negative inotropic effect. Other substances used to dissolve steroids such as methylene chloride and propylene glycol also markedly depress the contractile force of the papillary muscle. Therefore, ethanol is not unique among steroid solvents in depressing cardiac contractility.

Ethanol, however, appeared to decrease the responsiveness of cardiac muscle to corticosteroids. This can be inferred from the fact that the steroid treated muscles showed the same degree of depression as the ethanol treated muscles in almost all cases. If the ethanol merely depressed contractility, one would expect the steroid treated muscles to show less depression than their corresponding vehicle controls, particularly in the cases of dexamethasone and hydrocortisone.

This study calls attention to the fact that the manner in which corticosteroids are solubilized may play an important role in determining their eventual effect. Certainly, if the vehicle exerts an effect opposite in direction to that of the steroid, the steroid effect may be masked by the vehicle. This appears to be the case with the inotropic effect of corticosteroids dissolved in ethanol.

The mechanism for the enhancement of corticosteroid induced inotropic responses in a low calcium medium remains unanswered. Since lowering the sodium or the potassium concentration of the medium did not enhance inotropic responsiveness to corticosteroids, some specific role of calcium (*e.g.*, in relation to excitation-contraction coupling) may explain this phenomenon. No obvious correlation of inotropic activity with mineralocorticoid, glucocorticoid or anti-ouabain potency exists. Therefore, it seems likely that the inotropic effect exerted by some corticosteroids is brought about by mechanisms other than those involved in the more classic actions of these hormones.

Summary. A series of 9 corticosteroids dissolved in ethanol were studied in the isolated cat papillary muscle preparation maintained at 27°C in a reduced calcium buffer. Only aldosterone exerted a significant positive inotropic effect. The magnitude of this positive inotropic effect was larger than previously observed at 37°C in a normal calcium buffer. The ethanol was markedly depressant to the papillary muscles. Hydrocortisone and dexamethasone, in aqueous solutions, also exerted positive inotropic effects, whereas prednisolone and methylprednisolone were ineffective. Lowering the sodium or the potassium concentration of the medium did not result in significant inotropic responses to aldosterone or hydrocortisone.

1. Emele, J. F., Bonnycastle, D. D., Am. J. Physiol., 1956, v185, 103.

2. Tanz, R. D., Kerby, D. F., J. Pharmacol. Exp. Therap., 1961, v131, 56.

3. Loubatieres, A., Sassine, A., C. R. Acad. Sci. (Paris), 1966, v255, 373.

4. Lefer, A. M., Sayers, G., Am. J. Physiol., 1965, v208, 649.

5. Sayers, G., Lefer, A. M., Nadzam, G. R., Endocrinology, 1966, v78, 211.

6. Lefer, A. M., J. Pharmacol. Exp. Therap., 1966, v151, 294.

Received December 2, 1966. P.S.E.B.M., 1967, v125.

Comparison of Endogenous and Exogenous Creatinine Clearances in Man.* (32050)

RALPH GOLDMAN, RICHARD A. YADLEY, AND DAVID S. NOUROK
(With the technical assistance of Pedro Fiorello)
Department of Medicine, University of California Center for the Health Sciences, Los Angeles

Shannon(1) demonstrated that the excretion of exogenous creatinine in man is greater than can be expected from glomerular filtration alone. He concluded from these and

* Supported in part by USPHS HE-07852, Los Angeles County Heart Assn. and Southern California Chapter, National Kidney Foundation.

