few weak responses to rabbit seminal plasma were obtained. This challenges the widely accepted view that sperm of all species is a sequestered antigen which is antigenic when introduced into the systemic circulation. These results agree with those of Edwards (9). More unexpectedly, human sperm was not found to be antigenic since all reactions could be abolished with HSP. These findings support the view of Weil(10) that the predominant antigen in semen is in the seminal plasma and that reactions with sperm are due to their coating with this antigen. Hathaway and Hartree(11) found that Ouchterlony reactions to ram sperm *vs* rabbit antisera were abolished by prior addition of ram seminal plasma. This indicates that ram as well as human sperm is non-antigenic in the rabbit.

Summary. Rabbits were immunized with rabbit or human seminal plasma and/or sperm in complete adjuvant. Sperm counts and semen volumes were not depressed nor were testicular changes produced. Antibody studies using hemagglutination and Ouchterlony technics indicated that: a) rabbit sperm was not antigenic in rabbits, b) rabbit seminal plasma was weakly antigenic in some rabbits, non-antigenic in others, c) human sperm was not antigenic in rabbits, d) human seminal plasma was antigenic in rabbits, and e) human kidney shared a common antigen with human seminal plasma.

1. Voisin, G., Delaunay, A., Barber, M., *Ann. Inst. Pasteur,* 1951, v81, 48.
2. Freund, J., Lipton, M. M., Thompson, G. E., *J. Exp. Med.,* 1953, v97, 711.
3. ———, PROC. SOC. EXP. BIOL. AND MED., 1954, v87, 408.
4. Weil, A. J., Finkler, A. E., *ibid.,* 1959, v102, 624.
5. Katsh, S., Katsh, G. F., *Fert. & Sterility,* 1961, v12, 522.
6. Kibrick, S., Belding, S. L., Merrill, B., *ibid.,* 1952, v3, 430.
7. Ingraham, J. S., PROC. SOC. EXP. BIOL. AND MED., 1958, v99, 452.
8. Stevens, K. M., Fost, C. A., Ison, C. M., *ibid.,* 1963, v113, 188.
9. Edwards, R. G., *J. Reprod. Fertil.,* 1960, v1, 385.
10. Weil, A. J., *Fert. & Sterility,* 1961, v12, 538.
11. Hathaway, R. R., Hartree, E. F., *J. Reprod. Fertil.,* 1963, v5, 225.

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Differences in Levels of Circulating Vasopressor Materials in Dogs with Acute and Chronic Renal Hypertension.* (29514)

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It was shown previously in this laboratory that the blood of rats with acute renal hypertension (1-14 days) yielded a vasopressor response when injected into normal recipient rats(1). In contrast, no pressor response could be obtained when blood from rats with chronic renal hypertension was tested in the same manner.

This paper concerns findings of a similar study in dogs with renal hypertension, which essentially confirm the results of our previous observations in rats.

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Methods. Method of producing hypertension. Eight dogs weighing from 15 to 20 kg were rendered hypertensive by constricting the main renal arteries with Goldblatt clamps. In 5 dogs both renal arteries were constricted and in 3 dogs the right kidney was resected and a clamp was applied to the left main renal artery. All operative procedures were done in one stage. The surgery was performed by Dr. Lester Persky, Professor of Urology, Western Reserve University.

Blood pressure recording. Blood pressure was recorded on a Hg. manometer by means of direct puncture of the femoral artery.

Readings were usually taken at intervals of a few days during the first 3 weeks after renal clamping, then at weekly intervals until 2 months had elapsed. Subsequent pressures in the chronic hypertensive dogs were measured at approximately monthly intervals.

Test for pressor activity. The dogs were tested for pressor activity by injecting 1 cc of their plasma into the circulation of normal rats. The plasma was obtained from femoral arterial blood and was injected immediately. In several instances the plasma samples were refrigerated and retested after a few to several weeks.

Recipient rats weighing about 180 g were anesthetized with 2% sodium pentobarbital given intraperitoneally in dosage of 0.2 cc/100 g body weight. No. 50 polyethylene catheters were inserted into the femoral artery for blood pressure determinations and into the femoral vein for injections of plasma or angiotensin. Blood pressures in recipient rats so prepared ranged from 98 to 120 mm Hg. prior to testing.

