can be detected in fetal adrenal glands at this stage of embryonic life. On the other hand, the relatively high concentration of progesterone may be due to accumulation of lipids in the fetal cortex. However, histochemical studies indicate that lipoid material is present in only relatively small amounts in the fetal cortex, but these amounts may be still higher than that found in other fetal organs. In view of this finding it is of interest that Riegel *et al.*(12) found a relatively high concentration of radioactivity in the adrenals of mice after administration of $C^{11}$-21-progesterone.

*Summary.* $C^{14}$-4-progesterone was administered intramuscularly to pregnant women who were scheduled for therapeutic abortion. Distribution of radioactivity in maternal and fetal tissue removed at various time intervals following injections was determined. The highest concentration of radioactivity was found in the maternal fat. A rough calculation revealed that it contained about 17.7% of the administered radioactivity 12 hours, 33.7% 24 hours, and 19.6% 48 hours after administration of the labelled hormone. These findings indicate that progesterone and/or its metabolites diffuse promptly from the blood circulation into the fat compartment of the body. Only moderate amounts of radioactivity were found in the myometrium and the decidua, the 2 principal target organs of progesterone. Low to moderate concentrations of radioactivity were detected in the organs which serve as principal sources of the hormone, the corpus luteum and the placenta. Radioactivity was present in all fetal tissues in relatively small amounts with the exception of the fetal brain, and the testes.

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## Assay and Some Properties of Kidney Transamidinase.* (23132)

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Transamidination in mammalian kidney slices and homogenates was first reported by Borsook and Dubnoff(1). The reaction has since been confirmed by other investigators with the use of isotopic technics(2-5).

This paper reports a simple assay for tissue transamidinase activity. The assay was used to determine the following effects: addition of sulfhydryl inhibitors both *in vivo* and *in vitro*, substitution of canavanine for arginine as one of the substrates, *in vitro*, and substitution of a protein-free diet for a complete diet in the rat.

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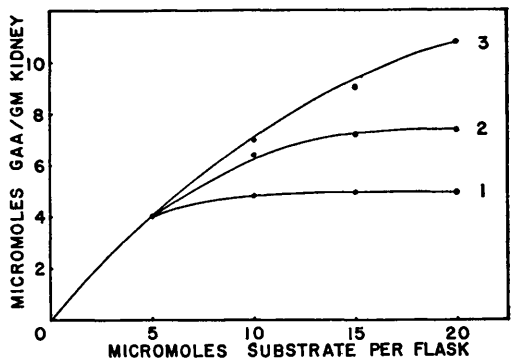


FIG. 1. Guanidinoacetic acid production as a function of varying concentrations of arginine and glycine. In series 1, arginine conc. was varied with constant glycine conc. of 5 μmoles/flask. Glycine was varied with arginine constant at 5 μmoles/flask in series 2. In series 3, equal molar amounts of arginine and glycine were used. The reaction flasks contained: 0.5 ml 0.067 M Sorensen phosphate buffer, pH 7.4; 0.5 ml 4% pork kidney homogenate in buffer (20 mg of wet kidney); and 0.5 ml arginine-glycine solution. Initial conc. in reaction flasks were: buffer, 0.067 M; pork kidney, 1.33%; and, in series one: glycine, 3.3 × $10^{-3}$M, and arginine, (3.3, 6.7, 9.9 and 13.3) × $10^{-3}$M; in series 2: arginine, 3.3 × $10^{-3}$M, and glycine, (3.3, 6.7, 9.9 and 13.3) × $10^{-3}$M; and in series 3: arginine and glycine each (3.3, 6.7, 9.9 and 13.3) × $10^{-3}$M. Incubation was for 1 hr at 37°. Guanidinoacetic acid was determined as under "Methods and materials."