subsequent studies(2) that there is active tubular secretion of creatinine by a mechanism having an absolute limitation of transport capacity. Although this concept is generally held(3), there are several experimental observations which are inconsistent with the proposed mechanism for tubular secretion. Many investigators† have shown that the true endogenous creatinine:inulin clearance ratio is only slightly greater than 1.0, and significantly less than the ratio of 1.4 which is derived from the exogenous clearance data. Miller and Winkler(5) demonstrated that the low endogenous creatinine:inulin clearance ratio increased abruptly following infusion of exogenous creatinine, an observation conconfirmed by Shannon and Ranges(2). In addition, Shannon's data showed that while the creatinine:inulin clearance ratio fell with a rising serum creatinine, it did not subsequently rise with a falling serum creatinine, which it should have done if a Tm (creatinine) were operative(1). If at this point there was a rapid infusion of exogenous creatinine, the high creatinine:inulin clearance ratio could be restored(2). These data support the contention of Miller and Winkler(5) that the secretion of exogenous creatinine by the human kidney is a response to a special stimulus rather than a continuous physiological process. The following experiments were designed to explore the difference between the excretion of endogenous and exogenous creatinine, and to determine the amount of exogenous creatinine required to alter this component of tubular function.

Materials and methods. Simultaneous inulin and creatinine clearances were performed in normal subjects and patients with renal functional impairment. The 9 normal control subjects consisted of hospital personnel and patients without evidence of renal involvement. The 6 patients had various types of renal lesions and varying levels of function. Each subject was hydrated with 1000 ml of water prior to the test. The inulin prime was then given and the sustaining solution started. Constancy of infusion was insured by the use of an infusion pump. When the urine output had stabilized at a maximum flow rate, the inulin and endogenous creatinine clearance periods were started. Each period was approximately 15 minutes in length. The blood-to-urine delay time was estimated at 5 minutes. Blood specimens were obtained 5 minutes before the beginning and end of each urine collection period, and the interpolated mean value used to calculate the clearance. At the completion of the third period creatinine was added to the inulin sustaining solution. Six more clearance periods were then obtained with rising serum creatinine levels. No subject was catheterized, although 2 uremic patients already had catheters in place. Inulin in serum and urine was determined by the method of Schreiner(6). The creatinine in the plasma filtrate and urine was adsorbed onto Lloyd's reagent according to a method derived from Hare(7), then eluted at the time of color development in order to determine only true creatinine. The plasma filtrate for creatinine was prepared by the method of Brod and Sirota(8).

Results. The average rate of creatinine infusion was about 20 mg per minute. Assuming a total body water of 50 kg, and not correcting for the increased creatinine loss, the increment in serum creatinine should be on the order of 0.04 mg% per minute, or 0.6 mg% per 15-minute period. Examination of Table I indicates that the predicted and actual blood levels were in reasonable agreement.

The inulin clearances were essentially constant throughout each study, as were the endogenous creatinine clearances. However, immediately following the infusion of exogenous creatinine in the normal subjects there was an abrupt increase in the creatinine clearance, at a time when the serum creatinine had increased only 10%. The creatinine clearance increased further during the second 15-minute period, then gradually declined during the subsequent 4 periods, remaining well above the endogenous clearance levels. With the exception of one patient (C) who had the highest clearance of any of the patients, there was no significant increase in the creatinine clearance in any individual with renal insufficiency following creatinine infusion.

† See Table VII of Doolan, Alpen and Theil(4).

The normal ratio of endogenous creatinine clearance to inulin clearance (C_{Cr}:C_{In}) averaged 1.11. This increased to 1.35 as soon as exogenous creatinine was introduced, reached 1.73 within 30 minutes, then gradually fell to 1.40 at 90 minutes. There was a several minute lag between the termination of period 3 and the actual arrival of exogenous creatinine in the blood stream after it was added to the infusion bottle. Therefore, although the serum creatinine and creatinine clearance values are calculated as though the whole period was involved, part of the period was still at control levels, and the rise in creatinine clearance more rapid than the calculations would indicate. Thus, the ratio after the onset of actual infusion must have been higher, suggesting that the maximal response was almost

TABLE I. Comparison of Creatinine and Inulin Clearances in Normal Subjects and Uremic Patients During Slow Infusion of Creatinine.