When the blood pressure of the recipient rat had been stable for several minutes, 1 cc of plasma from a hypertensive dog was injected into the venous catheter over a period of 30 seconds. The response of the recipient was measured in mm Hg. rise in blood pressure. As a control either 1 cc of plasma from a normal dog (negative control) or plasma from a dog with acute renal hypertension (positive control) was used. The 2 plasmas to be tested in each recipient rat were injected within approximately 5 minutes of each other. Negative controls were usually paired with plasma of acute hypertensive dogs and positive controls were paired with plasma of chronic hypertensive dogs. Each recipient received only 2 injections, one from the hypertensive dog and one from the control dog. Either the hypertensive or control plasma was given first and the order of injection did not influence the results. In a positive pressor response, the elevation of pressure began within several seconds after injection of plasma, reached a peak within one minute, then returned gradually to the initial baseline within 5 minutes. This simulates the type of pressor response which is observed following intravenous injection of angiotensin. The recipients usually had rises of 20-30 mm Hg. in response to injections of .005-.01 μg angiotensin.

Assays for vasopressor material were performed prior to renal artery clamping and subsequently at intervals from 2 days to 10 months during both the acute and chronic phases of renal hypertension.

Results. Table I shows the data on 8 dogs whose blood was tested for vasopressor effect during the acute phase (first 2 weeks) of renal hypertension. The postoperative blood pressures during the first 2 weeks ranged from 108 to 180 mm Hg. Three dogs (nos. 1, 2 and 3) died in uremia, 2 at 6 days and one at 2 weeks, probably because renal artery constriction was too severe. Pressor material was readily demonstrated in these 3 dogs, although in 2 animals only one test was done.

In dog no. 4 which died in uremia 5 weeks after renal artery clamping, the assays were positive from the 3rd to 17th postoperative day, then became negative at 3 and 4 weeks. Four dogs (nos. 5-8) with renal artery clamps survived the stage of acute hypertension. These 4 animals showed definite pressor activity during the first 7 days after clamping but after 2 weeks such activity was present in only one animal and this dog became negative after 3 weeks. In the period from 2 or 3 weeks to 10 months after renal artery clamping the blood pressures of these dogs ranged from 160 to 200 mm Hg. However, on repeated tests at weekly or monthly intervals their plasma failed to give any pressor reaction in recipient rats (Fig. 1), although the latter responded to angiotensin. Also

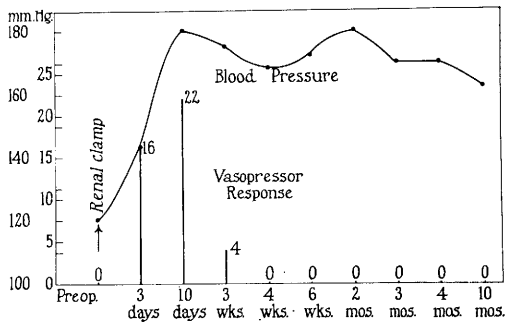

FIG. 1. Blood pressures and vasopressor responses in hypertensive dog (no. 8).

TABLE I. Assay for Vasopressor Material in Dogs with Acute Renal Hypertension.

Dog No.	Pre-op. B.P.	Operation	Post-op. B.P.	Time after operation (days)	Pressor response in assay rats (mm Hg)
1	100	Bilateral clamp	130	2	16
2	110	" "	n.r.*	5	26
3	90	Rt. kidney out, left clamp	128	3	20
			130	7	14
			160	10	31
4	115	Bilateral clamp	130	3	16
			180	7	18
			140	10	6
			130	14	4
5	120	" "	120	3	16
			142	10	22
			175	14	6
6	120	Rt. kidney out, left clamp	n.r.	3	16
			150	6	12
			n.r.	10	0
			200	16	0
7	120	Bilateral clamp	130	7	6
			140	14	0
8	120	Rt. kidney out, left clamp	112	5	8
			108	7	20
			130	14	0

* n.r. = not recorded.

plasma from dogs in the acute stage of hypertension which served as 'positive' controls invariably gave good pressor responses when tested. A total of 30 assays of plasma from the 4 dogs with chronic renal hypertension was performed and were all negative.