*Methods and materials.* Canavanine was isolated from Jack bean meal by the method of Kitagawa(6), and also was purchased from Nutritional Biochemicals Corp. Dimercaprol was purchased from Hynson, Westcott, and Dunning, Inc., Baltimore, Md. A crude liver arginase preparation was used to remove arginine from the incubation mixture prior to guanidinoacetic acid analysis. Thirty g fresh rabbit liver was homogenized with 70 ml cold water in a Waring Blendor and the homogenate dialyzed against cold water for 2 days at 4°C. The homogenate was centrifuged in the cold for 15 minutes at approximately 1000 relative centrifugal force and the supernatant fluid decanted and saved. The preparation could be kept frozen for months without loss of activity and had neither transamidinase nor transmethylase(7) activity. All solutions used in the incubation procedure were made up with 0.067 M phosphate buffer, pH 7.4. The assay was as follows: 0.5 ml of 4% kidney homogenate was added to 0.5 ml 0.04 M glycine solution and 0.5 ml 0.04 M arginine solution in a 25 ml Erlenmeyer flask. Incubation was for 1 hour at 37°C in a Dubnoff type metabolic incubator(8) with an air phase over the flasks. Prior to and after the incubation, 0.5 ml aliquots of the incubation mixture were removed, placed in a 15 ml centrifuge tube, and heated in a boiling water bath 10 minutes. After cooling, 0.5 ml of the liver arginase preparation was added and the mixture incubated for 1½ hours at 37°C. Protein was removed by the addition of 2.0 ml 0.3 N $Ba(OH)_2$ in phthalate buffer, pH 5.0, and 2.0 ml 5% $ZnSO_4 \cdot 7H_2O$. Two ml of the filtrate was used for colorimetric determination of guanidinoacetic acid(9).

*Development of the assay.* Effects of varying concentrations of arginine and glycine on guanidinoacetic acid production are shown in Fig. 1. With a constant amount of glycine at 5 μmoles/flask (series 1) a maximum guanidinoacetic acid production of 5 μmoles/gram kidney was obtained with 10 μmoles of arginine. With arginine constant at 5 μmoles/flask (series 2) a maximum production of 7.5 μmoles guanidinoacetic acid/gram kidney was obtained with 15 μmoles of glycine. Maximum production of guanidinoacetic acid was obtained with equal molar amounts of arginine and glycine (series 3). There was no production of guanidinoacetic acid by the kidney homogenate without added substrate. The effect of incubation time at various equal molar concentrations of arginine and glycine on guanidinoacetic acid production is shown in Fig. 2. The enzyme was still active after 3 hours incubation. It was assumed that the enzyme was sufficiently saturated with substrate after one hour of incubation with an initial amount of 20 μmoles each of arginine and glycine/flask (series 3). Forty μmoles of arginine/flask was an amount too great to be removed conveniently by the arginase preparation for guanidinoacetic acid analysis. The optimum temperature for transamidination by kidney homogenate was found to be 47°C. The effect of varying amounts of pork, rat, and rabbit kidney homogenate upon guanidinoacetic acid production was determined. 0.25, 0.5, or 1.0 ml of 4% homogenate in the assay resulted in a constant production per g kidney and was 9, 26, and 3

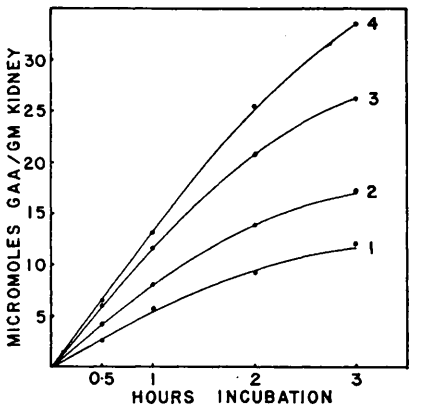


FIG. 2. Production of guanidinoacetic acid as a function of incubation time at different equal molar levels of arginine and glycine. Each flask contained 1 ml 0.067 M phosphate buffer, pH 7.4, 1 ml of 4% pork kidney homogenate, and 1 ml equimolar arginine-glycine solutions. Flasks 1, 2, 3, and 4 contained 4, 10, 20, and 40 μmoles each of arginine and glycine respectively. Initial conc. were: buffer, 0.067 M; pork kidney, 1.33%; and arginine and glycine each, (2.6, 6.7, 13.3 or 26.7) $\times 10^{-3}$M. Incubation was at 37°C. Guanidinoacetic acid was determined as under "Methods and materials."

μmoles hour for pig, rat, and rabbit respectively. Borsook and Dubnoff report by their procedure 4.6 and 3.4 μmoles/g kidney/hour for rabbit and rat respectively(1). Transamidinase activity was found only in kidneys. Twenty per cent homogenates of spleen, liver, blood, heart, muscle, testes, and brain from a normal rat had no activity. Borsook and Dubnoff also found no activity in these tissues.

