9. Cohn, M., Simmonds, S., Chandler, J. P., Du-Vigneaud, V., *ibid.*, 1946, v162, 343.
10. Roche, M., Benedict, J. D., Yü, T. F., Bien, E. J., Stetten, D., *Metabolism*, 1952, v1, 13.
11. Tower, S. S., *J. Comp. Neurol.*, 1937, v67, 109.
12. ———, *ibid.*, 1937, v67, 241.

Received May 16, 1962. P.S.E.B.M., 1962, v110.

Changes in Corticosterone Levels after Severe Stress in the Rat. (27620)

R. F. TIMMER (Introduced by E. G. Rennels)
Department of Anatomy, University of Colorado Medical School, Denver

Many measurements of free corticosteroids after various forms of stress have been noted in the literature(1). Rennels and Timmer (2) noted that free plasma corticosterone levels in the rat reached a peak level about one hour after scalding and remained elevated for 24 hours. Knigge *et al.*(3), using similar stress and adrenal vein cannulation technics, reported normal values 4 hours after scalding and a marked decrease in corticosterone secretion 12-36 hours after scalding. Rennels and Timmer(2) found maximum hemoconcentration as measured by hematocrit values occurred at one to 3 hours after stress. Marked reduction in blood volume following burns, which is due mainly to increased capillary permeability with resultant plasma loss, has been well documented(4). The extensive plasma loss occurring after burns raised the question as to how accurately the concentration of corticosterone in the blood plasma actually reflects adrenal corticosteroid output. For these reasons the author collected "burn tissue fluid" and measured its concentration of free corticosterone. The substance measured in burn tissue fluid was presumed to be corticosterone on the basis of the work by Silber, *et al.*(5) and will be referred to as such although its identity has not been rigidly established in burn tissue fluid. Previous reports have indicated that after periods longer than 24 hours hematocrit values returned toward normal levels accompanied by a marked reduction in edematous fluid volume(4). In the present study measurements were made of hematocrit values, free plasma corticosterone levels and free corticosterone in "burn tissue fluid" at time periods up to 168 hours after the burn stress.

Methods. Male Sprague-Dawley rats, weighing approximately 230 g were anesthetized with sodium pentobarbital (4.5 mg/100 g body weight) and immersed except for the head in 70°C water for 5 seconds. Animals were decapitated at one-half, 3, 12, 24, 48, 72, 96, 120 and 168 hours after burning. The blood of each animal was collected in heparinized tubes, immediately centrifuged, and determinations of free plasma corticosterone were made using the method of Guillemin *et al.*(6) with specificity of corticosterone established by Silber *et al.*(5) and Peterson (7). Hematocrits of individual animals were taken at time of decapitation. "Burn tissue fluid" was collected from the edematous feet of burned animals after decapitation by applying pressure to these areas and collecting the fluid in a heparinized tube. "Burn tissue fluid" was defined as all fluid obtained by applying pressure to the edematous feet, recognizing that most fluid obtained was extracellular, with possibly some intracellular fluid and small amounts of blood. Appropriate control animals were maintained for all groups.

Results. The highest level of free plasma corticosterone (52.5 μg/100 ml of plasma) was found in burned animals 3 hours after stress (Table I). All groups killed at time periods greater than 3 hours showed a progressive decline in concentration of plasma corticosterone. Approximately 48 hours after stress the animals had returned to resting levels (12 μg/100 ml plasma) of free plasma corticosterone. With one exception (72 hours) only slight variations from this level were found during the next 120 hours. Hematocrit values of burned animals showed a peak

TABLE I. Plasma Levels and "Tissue Fluid" Corticosterone Following Stress.

Hr after stress	No. of animals	Plasma vol (ml)*	Hemato-crit	Plasma corticosterone (μg/100 ml)	Tissue fluid (ml)†	Tissue fluid corticosterone (μg/100 ml)
Control	10	2.8	41	15.0 ± .8‡	0	—
½	10	1.7	51	45.0 ± 1.3	1.5	132.8 ± 4.6
3	10	1.0	59	52.5 ± 1.1	4.0	204.0 ± 5.8
12	10	1.1	55	32.0 ± 1.0	4.5	91.6 ± 4.1
24	10	1.5	46	25.6 ± 1.0	3.0	38.2 ± 6.1
48	10	3.2	36	12.0 ± 1.2	4.0	5.0 ± 1.4
72	9	2.8	38	25.6 ± .9	3.5	23.0 ± 2.6
96	9	2.7	33	14.5 ± 1.1	2.0	9.0 ± 2.0
120	9	2.1	36	12.0 ± 1.3	1.5	14.5 ± 2.6
168	10	2.6	40	14.5 ± 1.0	0	—

* Avg plasma volume per animal.
† Total volume "tissue fluid" obtained per group of animals.
‡ Stand. error.

hemoconcentration at 3 hours after burning. After 3 hours, all groups indicated a gradual decrease in hemoconcentration, returning to below normal values 48 hours after stress. This situation seemed to persist in all subsequent time intervals. A confirmatory expression of hematocrit values was noted in the average plasma volume obtained from each animal (Table I). The smallest plasma volume was obtained from animals 3 and 12 hours after stress when hematocrits were high. Peak hemoconcentration occurred simultaneously with the maximum level of free plasma corticosterone at 3 hours after burning. There was a gradual return toward normal values of both hemoconcentration and plasma corticosterone. Both reached normal levels at approximately 48 hours after stress and both remained at or below normal levels through 168 hours (Fig. 1).

