies were carried out on 3 groups of rats on the days specified. In Group 1, biopsies were performed on days 0, 6, and 9, resulting in a successive lowering of the arginase level (comparable with the effect shown by the biopsy controls in Fig. 1). In Group 2, in an otherwise identical experiment, the biopsy at 6 days was replaced by a sham operation, consisting of the usual incision and exterioration of the liver, without, however, injuring the liver tissue itself. In this case, it was found that the enzyme level after 9 days was practically the same ("p" > 0.1) as that on the day of the first biopsy, while in Group 1 where three biopsies had been carried out, the

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TABLE I

THE EFFECTS OF VARYING LIVER DAMAGE AND SURGICAL TREATMENT ON THE LEVEL OF HEPATIC ARGINASE

Experimental animals	*Arginase units/mg wet wt and standard errors*		
	1st liver biopsy (without previous biopsy)	*2nd liver biopsy (6 days after 1st biopsy)*	*3rd liver biopsy (3 days after 2nd biopsy)*
Group 1	105.4 ± 3.4	98.4 ± 2.8	93.7 ± 3.4
Group 2	113.7 ± 3.7	—*	111.6 ± 5.9
Group 3	107.0 ± 2.7	96.4 ± 4.7**	113.6 ± 3.2

* Sham operation in place of 2nd biopsy;
** Extensive liver damage inflicted during 2nd biopsy.

arginase level after 9 days proved to be significantly depressed ("p" = 0.03). It can, therefore, be concluded that the depression of arginase was probably caused by the removal of small amounts of tissue rather than by the operational shock. When, however, biopsies were carried out in which extensive liver damage was inflicted deliberately (Group 3), the arginase activity, far from being depressed, was increased. This is shown in Group 3, where the usual slight depression of arginase occurred after the first biopsy, which, however, was followed by a significant rise ("p" = 0.02) after the extensive liver injury during the second biopsy. This increase in arginase activity might be due to a stimulation of the regenerative activity of the liver tissue, a view which is in accordance with that of ROSENTHAL, ROGERS, VARS AND FERGUSON[19].

DISCUSSION

There can be little doubt that adrenalectomy caused a profound decrease in the activity of hepatic arginase. Injections of cortisone, on the other hand, stimulated arginase activity in normal rats and, when given to adrenalectomised rats, restored the activity almost to the normal level.

Certain precautions have been taken to ensure results as unambiguous as possible. The use of the biopsy technique, where the experimental animal serves as its own control, has contributed much to reduce the wide range of biological variations usually inherent in hormone experiments. Also, the use of larger doses of cortisone than generally given may have intensified the stimulating effect of the hormone on the enzyme in the present experiments. Furthermore, since the activity of arginase was expressed in units/mg wet weight, it was important to investigate the effect of adrenal hormones on the water content of the liver tissue: however, no significant difference could be detected between the water content of the liver tissue of normal rats and that of adrenalectomised animals. Another important factor was the nutritional state of the experimental animals. KOCHAKIAN AND ROBERTSON[18] reported that subcutaneous implantation of cortisone in fasting mice was followed by an increase in both urea excretion and hepatic arginase activity, while ROSENTHAL, ROGERS, VARS AND FERGUSON[19] claim that such effects could result from fasting alone. In the present work all the experimental animals were fed on ordinary laboratory diet and maintained their weights during the experimental period.

Various theories have been put forward as explanations for stimulating effects

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of hormones on enzymes. A direct activation of an enzyme by a hormone is difficult to demonstrate since *in vitro* experiments in which hormones are added to enzyme preparations have rarely been successful. Experiments of this kind have also been carried out in this work: a highly purified arginase preparation was incubated with cortisone suspensions: in another experiment it was incubated with serum taken from animals which had been injected with cortisone shortly before withdrawing blood samples. In neither case could a change in the activity of the enzyme be observed. At any rate, such an activating effect on arginase would not constitute a specific action since cortisone is known to increase the activity of other enzymes as well. Liver esterase activity, for instance, as shown in this work, is also stimulated by cortisone treatment, though not to the same extent.