Period		1	2	3	4	5	6	7	8	9
Normal subjects: Mean and S.D.										
S_{Cr}*		.88 (.14)	.86 (.18)	.88 (.19)	.96 (.26)	1.52 (.36)	2.05 (.58)	2.51 (.81)	2.89 (.97)	3.23 (1.17)
C_{Cr}†		151.9 (28.9)	155.8 (33.4)	145.0 (24.3)	181.0 (29.3)	230.4 (40.5)	220.8 (26.7)	212.2 (21.0)	203.8 (21.1)	199.2 (31.1)
C_{In}		139.8 (29.8)	139.0 (35.6)	133.7 (29.1)	137.2 (30.4)	137.0 (32.2)	141.7 (30.5)	140.1 (27.3)	139.9 (32.0)	143.5 (29.1)
C_{Cr}/C_{In}		1.09 (.084)	1.13 (.108)	1.10 (.170)	1.35 (.234)	1.73 (.396)	1.60 (.271)	1.55 (.236)	1.51 (.324)	1.40 (.126)
Uremic patients										
S_{Cr}	H	13.84	13.78	13.65	14.08	14.58	15.09	15.36	15.52	15.81
	P	6.77	6.73	6.80	6.88	7.12	7.39	7.58	7.78	7.99
	K	7.31	7.39	7.25	7.50	8.00	8.59	8.67	8.83	8.88
	A	6.99	6.82	7.13	7.55	8.07	8.11	8.04	8.07	8.30
	S	2.94	3.00	3.05	3.31	3.63	4.05	4.67	5.31	5.75
	C	1.04	1.02	1.26	1.67	2.06	2.33	2.60	2.89	3.19
C_{Cr}	H	5.5	5.5	5.4	5.8	5.8	5.7	5.7	5.9	6.1
	P	6.4	6.7	6.6	6.5	6.5	6.4	6.3	6.2	6.1
	K		14.1	20.8	18.1	14.8	14.7	15.4	15.6	16.2
	A	18.3	18.3	17.8	17.2	17.1	18.2	19.2	16.7	14.4
	S	30.0	30.8	31.4	32.3	33.3	34.2	34.7	33.5	32.0
	C	57.1	57.7	56.6	60.2	62.7	61.6	60.3	62.6	66.6
C_{In}	H	4.3	4.3	4.4	4.6	4.4	4.2	4.3	4.2	4.4
	P	4.8	4.8	4.6	4.4	4.4	4.3	4.2	4.0	4.0
	K		8.4	11.7	10.0	8.5	8.9	8.9	8.5	8.0
	A	10.6	10.8	10.7	10.5	10.2	10.3	10.3	8.6	7.3
	S	18.9	20.0	21.2	21.0	20.3	20.4	21.8	20.7	19.4
	C	38.8	44.2	40.3	40.2	40.8	39.6	38.4	38.7	40.0
C_{Cr}/C_{In}	H	1.26	1.26	1.23	1.27	1.30	1.34	1.34	1.38	1.38
	P	1.35	1.40	1.43	1.48	1.47	1.48	1.52	1.55	1.56
	K		1.70	1.78	1.81	1.75	1.65	1.73	1.84	2.02
	A	1.73	1.71	1.66	1.64	1.68	1.77	1.86	1.94	1.97
	S	1.59	1.54	1.48	1.54	1.64	1.68	1.60	1.62	1.65
	C	1.47	1.31	1.40	1.50	1.54	1.56	1.57	1.62	1.66
Mean C_{Cr}/C_{In}		1.52	1.49	1.50	1.54	1.56	1.58	1.60	1.66	1.71
S.D.		.198	.194	.196	.179	.163	.154	.179	.202	.245

* Values represent "true creatinine" expressed in mg %.

† All clearances adjusted to standard surface area of 1.73 M^2 and expressed in ml/min.

Patient H, 33, cortical necrosis; Patient P, 72, chronic pyelonephritis; Patient K, 18, chronic glomerulonephritis; Patient A, 51, chronic glomerulonephritis; Patient S, 57, chronic pyelonephritis; Patient C, 50, lupus nephritis.

All patients and all controls (with one exception) were males. Responses of the female control were identical with the others.

