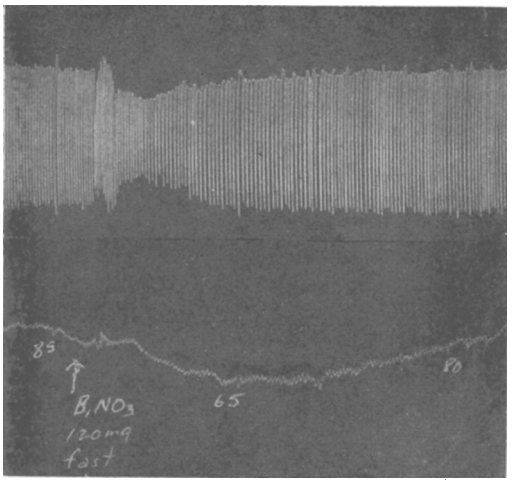

FIG. 2.
Top, Respiration. Bottom, Blood Pressure.

isolated frog heart and Smith *et al.*[22] on the isolated turtle heart indicates that thiamine has also a direct toxic effect on the myocardium. Furthermore, irregularities in the electrocardiogram of dogs after the administration of large doses of thiamine[22] shows that the mammalian heart is affected.

Smith *et al.*[22] found that, even after vagotomy or atropinization, there was a prolonged peripheral blood pressure fall. Haimovici and Pick[27] reported that thiamine caused vasodilatation which counteracted the vasoconstrictor action of nicotine on the perfused frog hind limb preparation. Thus it is probable that thiamine causes vasodilatation by direct action on the peripheral vascular musculature.

Summary. There is little difference in the lethal dose of either thiamine hydrochloride or mononitrate and the symptoms of toxicity are the same for both forms of the drug. The toxic effects of both forms of the vitamin on respiration and blood pressure are due to their thiamine content and not to the pH of the injected solutions or to the substituent groups on the nitrogen of the thiazole nucleus. In lethal doses either form of the vitamin causes death by a direct paralyzing action on the respiratory center followed by cardiac failure.

The author wishes to thank Merck and Co. for the generous supply of thiamine mononitrate used in this study.

27 Haimovici, H., and Pick, E. P., PROC. SOC. EXP. BIOL. AND MED., 1946, **62**, 234.

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Influence of Nephrectomy and Renal Pedicle Ligation on the Activity of Liver Arginase in Rats.*

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Intermediary nitrogen metabolism has been reported to be impaired in dogs with reduced or abolished kidney function.[1] This deficiency is manifest in the fact that 30 to 75% of fed nitrogen is not transformed into urea.

Consideration was given to the possibility that renal transformation of citrulline into arginine[2,3] might represent a reserve mechanism for the urea cycle in the liver and that curtailment of this renal reserve might be related to the observed deficiency.

The hypothesis that an increase in arginase activity might compensate—at least in part—for the lesser concentration of arginine and ornithin by speeding up the turnover of the urea cycle led to a series of experiments. They include determinations of liver arginase

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† James Hudson Brown Junior Fellow.

1 Mylon, E., Smith, E. R., and Goldstein, P., *Am. J. Physiol.*, 1948, in press.

2 Borsook, H., and Dubnoff, J. W., *J. Biol. Chem.*, 1941, **141**, 717.

3 Cohen, P. P., and Hayano, M., *J. Biol. Chem.*, 1946, **166**, 239, 251.

activity in normal, fasting, nephrectomized and renal artery ligated rats.

The results are contained in the report that follows:

Materials and Methods. Male albino and black hooded rats weighing from 250 to 400 g were standardized on a Purina Chow diet.

Removal of the kidneys as well as ligation of the renal pedicles was performed through a midline abdominal incision under light nembutal anesthesia (4 mg/100 g wt). Appropriate sham operations were carried out in all normal rats prior to the fasting. Nephrectomized animals and renal artery ligated animals were fasted after the operative procedures.

Liver arginase activity was determined by a slight modification of the method of Hunter and Downs.[4] The procedure follows:

The livers of lightly nembutalized rats were removed and weighed. In many instances a small piece of liver was dried to constant weight at 110° C. The remainder was placed in a Waring blendor with 50 ml of ice cold normal saline.

Homogenization was carried out in 2½ minute periods. After the first of these, the glass container was cooled in a deep freezer for 7 to 8 minutes so that the temperature of the homogenate did not rise above 40° C during the entire 5 minute procedure. A high speed homogenizer designed for 50-70 ml was used.

Aliquots of 5 ml of the homogenate were activated, diluted and incubated with a buffered arginine solution.

Urea determinations were made by the urease-aeration method of Van Slyke and Cullen;[5] commercial urease "Arlington" was used. All calculations were based on the "arginase unit" as described by Hunter and Downs.[4]

Results. Normal rats. The highest liver arginase activity in 18 normal rats was 300 units per g wet weight, the lowest, 233 units and the average 268 ± 23.1‡ units. The average arginase activity in the total liver was 3605 units. The relationship of liver weight to body weight and liver dry weight to wet weight was rather uniform; 3.73% of the body weight was the average weight of the liver and 28.6% of the wet weight was the average dry weight.

