# Effect of ACTH Administration on Certain Enzyme Systems in Rat Liver.* (20007)

SYLVIA F. HUNTER. (Introduced by F. Bernheim.)

*From the Department of Physiology and Pharmacology, Duke University Medical School, Durham, N. C.*

According to Russell and Wilhelmi(1), Jensen and Gray(2), and Umbreit and Tonhazy(3), adrenal cortical hormones affect the activity of D-amino acid oxidase. The proline oxidase of kidney(3) and arginase(4) are also influenced by the hormones. With the exception of a study on creatine synthesis by Umbreit and Tonhazy(3) in which they attributed their results to the effect of cortisone on the oxidation of D-methionine to the keto analog, no work has been reported on the effect of increased cortical activity on other energy requiring systems, including other methylations. The following is a report on the effect of ACTH on several such systems in rat liver including the effect on fatty acid oxidation.

*Experimental.* 1.0 mg ACTH (Corticotropin, Wilson) was injected daily into male rats weighing 150-300 g for 7-10 days. They, and a control of the same weight, were killed by a blow on the head, and subsequent bleeding. Liver slices were incubated in a Dubnoff shaker at 37°C in Krebs-bicarbonate solution and 95% $O_2$-5% $CO_2$ gas mixture. The end products of the methylation of nicotinamide and guanidoacetic by methionine and the formation of methionine itself from homocysteine and betaine were estimated according to the methods described in the original papers(5-7). Urea was estimated according to the method of Barker(8), tyrosine from phenylalanine by the method of Theis and Benedict(9) and uric acid from xanthine by the method of Folin (10).

The production of acetoacetate from octanoate was determined in 3 groups of rats by the method of Greenberg and Lester(11). Slices were incubated from animals which (A) had been fasted for 72 hours and then injected with 1.0 mg ACTH hourly for 3 hours before killing; (B) fed *ad libitum* and injected in the same way; and (C) fed *ad libitum* and injected with 1.0 mg ACTH daily for 8 days. Table I shows the effect of ACTH administration for 7-10 days on the methylation of 3 compounds. The methylation of nicotinamide was inhibited, that of homocysteine unaffected, and that of guanidoacetic acid increased. L-methionine was used and the color given by the keto acid when methionine was incubated alone was subtracted from that given by methionine plus creatine formed from the guanidoacetic acid. This differential effect of ACTH on the 3 methylations indicates that separate systems are involved.

Table I shows that the conversion of phenylalanine to tyrosine was depressed in the livers of ACTH-treated animals. Uric acid production from xanthine anaerobically with pyruvate as the hydrogen acceptor(12) was somewhat decreased whereas urea production from ammonium sulfate or glutamine was not affected.

Table I also shows that endogenous acetoacetate production as well as the oxidation of added octanoate was increased by ACTH injected for 7-10 days. The acute effects of ACTH were only seen when the rats were starved for 72 hours. These results on the fatty acid oxidase activity are in agreement with those of Shipley(13), Campbell and Davison(14) on the ketogenic effect of pituitary extracts, of Lipsett and Moore(15) on the effect of cortisone, and the *in vivo* results of Bennett *et al.*(16) on the ketonemia produced by ACTH and by growth hormone. They are in disagreement with the experiments of Wilhelmi and Bondy(17) on the effect of ACTH and growth hormone on endogenous ketone production by liver slices.

*Discussion.* With the exception of group A animals in the experiments on ketone production, the effects of ACTH were only evident after a relatively prolonged treatment.

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TABLE I. Effect of ACTH on Certain Reactions in Rat Liver. 250 mg (wet wt) of slices were incubated 3 hr at 37°. Final concentrations of substrates were as follows: $2 \times 10^{-4}$ M nicotinamide (NA), $3.3 \times 10^{-4}$ M l-methionine (M), $1 \times 10^{-3}$ M homocysteine (HC), $1.1 \times 10^{-2}$ M betaine (B), $5 \times 10^{-4}$ M guanidoacetic acid (GAA), $3.03 \times 10^{-3}$ M l-phenylalanine (PA), and $7.3 \times 10^{-3}$ M Na octanoate (OCT).