Table I lists values for "tissue fluid corticosterone" and total volume of fluid obtained from pooling of all animals in each group. The greatest total amount of fluid was obtained 12 hours after scalding. All subsequent burned groups indicated a gradual decrease in total volume of fluid obtained. High values for tissue fluid corticosterone were found ½ and 3 hours after stress. The time periods after 3 hours showed a gradual decrease in tissue fluid corticosterone concentration. The 48-hour levels were lowest and increased slightly in later time periods (Fig. 1). No fluid was obtainable at 168 hours after burn.

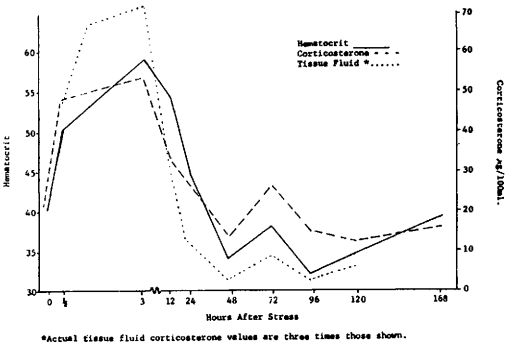

FIG. 1. Time relationship of plasma corticosterone, tissue fluid corticosterone and hematocrit after stress.

Discussion. In previous work by Rennels and Timmer(2), absolute peak values of free corticosterone were somewhat less than those found in this experiment although time relationships of peak corticosterone levels were approximately the same. Possibly the different values obtained were due to the use of rats of a different strain and age. Changes in hematocrit were similar in all respects to our previous findings. Average plasma volume per animal was greater in this experiment due to increased size of the animals (230 g *vs* 155 g).

In general, the levels of free plasma corticosterone found at time intervals after 24 hours were similar to control values. The group 72 hours after stress indicated a slight elevation which appeared to be real. From these data it appears that scalding exerts approximately a 24-hour effect on the elevation of plasma adrenal steroids. This period seems

critical in our experience because if the animal is alive 24 hours after a burn, it will most likely survive for a considerably longer period. The possible relationships of the return to normal of hematocrit values, plasma corticosterone, and tissue fluid corticosterone levels at 24 hours after scalding with survival of animals subsequent to this period involves speculation.

The levels of tissue fluid corticosterone indicated a remarkable loss of corticosterone through the capillary bed into the surrounding tissues during the first one-half to 3 hours after stress. Under these conditions it appears that the rapid rise in tissue fluid corticosterone in the 3 hours after stress follows the marked increase in edema fluid which is expressed partly by the change in hematocrit. Other materials have been noted in the literature to pass with plasma through the capillary bed in burned subjects(8). After 12 hours, the level of tissue fluid corticosterone drops, reaching a minimum value 48 hours after stress. Whether the decline in tissue fluid corticosterone is due to return of corticosterone to the vascular compartment is not known.

The simultaneous rise of plasma levels of corticosterone and tissue fluid corticosterone as noted in Fig. 1 might lead to the assumption that adrenal corticosterone output would reach a maximum rate 3 hours after scalding. Knigge *et al.*(3) using a similar form of stress noted a marked elevation in corticosterone production when measured in blood from adrenal vein cannulation during the first 2 hours but below normal values were noted 4 hours after burn. Whether the quantities they measured would account for the values observed in this experiment is uncertain because of changes in volume in both experiments. The decline of plasma and tissue fluid corticosterone values at 12 hours after scalding suggests a drop in adrenal output of corticosterone occurring about 12-24 hours after stress with a possible slight increase occurring at 72 hours after burn.

Yates *et al.*(9) noted that the circulating level of corticosterone appears to control the amount of ACTH released after a stress presets the mechanism for corticosterone requirements in animals. Thus, to reach and maintain necessary levels of circulating corticosterone, large amounts of ACTH may need to be secreted to obtain the observed corticosterone levels in burned animals. This concept is in accord with the reports by Holub, *et al.*(10) and Timmer(11) that the pituitary content of ACTH is markedly reduced by scalding.

Another factor which might affect corticosterone levels in burned animals involves the bound fraction of corticosterone. The wide variations in circulating proteins of burned subjects(12) may cause considerable changes in bound fractions of plasma corticosterone. It should be emphasized that the technics employed in this study did not permit measurement of protein bound corticosterone.