Thus the observed stimulating effects may have been due to a more general action of the hormone. It is generally recognised that administration of cortisone increases metabolic activities. There is, in fact, a marked correlation between the level of hepatic arginase and protein catabolism in rats (ROSENTHAL, ROGERS, VARS AND FERGUSON[19], ROSENTHAL AND VARS[20]), and this correlation has also been noted in other organisms (MUNRO[21], DOLPHIN AND FRIEDEN[22]). Moreover, this relationship was considered to be specific by ROSENTHAL AND VARS[20]: they observed that the rise in hepatic arginase associated with increased N-excretion in fasting rats was not accompanied by comparable changes in the total protein content of the liver or in the levels of other enzymes investigated. This phenomenon is also in line with the view that arginase may be associated with rapidly-metabolising tissues such as neoplasms (BACH AND LASNITZKI[13]) or mammary gland (FOLLEY AND GREENBAUM[11]); ROSENTHAL, ROGERS, VARS AND FERGUSON[19] demonstrated a specific rise of arginase, accompanied by increased N-excretion, during the immediate premitotic phase in regenerating liver, though this observation is not supported by THOMSON AND MOSS[7].

The results shown in Table I point to a possible connection between arginase and the regenerative activity of liver tissue. Operational shock alone had little effect on arginase activity, and the biopsies which involved the removal of only very small quantities of liver tissue caused but a slight decrease. Gross injury to the liver tissue, on the other hand, caused a definite rise in the activity of the enzyme, from which it is evident that the biopsy technique used in this work caused sufficiently little damage to the liver not to evoke such a stimulating effect. Regenerating liver tissue has, in fact, long been known to possess a high arginase content (EDLBACHER AND MERZ[23]), and though the exact significance of this phenomenon is unknown, the possibility of a controlling influence of arginase on rapid tissue growth has been suggested (BACH AND LASNITZKI[13]).

An explanation of the observed facts becomes even more difficult when arginase is considered not only as a catalyst but also as a protein. An increase in arginase activity could therefore not only be the result of an activation of the enzyme but also of extra synthesis of catalytic protein. In this connection, the observation of ROSENTHAL, ROGERS, VARS AND FERGUSON[19] that the arginase level in the liver is influenced by the protein content of the diet is noteworthy.

Liver esterase was chosen as another hydrolytic enzyme for comparison with hepatic arginase. As with arginase, adrenalectomy was followed by a substantial fall in the liver esterase level; cortisone treatment, on the other hand, appeared to be much less effective in stimulating esterase activity than in the case of arginase, and

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one is tempted to conclude that the liver esterase level may be influenced by adrenal secretions other than cortisone. Repeated biopsies, which caused a downward trend in arginase activity, appeared to have little effect on liver esterase, though this finding is somewhat vitiated by the great variations between the individual animals.

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SUMMARY

1. Liver biopsies were carried out on fed adult rats before and after adrenalectomy and before and after cortisone injections. The effect of this treatment on the levels of hepatic arginase and esterase in extracts prepared from the excised tissue was investigated.

2. Adrenalectomy caused a substantial reduction in the arginase activity which was restored to the normal level after cortisone injections. The latter also stimulated the arginase activity in the liver of normal animals.

3. A slight fall in arginase activity was also observed in control experiments in which biopsies were carried out without previous adrenalectomy or hormone treatment. The fall was due to the repeated removal of very small amounts of tissue during biopsies, but not to operational shock. When extensive liver damage was inflicted deliberately, the arginase activity was subsequently stimulated. This was interpreted as being the result of extensive regenerating processes in the liver tissue, and is in accordance with similar observations by other workers.

4. The influence of cortisone on hepatic arginase is discussed in the light of the well known stimulating effect of adrenal hormones on tissue metabolism in general.

5. The hepatic esterase activity was also greatly reduced after adrenalectomy, but only minor changes were observed after cortisone injections.

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