TABLE II. Calculated Mean Tubular Secretion of Creatinine in Normal Subjects.

Period	P_{Cr} mg%	C_{In} ml/min	C_{Cr} ml/min	GF_{Cr} mg/min	$U_{Cr}V$ mg/min	T_{Cr} mg/min	C_{Cr}/C_{In}
			Data from present study				
1.	.88	140	152	1.23	1.34	.11	1.09
2.	.86	139	156	1.20	1.34	.14	1.13
3.	.88	134	145	1.18	1.28	.10	1.10
4.	.96	137	181	1.32	1.74	.42	1.35
5.	1.52	137	230	2.08	3.50	1.42	1.73
6.	2.05	142	221	2.91	4.54	1.63	1.60
7.	2.51	140	212	3.54	5.32	1.78	1.55
8.	2.89	140	204	4.04	5.89	1.85	1.51
9.	3.23	144	199	4.66	6.44	1.78	1.40
			Data calculated from Shannon(1) and Shannon and Ranges(2)				
	10.0	140	196	14.0	19.6	5.60	1.4
	100.0	140	154	140.0	154.0	14.0	1.1

P_{Cr} = Plasma creatinine; C_{In} = Inulin clearance; C_{Cr} = Creatinine clearance; GF_{Cr} = Glomerular filtrate creatinine; $U_{Cr}V$ = Urine creatinine; T_{Cr} = Tubular secretion of creatinine.

immediate. The patients with renal failure had a mean ratio of 1.50 before creatinine infusion and this ratio rose gradually to 1.71 by the end of the creatinine infusion. This increase may be an artifact, since only one patient (C) had a significant increase in creatinine clearance, while 3 patients (P, K, and A) had increased ratios because of a fall in the inulin clearance. As each of these patients had low inulin clearance values, the actual decrease in inulin clearance could be within the range of analytical error.

Discussion. The data support the numerous previous reports, recently reviewed by Doolan, Alpen and Theil(4), which show the endogenous creatinine:inulin clearance ratio in normal man to be on the order of 1.07. This is suggestive, but not conclusive, evidence for a slight tubular secretion of endogenous creatinine. Many of the early attempts to establish the ratio must be disregarded because true creatinine was not determined. Most investigators have found the endogenous creatinine:inulin clearance ratios in renal failure to be greater than the normal. Several studies in which true creatinine was determined have an average ratio of 1.31(4). The fact that in these studies the clearances are based on true and not apparent serum creatinine eliminates the question of the effect of the non-creatinine chromogen(9).

It is evident that a very small increment in the serum creatinine level initiates a sudden and marked increase in the excretion of creatinine, which is in excess of that required to maintain a constant creatinine:inulin clearance ratio (Table II). The possible mechanisms for the increased exogenous creatinine clearance are limited. The serum creatinine may be changed in such a way as to render it less completely quantitated by analytical methods or more susceptible to tubular transport. Shannon and Ranges explored the possibility, suggested by Abdon(10) and Rehberg (11), that administration of creatinine causes the production of creatine-phosphoric acid. They were unable to support this possibility. It is possible that the serum creatinine is altered so that it does not all give a Jaffe reaction, yet is excreted in the urine as the chromogen. Under these conditions the apparent serum creatinine would be low and the clearance relatively increased. Our own *in vitro* recovery experiments on serum to which creatinine had been added yielded the anticipated levels and seems to eliminate this mechanism. There are no data relevant to a possible change in creatinine which would alter tubular transport.