Summary. Fluorometric measurement of free plasma corticosterone in rats after sustaining a burn in 70°C water indicated an increased concentration at 3 hours after stress with a gradual return toward normal by 48 hours after stress. Hematocrit values and average plasma volumes indicated the greatest hemoconcentration at 3 hours after stress. Volume of tissue fluid obtained from edematous areas was largest at 12 hours after stress. Measurements of free corticosterone in "burn tissue fluid" indicated that the maximum value occurred at 3 hours after stress.

1. Guillemin, R., Clayton, G. W., Smith, J. D., Lipscomb, H. S., *Endocrinology,* 1958, v63, 349.

2. Rennels, E. G., Timmer, R. F., *Research in Burns,* Waverly Press, Inc., Baltimore Md., 1962, 367.

3. Knigge, K. M., Penrod, C. H., Schindler, W. J., *Am. J. Physiol.,* 1959, v196, 579.

4. Gregerson, M. I., Rawson, R. A., *Physiol. Reviews,* 1959, v39, 307.

5. Silber, R. H., Busch, R. D., Oslapas, R., *Clin. Chem.,* 1958, v4, 278.

6. Guillemin, R. G., Clayton, G. W., Lipscomb, H. W., *J. Lab. & Clin. Med.,* 1959, v53, 830.

7. Peterson, R. E., *J. Biol. Chem.,* 1957, v25, 225.

8. Beard, J. W., Blalock, A., *Arch. Surg.,* 1931, v22, 617.

9. Yates, F. E., Leeman, S. E., Glenister, D. W., Dallman, M. F., *Endocrinology,* 1961, v69, 67.

10. Holub, D. A., Kitay, J. I., Jailer, J. W., *ibid.*, 1959, v65, 968.

11. Timmer, R. F., *Texas Rep. on Biol. Med.*, 1960, v18, 309.

12. Nylen, B., Wallenias, G., *Acta Chir. Scand.*, 1961, v122, 97.

Received May 18, 1962. P.S.E.B.M., 1962, v110.

Natriuresis in Conscious Dogs during Arginine Vasopressin Infusion and after Oxytocin Injection.* (27621)

W. Y. CHAN† AND WILBUR H. SAWYER

Department of Pharmacology, College of Physicians and Surgeons, Columbia University, New York

Both vasopressin and oxytocin can enhance electrolyte excretion by conscious dogs(1-4). Brooks and Pickford(2) observed that low doses of arginine vasopressin cause natriuresis only if administered during water diuresis. Oxytocin, however, is natriuretic only when the rate of urine flow is low. We have confirmed both these observations(4). The peculiar relation of the rate of urine flow to natriuretic responses suggests that endogenous vasopressin may enhance the natriuretic response to oxytocin while it may inhibit the response to exogenous vasopressin. In this paper we describe the influence of prolonged infusion of arginine vasopressin on natriuretic responses of conscious hydrated dogs to oxytocin.

Materials and methods. All experiments were performed on 4 trained female dogs weighing between 15 and 21 kg. They were not fed but were allowed water *ad lib.* for 12 hours preceding each experiment. Diuresis was induced by oral administration of 40-50 ml/kg of water.

In the experiments on electrolyte excretion, arginine vasopressin was injected intravenously in a priming dose of 0.5 to 1.0 mU and then infused at 15-20 mU/hr. After the rates of urine flow and electrolyte excretion became stable, 100 to 150 mU of oxytocin were given intravenously. Urine was collected from an indwelling bladder catheter at 10-minute intervals. The bladder was rinsed with water and air at the end of each period. Sodium and potassium were measured by internal standard flame photometry.

Glomerular filtration rate was measured by exogenous creatinine clearance. Renal plasma flow was estimated by either para-aminohippurate or iodopyracet (Diodrast) clearance. The indicators were infused intravenously.

The hormones used were purified arginine vasopressin (Pitressin of bovine origin) supplied by the late Dr. D. A. McGinty of Parke, Davis and Co., and synthetic oxytocin (Syntocinon) supplied by Dr. R. Bircher of Sandoz Pharmaceuticals. Both preparations were standardized in this laboratory against USP Reference Standard by rat vasopressor or rat uterus assays.

Results and discussion. Low doses of arginine vasopressin cause a marked increase in rate of sodium excretion by conscious dogs during water diuresis(2,3,4). There is also a variable increase in potassium excretion. Vasopressin infusion, however, at 1 mU/kg $\times$ hr provokes a transient natriuresis that begins to subside even before antidiuresis becomes maximal (Fig. 1A). Sodium excretion returns to the control rate within an hour, although antidiuresis persists as long as the infusion is maintained (up to 4 hours). This indicates that the kidney adapts rapidly to the natriuretic influence of vasopressin, becoming refractory despite persistent antidiuresis. Such development of resistance to the natriuretic action could be of adaptive value to the organism, allowing maintained antidiuresis in response to endogenous vaso-

* Supported by grants from Nat. Inst. of Arthritis and Metab. Dis., U.S.P.H.S., and from the Lowell M. Palmer Foundation.

† Present address: Dept. of Biochemistry, Cornell Univ. Med. Coll., New York.