Creatine Synthesis by Liver Slices in Hyperthyroid Rats

Conditions of hyperthyroidism are always associated with variations in creatinic metabolism; nevertheless, it is not known whether these variations affect likewise the ability of liver to synthesize creatine.

In this work some results are reported concerning such an ability by liver slices in animals treated with thyroxine and antithyroid drugs.

Male rats weighing 110–130 gm. were injected intramuscularly with 0·25 mgm. of DL-thyroxine every day for 20 days (Groups *B*, *C*, *D* and *E* of Tables 1 and 2).

The animals of Group *C* also received 0·25 gm. methylthiouracil, those of Group *D* 0·10 gm. 2-carbethoxythio-1-methylglyoxaline, those of Group *E* 0·5 gm. DL-methionine. All the substances were given *per os* daily.

On the twentieth day of treatment, the animals were killed by draining the blood and the livers were removed aseptically. For each half-liver creatine content was determined by the direct method of Ennor and Stocken[1]. Soon after killing, the bicipital muscles of the femurs were removed; the muscles were then freed from fat and fascia and then dried in an air oven for 24 hr. at 105° C. After drying, the parts were put into a 4 per cent trichloracetic acid solution and finally the creatine content of this extract was determined by the Hawk method[2]. The results are shown in Table 1.

Table 1. Creatine Content in Liver and in Muscle

Animal groups	Creatine content: In muscle (mgm./gm. of fresh tissue)	Creatine content: In liver (μgm./gm. of fresh tissue)
*A**	18·6 ± 3·1	92 ± 12
B	13·7 ± 3·0	64 ± 10
C	14·9 ± 2·6	69 ± 8
D	16·6 ± 2·9	74 ± 16
E	14·2 ± 2·4	77 ± 13

* Control group. Each group consisted of 8 animals

The remaining half of each liver was cut into slices weighing each 0·3 gm. Ability of liver to synthesize creatine *in vitro* was then determined by the method of Borsook and Dubnoff[3], incubating the slices for 2 hr. at 37° C. and adding in the substrate 0·2 mgm. of guanidinacetic acid for each slice to half of them and 0·2 mgm. of guanidinacetic acid and 1 mgm. DL-methionine for each slice to the remaining ones. The results of this treatment are shown in Table 2.

It was therefore possible to show that the livers of hyperthyroid animals contained a smaller quantity of creatine than normal animals. As might be foreseen, creatine had lessened in the muscles of hyperthyroid animals. Antithyroid drugs prevent depression in muscular creatine-rate but not in hepatic creatine-rate, while methionine prevents depression in liver but not in muscle creatine-rate.

Table 2. Creatine Synthesis by Liver Slices

Animal groups	Synthesized creatine (μgm./gm. of fresh tissue): From guanidinacetic acid only	Synthesized creatine (μgm./gm. of fresh tissue): From guanidinacetic acid and methionine
*A**	58·6 ± 10·9	117·5 ± 19·4
B	40·3 ± 6·4	108·0 ± 11·2
C	46·1 ± 8·1	111·6 ± 12·0
D	49·4 ± 11·2	110·4 ± 9·1
E	45·0 ± 9·1	114·9 ± 13·1

* Control group. Each group consisted of eight animals

In vitro, liver slices from hyperthyroid animals show a remarkable loss of their ability to synthesize creatine when incubated with guanidinacetic acid only, while such an ability is nearly restored when incubated with methionine and guanidinacetic acid. Antithyroid drugs and methionine administered *in vivo* cause small changes only.

Therefore, it seems to be clear that the changes noted in creatine synthesis-rate by liver slices are associated with depression in liver methylizing functions, as previously observed in other experimental conditions[4,5].

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Dec. 6.

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Oxytocic Effect of some *d*-Lysergic Acid *cyclo*Alkylamides

Ergometrine (*d*-lysergic acid L(+)-propanolamide-(2)) and methylergometrine (methylergobasine, *d*-lysergic acid (+)-butanolamide-(2)) exhibit a pronounced oxytocic effect[1]. On the other hand, the oxytocic effect of *d*-lysergic acid diethylamide is very small[2]. It has been shown[3] that the pharmacological properties of ergot alkaloids are profoundly changed by a relatively slight modification of the molecular structure. Saturation of the double bond in ring *D* of the molecule of ergotamine and alkaloids of the ergotoxine group causes a loss of the oxytocic effect and potentiation of the sympatholytic activity. Prolongation of the side-chain of ergometrine causes in the case of methylergometrine a potentiation of the oxytocic effect.

In our experiments, an attempt was made to determine the oxytocic effect of a group of *d*-lysergic acid *cyclo*alkylamides. The synthesis of these compounds is being described elsewhere[4]. Their formulæ are shown in Table 1.

Preliminary tests were carried out on the isolated rabbit and guinea pig uterus. Quantitative determinations of the oxytocic effect were performed on anæsthetized rabbits by the method described by Rothlin[5]. The uterus *in situ* was connected through the opened abdominal cavity with a lever, by means of a fine thread, and the contractions of the uterus were registered on the drum. Blood pressure from the arteria carotis and respiration from the trachea were also registered. Ergometrine hydrogen maleate and *d*-lysergic acid diethylamide in the form of normal tartaric salt were used as standards. All the *d*-lysergic acid *cyclo*alkylamides tested were used in the form of acid maleic salts, and the doses were expressed as the amount of the alkaloid base.

Fig. 1 shows the oxytocic effect of *d*-lysergic acid *cyclo*pentylamide (C_5AL) compared with ergometrine as an example of our experiments.

In order to make an exact comparison of the effects of the compounds tested possible, intravenous in-