It is unlikely that the increased exogenous creatinine excretion is due to suppression of tubular reabsorption. In 2 normal subjects the increased creatinine excretion would have required the suppression of reabsorption of an amount of creatinine equal to the entire amount filtered. There is no evidence for tubular reabsorption of creatinine of this magnitude. The close identity of the normal

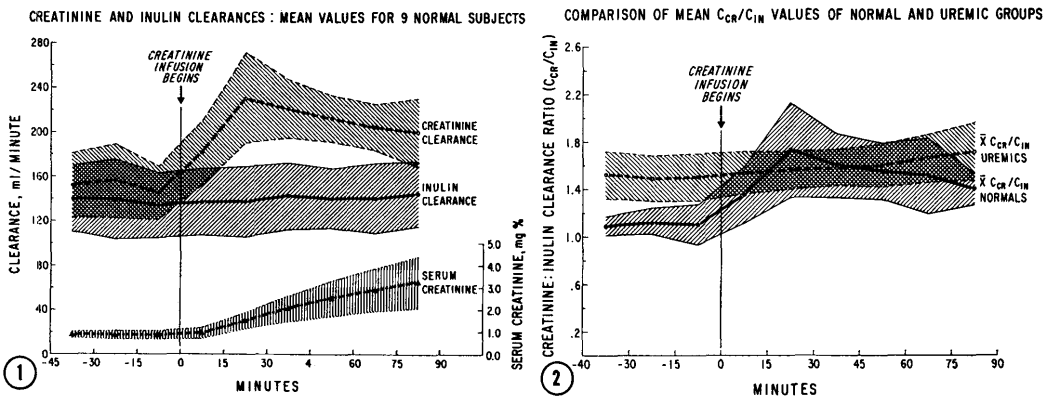

FIG. 1. Creatinine and inulin clearances: Mean values for 9 normal subjects. The shaded area includes one standard deviation.

FIG. 2. Comparison of mean C_{Cr}/C_{In} values of normal and uremic groups. The shaded area includes one standard deviation.

endogenous creatinine and inulin clearances makes it unlikely that there is ordinarily a significant tubular reabsorption of creatinine.

The evidence indicates that there is tubular secretion of creatinine, and it is probable that the increase in the clearance ratio is due to an increase in this component. This response stabilizes in 30 to 45 minutes. Examination of the data (Table II) suggests that a Tm is reached at about 1.8 mg per minute. However, with more rapid rates of infusion Shannon(1) showed a creatinine:inulin clearance ratio of 1.4 at a serum creatinine level of 10 mg% and 1.1 at 100 mg%. If these ratios are applied to a GFR of 140 ml per minute (comparable to our mean control GFR), the T_{cr} is 5.6 mg per minute at the first level and 14.0 mg per minute at the second. In another study(12) we found that following infusion of creatinine in rats there was a change in the creatinine:inulin clearance ratios similar to that seen in man. Rising serum creatinine concentrations produced high ratios initially, which eventually decreased and suggested that a Tm had been reached. However, the level of this apparent Tm was related to the rate of creatinine infusion. Thus, it is doubtful that the decreasing creatinine clearance is due to self-depression caused by the achievement of a Tm (creatinine).

These studies and the data cited are inconsistent with a simple rate-limited secretory mechanism. The fixed creatinine:inulin clearance ratio with decreasing serum concentrations noted by Shannon(1) in man and by ourselves in rats(12) suggests that there may be a gradient-time-limited mechanism(13). Further studies are necessary.

The high creatinine:inulin clearance ratio in renal insufficiency and the failure to respond to creatinine infusion have been reported previously(4,5). The mechanism for the increased creatinine excretion is probably similar to that seen in normal individuals. Once elevated, a marked further increase would not be expected after infusion. It is not evident why individual uremic subjects maintain different ratios. Possibly the persistently elevated serum endogenous creatinine concentration causes the response which varies in individual cases dependent upon the nature of the renal disease.

Summary. Nine normal individuals and 6 patients with varying levels of renal insufficiency were given intravenous infusions of creatinine. The endogenous creatinine clearance:inulin clearance ratio in the normal subjects was 1.11. The ratio of the exogenous creatinine clearance to inulin clearance rose quickly to 1.73 as soon as the creatinine infusion was started, then gradually fell to 1.40 after ninety minutes of infusion. The patients with renal insufficiency had an initial ratio of 1.50 and a late rise to 1.71 after ninety minutes. It seems most likely that a small increment in the serum creatinine concentration can cause an increased effectiveness of the

tubular mechanism for creatinine secretion. An alteration in the serum creatinine resulting in enhanced tubular transport, or reducing apparent serum creatinine concentration without affecting filtration, is less probable. The high pre-infusion ratio in patients with renal insufficiency, and the failure to respond to exogenous creatinine, suggests that the mechanism for increased tubular excretion may continue to function as long as the stimulus of an elevated serum creatinine persists.