*Sulfhydryl inhibitors.* 0.15 μmole of parachloromercuribenzoic acid produced complete inhibition of the system. One μmole of reduced glutathione, or 2 μmoles of cysteine, or 1 μmole of dimercaprol resulted in a 122, 105, and 78% reversal of the inhibition respectively. Addition of reduced glutathione to an uninhibited system resulted in a 138% production as compared to the control flask. Cysteine or dimercaprol did not increase production in the uninhibited system. These results indicated that transamidinase is a sulfhydryl enzyme(10). The *in vivo* inhibition studies were carried out on a 125 g albino rat. The rat was given a dose of 5 mg $HgCl_2$/kilo body weight by intramuscular injection of a solution containing 5 mg/ml in 0.9% NaCl. Seventy-two hours after the injection the rat was anesthetized with ether and 5 ml of blood removed by heart puncture for creatinine and urea determinations. The kidneys were removed; one was preserved in formalin for morphological examination and one was used for the enzyme assay. The cortices were found to be white and the medulla was congested. The serum creatinine and urea nitrogen was found to be 3.8 mg% and 85 mg% respectively, which indicated severe renal damage. A transamidinase assay showed an activity of 2.7 units as compared with a normal activity of 25 units. The addition of dimercaprol to the reaction flask did not restore the transamidinase activity.

TABLE I. Substitution of Canavanine for Arginine as Substrate for Transamidination.

| μmoles substrate/flask | | | μmoles guanidinoacetic acid formed/g rat kidney/hr |
|---|---|---|---|
| Glycine | Arginine | Canavanine | |
| 20 | | 20 | 22 |
| 20 | 20 | | 7 |
| 20 | 20 | 20 | 13 |
| 20 | 20 | 40 | 22 |

*Substitution of canavanine for arginine as the substrate.* Canavanine previously has been reported to be an amidine group donor for the transamidinase system(4,5,11). The data in Table I confirm these findings. Arginine was approximately 1/3 as effective as canavanine and appeared to inhibit canavanine as an amidine donor. The guanidinoacetic acid formed in these experiments was also identified by filter paper chromatography. Transamidination with canavanine as the substrate was found to be inhibited by PCMB and this inhibition was reversed with glutathione, cysteine, and dimercaprol as with arginine as the substrate.

*Effect of protein-free diet on kidney transaminidase.* Twelve weanling, albino rats were used to determine effect of a protein-free diet on kidney transaminidase. The complete diet consisted of: lactalbumin, 180 g, dl-methionine, 2.5 g, sucrose, 726.5 g, choline, 1 g, corn oil, 50 g, salt mixture(12), 40 g, and a vitamin preparation (Upjohn "Zymadrops"), 1.2 ml. The protein-free diet was the same except that lactalbumin was replaced by su-

TABLE II. Effect of a Protein-Free Diet on Kidney Transamidinase Activity of Weanling Rats.

| | Avg wt on day | | | Avg kidney | | | | |
|---|---|---|---|---|---|---|---|---|
| Group* | 0 | 12 | 27 | Wt | % N | Units activity | Total activity | Activity per mg N |
| 1 | 50 | 38 | | .3 | 2.5 | 3.9 | 1.1 | .15 |
| 2 | 48 | 33 | 60 | .4 | 2.6 | 13.5 | 5.4 | .51 |
| 3 | 50 | 67 | 63 | .4 | 2.9 | 3.4 | 1.4 | .12 |
| 4 | 52 | 77 | | .5 | 2.7 | 17.5 | 8.8 | .63 |

* Group 1 received the protein-free diet for 12 days. Group 2 received protein-free diet 12 days, followed by complete diet for 15 days. Group 3 received complete diet for 12 days, followed by protein-free diet for 15 days. Group 4 received a complete diet for 12 days. All animals were given food and water *ad libitum*. A unit activity is described as μmoles guanidinoacetic acid formed/g kidney (wet wt)/hr.

crose. Six weanling rats were fed the complete diet, and 6 were fed the protein-free diet for 12 days. On the twelfth day, 3 rats from each group were sacrificed and kidney transaminidase activities determined. A separate kidney homogenate was made for each rat, and an aliquot removed for micro-Kjeldahl nitrogen analysis. The dietary regimen of the remaining animals was then changed as follows: Rats which had been on the protein-free diet received the complete diet, and the rats which had been on the complete diet received the protein-free diet. Fifteen days later, the rats were sacrificed, and transaminidase and nitrogen analyses performed as above.