Thus in all 8 dogs with constricting clamps on the renal arteries, the plasma gave a definite pressor response when tested within one week after clamping. Three of 6 animals whose plasma was tested from 8 to 21 days had positive responses. After 3 weeks all assays for vasopressor material were negative even though the average mean blood pressures were higher than before.

Discussion. Pickering, Prinzmetal and Kelsall(2) could not recover renin from the blood of rabbits with chronic renal hypertension of 2 to 5 months duration. Peart, Robertson, and Grahame-Smith(3) were unable to demonstrate renin release into the renal vein in any of the rabbits which they studied from 3 to 259 days after onset of renal hypertension.

Haynes and Dexter(4) determined the renin content of plasma from dogs with acute and chronic renal hypertension. After removal of the angiotensinase, 8 to 10 cc samples of plasma were incubated with an excess of beef angiotensinogen and the resulting angiotensin was assayed for its pressor effect in cats. They found that the renin level reached a maximum about one week after constriction of the renal arteries, then gradually fell so that after a period of weeks or months renin was no longer detectable in the plasma.

Gollan, Richardson and Goldblatt(5) removed 700, 1500 and 800 cc of blood respectively from each of 3 dogs which were hypertensive for 24 to 72 hours, pooled the specimens and after chemically processing and concentrating the plasma found that the concentrate gave a good pressor response when injected into normal dogs. Also single samples of 70 to 450 cc of blood from each of 8 other dogs with renal hypertension of 2 days to 3 weeks duration were processed and tested in like manner. The rise in blood pressure varied from 0 to 40 mm Hg. and the average rise for the group was 16 mm Hg. Five dogs with chronic renal hypertension (2½ mo to 3 yr) were also studied and the plasma concentrates from 200 to 520 cc of blood yielded rises in pressure of 0 to 20 mm Hg. with an average of 9 mm. The au-

thors concluded that a large amount of blood equivalent to 1/5 to 1/3 of the total blood volume was needed to detect the presence of renin in the blood, especially later in the course of experimental renal hypertension.

Skeggs, Kahn and Shumway(6) obtained a dialysate of blood with an artificial kidney and then purified, concentrated and tested the dialysate for its pressor effect in rats. They found an average of 0.25 angiotensin unit/l dialysate in 5 dogs studied 4 to 15 days after hypertension was produced by renal artery clamps. However, in 6 dialyses involving 4 dogs with hypertension of 21 to 90 days duration they obtained an average of only 0.06 unit of angiotensin/l of dialysate, if dog #3 is excluded. This animal, which was dialyzed once and gave a value of 0.42 unit, had malignant hypertension and subsequently died in uremia.

Govaerts and Verniory(7) produced acute hypertension in 6 dogs by removing the right kidney and constricting the left main renal artery. Utilizing hind limb perfusion in bilaterally nephrectomized dogs for pressor assays and employing 50 cc samples of blood removed from the inferior vena cava just above the renal veins, they found increased amounts of vasoconstrictor material in dogs which were hypertensive from 3 to 31 days. In contrast there was no vasoconstrictor activity when blood from dogs with constriction of both renal arteries was tested 35 to 100 days after application of the clamps.

More recently Scornik and Paladini(8), using an improved technique which yielded 60 to 65% recovery of angiotensin from 50 cc samples of blood, obtained similar results in the acute and chronic phases of renal hypertension in dogs. The blood of 17 dogs tested during the first week of hypertension yielded an average value of 21 rat units of angiotensin/l. In 8 dogs with hypertension from 4½ to 40 months, assays of blood gave an average of 4.7 rat units of angiotensin/l which was equivalent to that of their normal control dogs.

The above experiments in dogs were selected because of substantial differences in the technique of preparation and assay of blood for pressor substances and because the investigators tested animals with both acute and chronic renal hypertension. However, the results as a whole generally support the conclusion that there is a distinct difference in the amount of vasopressor material which can be detected in the blood of dogs in the acute and chronic phases of renal hypertension. Pressor activity was demonstrated with relative ease in the first 2 or 3 weeks but only with great difficulty, if at all, in hypertension of longer duration.

Our rather simple and direct method of assay confirms this observation. In the dog as in the rat(1) we found a circulating pressor substance only in the acute stage of renal hypertension. In chronic hypertension such material is either no longer elaborated or, if elaborated, is in some way mostly neutralized or the amount produced becomes too small to be readily detected. Perhaps more refined methods for the recovery of angiotensin or renin will shed some light on these alternatives.

