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Renin Substrate and Angiotonase in Dogs' Lymph and Plasma.*

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Besides renin, two substances of protein nature appear to be primarily responsible for the regulation of the angiotonin (hypertensin) concentration in the blood: Renin substrate (hypertensinogen) and angiotonase (hypertensinase), precursor and inactivator respectively of angiotonin.[1,2,3] Both have been demonstrated in the blood of dogs but not as yet in other body fluids. The present communication deals with the occurrence of these two substances in lymph.

Methods. Lymph was obtained from the cervical lymph ducts of normal dogs anesthetized with sodium pentobarbital. From the same animals, blood was taken simultaneously for preparation of plasma samples. Heparin was used as anticoagulant.

For determination of renin substrate content, lymph and blood plasma samples were incubated in quantities varying from 0.1 to 1.0 cc with a constant amount of renin for 10 minutes at room temperature (approximately 20 to 28°C), and the vasoconstrictor activity of the angiotonin formed was measured using the Läwen-Trendelenburg toad preparation. In order to insure a complete conversion of the renin substrate into angiotonin, an excess of renin was used. It was assumed that the amount of angiotonin so formed was proportional to the quantity of its precursor, the renin substrate originally present. In preliminary experiments, the quantity of renin was determined, which, when incubated with 1.0 cc of plasma, produced a maximal vasoconstrictor effect. A fourfold amount was adopted as a standard quantity in the substrate assays.

For determination of angiotonase, angiotonin was incubated with 1.0 cc of lymph or plasma for one hour at 37°C. Care was taken to avoid hemolysis of the blood, in order to exclude contamination of the samples with angiotonase derived from red cells. As observed by Fasciolo *et al.*,[3] the latter contain approximately 100 times as much angiotonase as plasma. Since practically no renin is present under such conditions, it was possible to estimate the quantity of angiotonase from the amount of angiotonin inactivated during incubation. Thus, angiotonin activity was used as an indirect criterion of the concentration of both renin substrate and angiotonase.

For estimation of the angiotonin, the samples were diluted immediately after incubation to 100 cc with Ringer's solution containing 0.5% sodium citrate. Their vasoconstrictor activity was then tested on the toad preparation which was perfused with the same modified Ringer's solution. Neither lymph nor plasma alone, in the dilutions used, produced any significant change in the rate of flow. In order to avoid effects due to differences in the responsiveness of individual toads, lymph and plasma samples from each dog, along with a suitable control, were always assayed in the same toad. The order was varied to offset any effects of decreasing responsiveness in the toad preparation.

The renin preparation was extracted from acetone-treated hog kidney cortex with $NaCl/NaHCO_3$ according to the Wakerlin and

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2 Braun-Menendez, E., Fasciolo, J. C., Leloir, L. F., and Munoz, J. M., *Rev. Soc. Argent. de Biol.*, 1939, **15**, 420.

3 Fasciolo, J. C., Leloir, L. F., Munoz, J. M., and Braun-Menendez, E., *Rev. Soc. Argent. de Biol.*, 1940, **16**, 643.

TABLE I.
Renin Substrate in Lymph and Plasma.
Vasoconstrictor Effects after Incubation with Renin.

Amount body fluid* cc	No. of toads	% change in flow (toad perfusion) Mean value and standard error† A Lymph* + renin	B Plasma* + renin	C Renin control	Difference (A-B) and standard error†
1.00	7	—71 ± 3	—68 ± 5	— 1 ± 4	— 3 ± 6
0.50	7	—63 ± 5	—64 ± 6	+ 1 ± 10	+ 1 ± 8
0.20	8	—40 ± 4	—57 ± 4	+13 ± 4	+17 ± 6
0.15	3	—36 ± 2	—38 ± 7	+11 ± 2	+ 2 ± 7
0.10	7	—32 ± 5	—32 ± 5	+ 7 ± 7	0 ± 7

* Samples obtained from 12 dogs.

† Standard error of the mean (S_x) and standard error of the difference of the means (S_D) computed for small samples:[7] $S_x = \sqrt{\frac{\Sigma(x^2)}{N(N-1)}}$; $S_D = \sqrt{\frac{\Sigma(x_1^2) + \Sigma(x_2^2)}{(N_1-1) + (N_2-1) \times \frac{N_1 \times N_2}{N_1 + N_2}}}$

The difference (A — B) is considered significant only when $\geqq 3 \times S_D$.