The results of this experiment are shown in Table II. Kidneys from rats fed a protein-free diet for 12 days had only 24% as much activity per mg kidney nitrogen as kidneys from rats fed the complete diet during the same period. Kidneys from rats fed a protein-free diet 12 days followed by a complete diet for 15 days had 80% as much activity per mg kidney nitrogen as kidneys from the rats fed the complete diet for 12 days. Kidneys from rats fed a complete diet for 12 days followed by a protein-free diet for 15 days had 19% as much activity per mg nitrogen as kidneys from rats fed the complete diet for 12 days. These results indicate that transaminidase activity of rat kidney is dependent on dietary protein intake. This is in marked contrast to kidney D-amino acid oxidase, which shows little or no change of activity in a protein depleted rat(13-16).

*Summary and conclusions.* 1. An assay of transaminidase activity in mammalian kidneys was developed. 2. The enzyme was inhibited by p-chloromercuribenzoic acid. The inhibition could be reversed with reduced glutathione, cysteine, or dimercaprol. These results suggest that transaminidase is a sulfhydryl enzyme. 3. Kidneys from a mercury-poisoned rat showed reduced transaminidase activity. The activity could not be restored by addition of dimercaprol to the reaction flask. 4. Canavanine was found to be 3 times as effective as arginine as an amidine donor in the transamidinase system. 5. Kidney transaminidase activity in weanling rats fed a protein-free diet for 12 days was 24% of normal. The activity was restored to 80% of normal by feeding the rats a complete diet for 15 days following the feeding of a protein-free diet for 12 days. Thus, kidney transaminidase activity appears to be dependent on exogenous protein sources.

The authors wish to thank F. Bollum for helpful suggestions concerning liver arginase.

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## Cheyne-Stokes Breathing after Denervation of Carotid and Aortic Chemoreceptors and Sino-Aortic Pressoreceptors.* (23133)

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According to some theories concerning the mechanism of production of Cheyne-Stokes breathing oxygen-lack drive from chemoreceptors is a factor(1), while in other theories reflex drive is not mentioned(2). If Cheyne-Stokes breathing in any given instance is dependent upon a seesawing between the action of $CO_2$ on the respiratory center and oxygen lack drive exerted reflexly from chemoreceptors in the carotid and aortic bodies, it could not occur in animals having the chemoreceptors denervated, or if it should appear in an animal with IXth and Xth nerves intact it should be eliminated by denervation. In the course of studies on other aspects of control of respiration typical Cheyne-Stokes breathing was observed in 2 dogs having the sino-aortic zones denervated.

*Methods and results.* Each of the 2 dogs was anesthetized with sodium pentobarbital, 30 mg/kg, and had been bilaterally vagotomized at the upper cervical level. In the first experiment 500 ml of blood had been withdrawn from the dog which weighed 26 kg. Next, the region of the carotid bifurcation on each side was isolated by ligatures and excised. Shortly after this Cheyne-Stokes breathing developed. The respiratory record is shown in Fig. 1. Some of the apneic periods were as long as 40 seconds. The arrhythmia disappeared after a time to be replaced by rapid shallow breathing and returned after injection of an additional 5 mg of sodium pentobarbital per kg. The surgical procedures performed in this animal regularly result in elimination of the respiratory stimulation which is produced before denervation by 1 to 2 mg of NaCN injected intravenously; however, unfortunately, the cyanide test was not performed in this animal.

In the second dog (weighing 8 kg) the Cheyne-Stokes breathing appeared prior to isolation of the second one of the carotid zones. This animal showed a pronounced respiratory stimulation in response to 1 mg of NaCN, intravenously, before denervation. The carotid bodies and sinuses were isolated by means of ligatures placed on the appropriate arteries. The carotid arteries were cut open bilaterally to demonstrate that no blood supply to the bodies or sinuses remained. The Cheyne-Stokes respiration was a little more

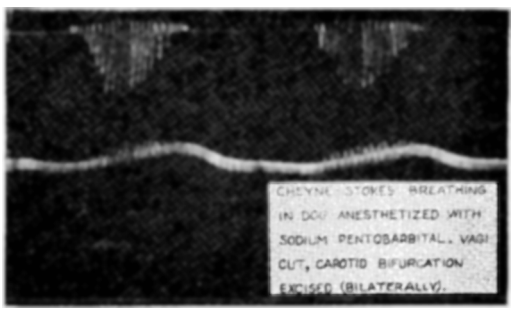


FIG. 1. Cheyne-Stokes breathing in dog having the vagi sectioned and carotid sinuses and bodies excised bilaterally. *Above:* Record of respiration. Downstroke indicates inspiration. *Below:* Blood pressure from femoral artery.

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