At present, however, this difference between the acute and chronic stages of experimental renal hypertension remains to be explained. Any theory on the pathogenesis of experimental renal hypertension based on circulating pressor agents should account for the difficulty in demonstrating a pressor agent in the blood when the hypertension is well established or in its chronic phase, and the relative ease of detection during the early period of pressure elevation. It seems likely that in chronic hypertension factors other than the level of circulating pressor agent are responsible for the high blood pressure. For example, it was suggested recently by McCubbin and Page(9) that a small quantity of renin or angiotensin might sustain high blood pressure by augmenting the neuropressor mechanism in vascular smooth muscle.

Summary. Small amounts of plasma from dogs with acute renal hypertension were shown to contain easily detectable amounts of a vasopressor substance for approximately the first 2 weeks after renal artery constriction. This is consistent with the view that a humoral mechanism plays a role in the origin

of the high blood pressure. With our technique the vasopressor material tended to disappear from the blood between 1 and 3 weeks after renal artery clamping and thereafter all assays of plasma for vasopressor activity were negative. Thus, in the dog as in the rat, and for reasons not clear, the acute and chronic stages of experimental renal hypertension differ sharply in respect to recovery of circulating pressor substance. While this finding does not necessarily exclude a humoral system from participating in the pathogenesis of chronic renal hypertension, it indicates that the level *per se* of circulating vasopressor material is not the critical factor in sustaining the high blood pressure in this stage.

1. Koletsky, S., Pritchard, W. H., *Circulation Res.*, 1963, v13, 552.
2. Pickering, G. W., Prinzmetal, M., Kelsall, A. R., *Clin. Sci.*, 1942, v4, 401.
3. Peart, W. S., Robertson, J. I. S., Grahame-Smith, D. G., *Circulation Res.*, 1961, v9, 1171.
4. Haynes, F. W., Dexter, L., *Am. J. Physiol.*, 1947, v150, 190.
5. Gollan, F., Richardson, E., Goldblatt, H., *J. Exp. Med.*, 1948, v88, 389.
6. Skeggs, L. T., Kahn, J. R., Shumway, N. P., *Circulation*, 1951, v3, 384.
7. Govaerts, P., Verniory, A., *Acta Med. Scand. Suppl.*, 1952, v266, 419.
8. Scornik, O. A., Paladini, A. C., *Am. J. Physiol.*, 1961, v201, 526.
9. McCubbin, J. W., Page, I. H., *Science*, 1963, v139, 210.

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Serum Vitamin E Levels in a Normal Adult Population in the Washington, D. C., Area. (29515)

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There is currently considerable interest in attempting to establish the human dietary requirement for vitamin E and, concomitantly, devising procedures for assessing the nutritional status with respect to this vitamin (1,2). The accumulation of information on the normal distribution of blood levels of vit. E (α-tocopherol) is a necessary step in this endeavor. We present here values for 132 normal adults residing in the area of Washington, D. C., which supplement data reported recently by Harris *et al*(3) for adults in Rochester, N. Y. These workers also summarized previous surveys of serum tocopherol values.

Methods. Subjects were new employees of the Nat. Inst. of Health who had passed a physical examination and were in apparent good health. Age range was 17-55 years and the division between Negroes and Caucasians was approximately 1:2. The survey was done during July and August, 1963. Blood was drawn by venipuncture in the Employee Health Service between 9 and 11 a.m.; subjects were not fasting. Eight to 10 samples daily were collected and taken to the laboratory for immediate analysis.

For the analysis of vit. E, 1.0 ml of serum was thoroughly mixed with 1.0 ml of redistilled 95% ethanol in a 15 ml glass-stoppered centrifuge tube. Three ml of petroleum ether (B.P. 40-60°C) were added and the tubes shaken rapidly by hand, or on a mechanical shaker, for 3 minutes. After centrifuging, 2 ml of the petroleum ether were carefully pipetted off and transferred to a 10 $\times$ 75 mm cuvette for the Coleman Junior Spectrophotometer. A reading at 450 mμ was made for total carotenoids. The tubes were placed in a 50°C water bath and the solvent evaporated with a stream of nitrogen. One-tenth ml of

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