TABLE I. Selective Effect of Cortisone and Thyroxine on Adrenal and Thyroid Hypertrophy Induced by Amphenone "B."*

Treatment	Final body wt (g)	Adrenal wt (mg)	Thyroid wt (mg)	Thymus wt (mg)
0	230 ± 13	67 ± 13	14 ± 2	827 ± 62
Amphenone B alone	180 ± 21	112 ± 18	50 ± 6	408 ± 177
Amphenone B + cortisone	150 ± 13	32 ± 8	33 ± 8	51 ± 11
Amphenone B + thyroxine	184 ± 26	111 ± 50	9 ± 1	545 ± 177

* Amphenone "B" given 32 mg daily *per os* in 0.5 cc H_2O for 14 days. Thyroxine (dl) given 32 μg daily subcut. 0.2 cc of .01 N NaOH for 16 days. Cortisone acetate given 2 mg daily subcut. in 0.2 cc saline suspension for 16 days. Cortisone and thyroxine begun 2 days before Amphenone "B." Each experimental group includes 10 animals.

corticoid production by the enlarged adrenal suggests that Amphenone "B" may be inducing a non-productive hypertrophy in the adrenal similar to that produced by thiouracil in the thyroid(4-5). In any case, it is clear that the availability of such compounds as Amphenone "B" will provide interesting experimental tools for the elucidation of the reciprocal relationship between the anterior pituitary and its various target organs.

Summary. Amphenone "B" induces a marked hypertrophy of both the thyroid and adrenal in the rat. Simultaneous administration of thyroxin and Amphenone "B" prevents the thyroid hypertrophy without reducing the adrenal enlargement. Combined cortisone and Amphenone "B" administration prevents the adrenal enlargement without altering the thyroid hypertrophy. Thus, Amphenone "B" exerts its adrenal and thyroid effect by acting through two distinct end-points in the anterior pituitary.

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Metabolism of Glycine.* (18822)

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The metabolism of glycine in normal mice has been studied after a single intravenous injection of glycine-2-C^{14} by following the specific activity of the uncombined glycine in the plasma, liver and skeletal muscle and by determining the specific activity of the expired CO_2. In this way it has been possible to estimate the size of the body pool of glycine, the turnover time and rate of turnover of body glycine, and the rate of oxidation of the methylene carbon atom of glycine to CO_2. A similar study of glucose metabolism in normal and diabetic rats was reported recently by Feller, *et al.*(1).

Experimental. Glycine-2-C^{14}† (1.5-2.0 μg per g body weight), in 0.1 ml of 0.85% saline solution, was injected intravenously into young, adult, Rockland strain white mice. The specific activity of the glycine was 10,000 cpm per μg. The respiratory CO_2 was absorbed in sodium hydroxide solution and then precipitated as $BaCO_3$ for radioactivity meas-

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† Obtained from Oak Ridge National Laboratory on allocation of the U. S. Atomic Energy Commission.

urements. After the appropriate time the animals were sacrificed and deproteinized extracts of plasma, liver and thigh muscles were prepared; the tungstic acid procedure of Schurr, *et al.*(2) was used except that boiling was omitted and the entire process carried out rapidly below 5°. An aliquot of each tissue extract was mixed with phosphate buffer and ninhydrin solution and the mixture was then made up to 8 ml and distilled, as described by Alexander, *et al.*(3). Under these conditions the uncombined glycine is decomposed and the radioactive methylene carbon is converted to formaldehyde which collects in the distillate. The danger of carrying over non-volatile material which might be radioactive was minimized by using a spray trap and by collecting only the first 6 ml of distillate. In order to determine the formaldehyde concentration in the distillate, a 1.7 ml aliquot was mixed with 0.2 ml of 5% aqueous chromotropic acid solution and the mixture was chilled while the total volume was brought to exactly 3 ml by adding concentrated sulfuric acid. The resulting solution was heated in a boiling water bath for 35 minutes and the optical density was measured at 570 mμ in a Beckman spectrophotometer as recommended by MacFadyen(4). In order to measure the radioactivity of the formaldehyde in the distillate, a 2 ml aliquot was mixed with exactly 1 mg of non-radioactive formaldehyde and the mixture was precipitated by the addition of 10 ml of 0.4% dimedon solution. The mixture was allowed to stand for at least 3 hours and was then filtered onto a paper disc and mounted on a brass holder, substantially as described by Henriques, *et al.*(5). The weight of the sample was determined‡ and the total radioactivity measured was corrected for the difference between the weight of the sample and the theoretical weight of dimedon precipitate from 1 mg of formaldehyde (9.74 mg). The specific activity of the formaldehyde divided by 2.50 gives the specific activity of the uncombined glycine originally present in the tissue extract. All radioactive samples were counted for a time sufficient to bring the statistical error to less than 10 percent and were corrected for self absorption(6).