TABLE II.
Angiotonase in Lymph and Plasma.
Vasoconstrictor Effects of Angiotonin after Incubation with These Fluids.

Fluids incubated	No. of toads	% change in flow (toad perfusion) Mean and standard error	Difference (A-B) and standard error
A: Angiotonin + lymph*	12	—38 ± 5	—24 ± 6
B: Angiotonin + plasma*		—14 ± 3	
C: Angiotonin + control*		—71 ± 2	

* Samples obtained from 6 dogs.

Johnson modification of Grossman's method.[4,5] For each renin substrate assay, 0.2 cc of a standard renin solution containing 1.2 mg nitrogen per cc was used. Five cc of this solution produced a rise in blood pressure of 60 mm Hg. in 2.5 minutes, when injected intravenously into a normal unanesthetized dog.

Purified angiotonin preparations containing approximately 5 units per cc,[6] were most kindly supplied by Dr. I. H. Page. In each angiotonase assay, the smallest quantity of angiotonin was used which, when tested alone, produced a decrease in flow of from 60 to 80%.

Results. The experimental data obtained for renin substrate are summarized in Table I. For a given dose level, lymph and plasma, after incubation with renin, produced practically the same degree of vasoconstriction. With decreasing concentrations the effect declined at the same rate for both fluids. If it be assumed that in lymph the same mechanism is involved in causing the observed vascular effects as in plasma, it is apparent that renin substrate is present in similar concentrations in both.

However, the concentrations of angiotonase were found to be considerably different in these body fluids. Table II indicates that 1.0 cc of plasma contained enough angiotonase to inactivate most of the angiotonin present. Under identical conditions, 1.0 cc of lymph, on the other hand, neutralized a considerably smaller fraction of the angiotonin, the pressor action being reduced only to approximately one-half of that of the angiotonin control. If the inactivation of

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[5] Grossman, E. B., PROC. SOC. EXP. BIOL. AND MED., 1938, **39**, 40.

[6] Page, I. H., personal communication.

[7] Arkin, H., and Colton, R. R., *Outline of Statistical Methods*, New York, 1939, 126.

angiotonin by lymph and plasma involves the same mechanism, it is evident that the concentration of angiotonase in lymph is considerably lower than in plasma. It is not known whether the relatively much higher angiotonase content of the red blood cells[3] is responsible for the higher concentration of this substance in plasma, as compared to lymph.

Summary. Cervical lymph and plasma from normal dogs were compared as to their contents of renin substrate and angiotonase. It was observed that renin substrate occurs in similar concentrations in both lymph and plasma, but that the quantity of angiotonase present in lymph is considerably less than that in plasma.

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Action of Testosterone and Prolactin on the Corpora Lutea of the Rat.*

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In previous studies[1,2] it was shown that under certain experimental conditions the continuous administration of testosterone propionate (total dose, 20 to 40 mg) to female rats led to the formation of hypertrophied corpora lutea such as normally occurs during pregnancy. These structures likewise were found to have an active function, since deciduomata could be produced in the uteri while they were present. At that time we felt that the hypertrophy and function of these corpora resulted from an excessive secretion of prolactin due to a direct effect of the testosterone on the anterior hypophysis.

The present report deals with a series of experiments designed to determine (1) whether large dosages of prolactin lead to hypertrophy of corpora lutea, and (2) whether this luteal hypertrophy is necessary for active function, as indicated by the production of deciduomata.

Fifty-nine adult female rats of the Stanford strain, averaging 110 days of age, were employed. Daily vaginal smears of each animal were studied for 28 days previous to injection, and the first injection was always given during late estrus. The injections were administered subcutaneously for a period of 10 days. The testosterone propionate† was dissolved in sesame oil in varying concentrations and given in single daily doses of from 0.1 cc to 0.05 cc, according to the total dosage indicated in Table I. The prolactin was made up in saline solutions of varying concentrations and from 2 to 4 injections were given daily, but each consisted of 0.2 cc. The control animals received equivalent amounts of sesame oil or saline solution.

On the 5th day the animals were anesthetized, the abdomen opened, and a silk thread introduced for a distance of about 1 cm through the lumen of one of the uterine horns. The rats were sacrificed on the 11th day and the ovaries, together with sections of the uteri taken at the level of the traumatization with

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2 Laqueur, G. L., and Fluhmann, C. F., *Endocrinology*, 1942, **30**, 93.

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