Fasting normal rats: After 48 to 72 hours of fasting the liver arginase activity had only slightly decreased; the highest value in 12 animals was 290.5 units/g wet weight, the lowest 222.1 and the average 256 ± 25.9‡ units. The marked decrease in liver weight on fasting, 32.4%, was the main factor, therefore, in the reduction of the total liver arginase to 2375 units.

Nephrectomized rats: Arginase activity in liver, 48 hours after bilateral nephrectomy was in the range of that of normal fasting rats averaging 261.5 units per g wet weight.

In contrast, 72 hours after nephrectomy a marked increase of the arginase activity was found; the highest value in 8 experiments was 447 units per g wet weight, the lowest 331 units, and the average 373.7 ± 46.2‡ units. This increase observed in nephrectomized fasting rats as compared to the controls, *i.e.* normal fasting rats, is highly significant (p value <0.001, Fisher's tables).

While the percentage of dry weight to wet weight in the liver was the same as in normal rats, its decrease in weight was only 22% during the 72 hours following the nephrectomy as compared with a loss of 32.4% observed in the normal group fasted for the same period. The high value for total liver arginase, 3487 units, obviously is based on two additive factors: One the significant increase of arginase units per g wet weight, and the other, the smaller loss in the liver weight after nephrectomy.

Rats with bilaterally ligated renal pedicles: The severity of this intervention, indicated by the death of all animals of the first series before the end of the second day required termination of the experiments 24 hours after ligation. Despite this short experimental period the arginase units per g wet weight

[4] Hunter, A., and Downs, C. E., *J. Biol. Chem.*, 1944, **155**, 173.

[5] Van Slyke, D. D., and Cullen, G. E., *J. Biol. Chem.*, 1914, **19**, 211; *J. Biol. Chem.*, 1916, **24**, 117.

‡ Standard deviation of the mean.

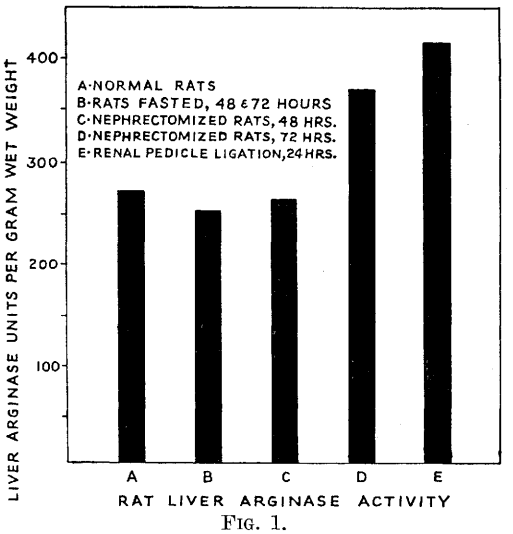

FIG. 1.

Comparison of liver arginase activity of normal, fasting, and nephrectomized rats, and rats after bilateral renal pedicle ligation.

were extremely high, 466.4 units as a maximum, 351.5 as a minimum, with an average of 412.4 ± 51.6 units.§ Fig. 1 is a survey of the results obtained.

Discussion. Liver arginase activity of normal rats on a high protein diet,[6] of hypophysectomized and of adrenalectomized rats injected with cortical steroids[7-9] has been reported to be markedly elevated. The question arises whether the change in liver arginase activity as reported in this paper is related to a similar increase in nitrogen metabolism. This has been found not to be the case. The increase in blood urea-N on the third day following nephrectomy would indicate that only 1.438 g of protein were metabolized¶ by a 250 g rat, an amount which corresponds to less than one half of the daily intake on Purina food. The nitrogen metabolism of a normal rat on this diet, therefore, is significantly higher than that of a fasting nephrectomized rat. This makes it difficult to assume that the increased liver arginase activity following removal of the kidneys is associated with an elevation of the nitrogen metabolism.

The possibility is considered that elimination of kidney function limits one or several components of the urea cycle, and that under these circumstances, increase in arginase activity may be a compensatory mechanism. It must be emphasized, however, that the deficiency in liver arginine or ornithin in the above conditions is an assumption.

Summary. Liver arginase activity is significantly increased after bilateral nephrectomy and also after ligation of the renal arteries in rats. This reaches a maximum within 24 hours after ligation of the arteries as compared with 72 hours after removal of the kidneys. The possibility is considered that the marked increase in arginase activity is a compensatory mechanism for a deficiency in renal arginine formation.

§ Standard deviation of the mean.

6 Lightbody, H. D., and Kleinman, A., PROC. SOC. EXP. BIOL. AND MED., 1941, **46**, 472.

7 Fraenkel-Conrat, H., and Evans, H. M., *Science*, 1942, **95**, 305.

8 Fraenkel-Conrat, H., Simpson, M. E., and Evans, H. M., *J. Biol. Chem.*, 1943, **147**, 99.

9 Folley, S. J., and Greenbaum, A. L., *Biochem. J.*, 1946, **40**, 46,

¶ Total urea formation was calculated from the increase of blood urea per day, assuming it is equally distributed in body water (75% of the body weight).