Results. The specific activity of the carbon in the expired CO_2 and of the uncombined glycine in liver, muscle and plasma are shown in Fig 1. Each point on the liver and muscle curves represents the average of measurements on 3 mice. Each point on the plasma curve represents the average of 2 determinations, each of which was made on pooled plasma from 2 mice. Each point on the expired CO_2 curve represents the average of measurements on 4 mice. The vertical or sloping line through each point indicates the range of the values whose average is represented by the point.

Determination of the size of the body pool of glycine. As shown in Fig. 1, the decrease in the specific activity of the uncombined glycine becomes approximately logarithmic about 15 minutes after the glycine injection. Extrapolation of these curves to zero time as suggested by Feller, *et al.*(1) is considered to indicate what the initial dilution of the labeled glycine would have been if there had been instantaneous mixing of the added glycine with the glycine in the body pool. This interpretation involves the assumption that the dilution of the labeled glycine was going on during the earlier part of the experiment in the same way as during the period of logarithmic decrease in specific activity. In the present experiments this extrapolation indicates that the size of the dynamic body pool of glycine is approximately 30 mg per 100 g body

2. Schurr, P. E., Thompson, H. T., Henderson, L. M., and Elvehjem, C. A., *J. Biol. Chem.*, 1950, v182, 29.

3. Alexander, B., Landwehr, G., and Seligman, A. M., *J. Biol. Chem.*, 1945, v160, 51.

4. MacFadyen, D. A., *J. Biol. Chem.*, 1945, v158, 107.

5. Henriques, F. C., Jr., Kistiakowsky, G. B., Margnetti, C., and Schneider, W. G., *Ind. Eng. Chem., Anal. Ed.*, 1946, v18, 349.

‡ Samples were dried at 80°C for 20 minutes. Prolonged heating at higher temperatures results in decomposition and sublimation of sample and decrease in specific activity of residue.

6. Calvin, M., Heidelberger, C., Reid, J. C., Tolbert, B. M., and Yankwich, P. E., *Isotopic carbon*, New York, 1949, pp. 317-318.

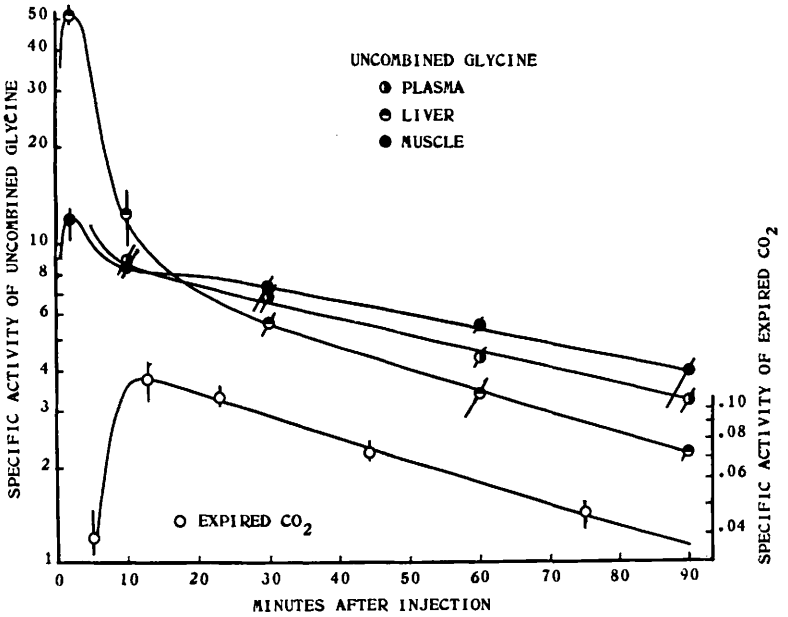

FIG. 1. Specific activity of expired CO_2 and of uncombined glycine in different tissues, following a single intravenous injection of glycine-2-C^{14}. The specific activity is expressed as percent of administered dose per mg of glycine or per mg of carbon in the expired CO_2. Each vertical or sloping line indicates the range of values whose avg is represented by the corresponding point.