1. Shannon, J. A., J. Clin. Invest., 1935, v14, 403.
2. Shannon, J. A., Ranges, H. A., ibid., 1941, v20, 169.
3. Pitts, R. F., Physiology of the Kidney and Body Fluids, Year Book Medical Publishers, Inc., 1963, 116.
4. Doolan, P. D., Alpen, E. L., Theil, G. B., Am. J. Med., 1962, v32, 65.
5. Miller, B. F., Winkler, A. W., J. Clin. Invest., 1938, v17, 31.
6. Schreiner, G. E., Proc. Soc. Exp. Biol. & Med., 1950, v74, 117.
7. Hare, R. S., ibid., 1950, v74, 148.
8. Brod, J., Sirota, J. H., J. Clin. Invest., 1948, v27, 645.
9. Miller, Z., Miller, B. F., Proc. Soc. Exp. Biol. & Med., 1951, v78, 471.
10. Abdon, N. O., K. Fysiogr. Sällsk, i Lund Forh., 1935, v5, 140 (Cited by Shannon and Ranges).
11. Rehberg, P. B., Internationale physiologenkongresses, 16 ste., Zurich, Kongressbericht I, 1938, p4 (Cited by Shannon and Ranges).
12. Goldman, R., Studies on the Clearance of Exogenous Creatinine by Rats. From Renal Metabolism and Epidemiology of Some Renal Diseases, National Kidney Foundation, 1964.
13. Pitts, R. F., Physiology of the Kidney and Body Fluids, Year Book Medical Publishers, Inc., 1963, 124.

Received December 6, 1966. P.S.E.B.M., 1967, v125.

Competition Between Thyroxine and TRF at the Pituitary Level in the Release of TSH.* (32051)

WYLIE VALE, ROGER BURGUS, AND ROGER GUILLEMIN
Department of Physiology, Baylor University College of Medicine, Texas Medical Center, Houston

In the first note in which we reported on the existence and early purification of a TSH releasing factor (TRF) of hypothalamic origin(1), we had shown that the TSH-releasing activity of a given dose of TRF could be decreased and eventually abolished by previous administration of increasing doses of thyroxine. Subsequently we also demonstrated that minute doses of thyroxine (T_4) added to the medium in which pituitary fragments were pre-incubated for 15 minutes would inhibit the effects of TRF added to the incubation system(2); also the administration of T_4 *in vivo* to the animal prior to removal of the pituitary for *in vitro* incubation was shown to decrease or inhibit the activity of TRF subsequently added to the incubating tissue. These results have been confirmed by others(3,4).

The results from *in vivo* experiments reported here show that some sort of a competition between thyroxine and purified TRF seems to exist at the level of the pituitary for the release of TSH, as increasing doses of TRF can overcome the blockade of TSH-release produced by increasing doses of thyroxine. In the course of these studies, we became intrigued by the possibility that the molecule of TRF might contain iodine; this was studied by neutron activation analysis of a sample of highly purified TRF as reported below.

Materials and methods. A. All studies were conducted on rats, male, 40-50 g B.W., obtained from Cheek-Jones Farms, Houston. After 3 days of adaptation to the environment of the animal quarters, the animals received a single i.p. injection of 5 μc of ^{131}I. Three days later, they were submitted to the

* Supported by USPHS Research Grants AM 08290-03, HD 02577-01, Endocrinology Training Grant 5T1-GM-956-03 and FR 00254 to Common Research Computer Facility, Texas Medical Center, Houston.