| Reaction | Control: No. of animals | Control | | | After ACTH: No. of animals | After ACTH | | | P significant at: 1% | P significant at: .01% |
|---|---|---|---|---|---|---|---|---|---|---|
| *Methyl nicotinamide* from NA and M as μg/g wet wt | 5 | NA 12.8 | NA+M 33.4 | | 8 | NA 3.3 | NA+M 12.1 | | + | ± |
| *Methionine* from HC and B as mg/g wet wt | 9 | HC .24 | HC+B 1.01 | | 7 | HC .23 | HC+B 1.01 | | — | — |
| *Creatine* from GAA and M as μg/g wet wt | 7 | M 55 | M+GAA 112 | Diff. 57 | 7 | M 49.7 | M+GAA 157.2 | Diff. 107.5 | + | ± |
| *Tyrosine* from PA as mg/g wet wt | 8 | No add. 1.18 | PA 3.86 | | 8 | No add. .79 | PA 2.04 | | + | + |
| *Acetoacetate* from OCT as μg acetone per g wet wt | | No add. | OCT | Diff. | | No add. | OCT | Diff. | | |
| Treatment A | 7 | 502 | 1239 | 737 | 7 | 927 | 2101 | 1174 | + | + |
| B | 4 | 314 | 731 | 417 | 4 | 511 | 726 | 215 | — | — |
| C | 3 | 231 | 748 | 517 | 4 | 433 | 1516 | 1077 | + | + |

Since this brings about changes in liver glycogen, fat and minerals, the hormone effect may be the result of these changes rather than a direct action on the enzyme systems. It is also possible that growth hormone (or another pituitary factor) which may be a contaminant in the preparation may be responsible for some of the results. Engel(18) has found that ketosis following ACTH administration is not primarily the result of stimulation of the adrenal gland, and Tepperman and Tepperman(19) showed that pretreatment of hypophysectomized rats with cortisone caused ketosis in liver slices when growth hormone was administered. In group A, fasting may be considered a stress, which might have conditioned the animal to the action of the "ketogenic" pituitary factor.

*Summary.* 1.0 mg ACTH was injected into rats for 8-10 days. Liver slices from these animals showed a decreased methylation of nicotinamide and an increased methylation of guanidoacetic acid. The methylation of homocysteine was unaffected. Tyrosine formation from phenylalanine was inhibited as was uric acid formation from xanthine. Urea production was normal. Endogenous ketone body production and the oxidation of octanoate was increased.

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## Nutrition of the Mouse. XII. Comparison of Several Salt Mixtures.* (20008)

MARIAN T. DOWLING AND PAUL F. FENTON.

*From the Department of Biology, Brown University, Providence, R. I.*

Although a wide variety of salt mixtures, many of these commercially available, is in use in nutritional experiments with mice, there is at the present time little information about the relative merits of these preparations. For this reason we decided to compare five salt mixtures fed at a 5% level as part of an otherwise complete synthetic diet.

*Method.* Weanling mice of the C57 and I strains were divided into 5 comparable groups, each receiving the same basal diet (Table I)

TABLE I. Composition of Diet in %.*

| | | | |
|---|---|---|---|
| Casein | 30 | Corn oil | 5 |
| Dextrin | 50 | Salt mixture | 5 |
| Crisco | 10 | | |

* Vitamins added in amounts described by Fenton and Carr(4).

but a different salt mixture. The following preparations were employed: Hubbell, Mendel and Wakeman(1), Wesson(2), U.S.P. XII No. 2, McCollum and Simmonds No. 185(3) and Fenton and Carr(4). The mice were housed in individual screen-bottom metal cages in a constant temperature room. Food and water were supplied *ad libitum*. The animals were weighed once each week and sacrificed when 135 days of age. At this time determinations of water, fat and ash content were carried out.

*Results and discussion.* The experimental results are summarized in Table II. Although variations among groups were encountered with respect to final body weight and total fat content, no striking effects of varying the salt mixture were seen. With each salt mixture employed there was, however, considerable difference between the 2 strains of mice with respect to final body weight and total carcass fat. The dry, fat-free weight of all groups of mice was virtually the same. Plotting the body fat content against percent body water showed the inverse linear relationship already reported by other investigators. The ash content of mice fed the diet containing Hubbell, Mendel and Wakeman salts was significantly higher than the ash content of any other group. This was true for both strains of mice. The values obtained for total ash content are in good agreement with those of Morris(5).

The most striking difference between the salt mixture described by Hubbell, Mendel and Wakeman and that described by Fenton and Carr lies in the Ca:P ratio. This ratio is close to unity in the Fenton and Carr mixture but is considerably higher in the Hubbell, Mendel and Wakeman preparation. For this reason a new salt mixture was prepared differing from the original Fenton and Carr preparation in that some of the calcium phosphate was replaced by calcium carbonate, thus retaining essentially the same calcium level but decreasing the phosphorus content. Two groups of 12 mice each were fed identical diets but one receiving the old while the other received the new salt preparation. The ash content of these mice when sacrificed at 135 days of age differed significantly, the new salt mixture giving a higher total body ash.

*Summary.* Five salt mixtures were tested for their ability to support growth as well as

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