weight. However, isotope dilution experiments in which a similar quantity of labeled glycine was added to chilled homogenates of whole mice indicated that the amount of uncombined glycine present in the mouse is approximately 10 mg per 100 g. It is evident that within 15 minutes after its injection, the labeled glycine not only mixes with the uncombined glycine present in the mouse but also comes to equilibrium with other compounds. Compounds with which it equilibrates may include the glycine in glutathione (7) and other physiologically active peptides (8), and perhaps serine(9-10). It has been shown by Friedberg and Greenberg(11) and more recently by Borsook, *et al.*(8) that intravenously administered amino acids are removed from the plasma very rapidly. In the present study, the initial rapid rise and the subsequent rapid decrease in the specific activity of the uncombined glycine in the liver indicate that this organ is particularly active in the effort of the organism to dispose of the "extra" glycine which has been injected. It is possible that in this initial readjustment some of the labeled glycine might be irreversibly removed from the glycine pool at a rate which would not be taken into account by the extrapolation to zero time. If this happened, the actual size of the glycine pool would be smaller than that indicated by the extrapolation procedure. In the present case, it is estimated§ that this effect introduces less than 5% error in the pool size.

Turnover time of the glycine pool. During

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8. Borsook, H., Deasy, C. L., Haagen-Smit, A. J., Keighley, G., and Lowy, P. H., *J. Biol. Chem.*, 1950, v187, 839.

9. Sakami, W., *J. Biol. Chem.*, 1949, v178, 519.

10. Goldsworthy, P. D., Winnick, T., and Greenberg, D. M., *J. Biol. Chem.*, 1950, v180, 341.

11. Friedberg, F., and Greenberg, D. M., *J. Biol. Chem.*, 1947, v168, 405.

§ If 60 μg of glycine, (the quantity injected in these experiments), was destroyed in the viscera at a time when the specific activity of uncombined glycine was 500 cpm per μg, (higher than that observed in any of these experiments), this would result in the removal of only 30,000 cpm from the glycine pool, *i.e.*, approximately 5% of the 600,000 cpm injected.

the period of logarithmic decrease the times for the specific activity of the glycine to decrease by one-half ($t_{1/2}$) are 45, 60 and 75 minutes for liver, plasma and muscle respectively. The mean $t_{1/2}$ for the glycine pool is probably close to 1 hour. The time for the turnover of the pool (t_t) is given by the expression $t_t = 1.44\ t_{1/2}(1)$ and in this case is approximately 1.5 hours.

Rate of turnover of glycine. Since 30 mg of "glycine" per 100 g is turned over in 1.5 hours, the rate of turnover is approximately 20 mg per 100 g per hour. Except for the most active protein components, the exchange of the labeled glycine with proteins is still in the phase during which glycine of high specific activity is being incorporated and glycine of low specific activity is being liberated. Consequently the turnover rate measured in these experiments includes the exchange of the labeled glycine pool with proteins and is probably close to the actual turnover rate of the glycine pool in the animal. Turnover as used here includes all of the processes by which molecules are removed from the glycine pool and other molecules are added to it. The slopes of the curves in Fig. 1 indicate that the rate of turnover of glycine in liver is much greater than it is in skeletal muscle; moreover, the transfer of glycine between the plasma and the tissues is not quite fast enough to bring the specific activity of the glycine in all parts of the body pool to the same value at a given instant. However, the interchange of glycine between tissues is undoubtedly too rapid to permit the calculation of rates of turnover in individual organs.

Oxidation of glycine to CO_2 The rate of oxidation of glycine to yield CO_2 may be estimated by dividing the amount of radioactivity expired in a given time interval by the mean specific activity of the glycine in the animal during the same time interval, or by the method suggested by Feller, *et al.*(1). Application of either method to the present data indicates that the rate of oxidation of glycine to CO_2 is approximately 3 mg per 100 g per hour. As a result of this oxidation the methylene carbon of glycine contributes approximately 0.2% of the carbon in the respiratory CO_2. It is evident that these mice were synthesizing most of the glycine required in their metabolism. With the semi-synthetic diet used(12) they received in their food not more than 7 mg of glycine per 100 g per day, whereas oxidation at the rate indicated in these experiments would consume approximately 72 mg of glycine per 100 g in 24 hours. This may provide at least a partial explanation for the finding that even 90 minutes after injection of the labeled glycine the specific activity of the uncombined glycine in various tissues did not approach a common value.

Summary. The metabolism of glycine in mice has been studied after a single injection of glycine-2-C^{14} by following the specific activity of the uncombined glycine in plasma, liver and skeletal muscle, as well as the specific activity of the respiratory CO_2. The size of the dynamic pool of glycine was found to be 30 mg per 100 g of which 10 mg per 100 g was uncombined glycine; the turnover time of the glycine pool was 1.5 hours and the rate of turnover 20 mg per 100 g per hour. The amount of glycine oxidized to yield CO_2 in the breath was found to be approximately 10 times the amount of glycine ingested in the food.

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