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THE BIOASSAY OF ADRENAL CORTICOIDS IN THE URINE OF PATIENTS WITH CONGESTIVE HEART FAILURE

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Futcher and Schroeder (1) and Warren and Stead (2) suggested that changes in endocrine activity might be present in congestive heart failure and might explain some of the symptoms such as edema and sodium retention. Merrill, Morrison, and Brannon (3) found an increased concentration of renin in the renal venous blood. No other reports of endocrine changes in congestive heart failure appear in the literature. Since sodium and water retention are known to occur following administration of adrenal cortical hormones, especially desoxycorticosterone, studies were undertaken to determine if the urine of patients in congestive heart failure contained increased amounts of corticoid substances.

METHODS

Ten male patients with congestive heart failure were selected at random from the Gallinger Municipal Hospital. These represented four cases of hypertensive heart disease, one of rheumatic heart disease, four of arteriosclerotic heart disease, and one of chronic cor pulmonale associated with asthma. Their ages ranged from 28 to 70 years, and all but two were negroes. The study was restricted to males to eliminate any possible interference due to changes in the menstrual cycle. Six medical students, none of whom had any evidence of heart disease, were used as control subjects. Twenty-four hour samples of urine were collected without preservatives. In the case of the heart failure patients these were collected as soon after admission as possible, usually within the first 48 hours. The first stage of extraction was carried through in all instances within 12 hours after the collection was made.

Urinary corticoids were extracted by the method of Venning, Kazmin, and Bell (4); and the extracted material was stored in the refrigerator until used. Before use this material was taken up in 6 cc. of 10 per cent ethanol so that each cubic centimeter of the resulting solution represented a four-hour excretion of corticoids.

This material was assayed on young male rats of the Sprague-Dawley strain, using the survival time of adrenalectomized rats as one method of assay (5-7). The animals ranged in weight from 70 to 120 gm. Immediately after adrenalectomy these rats were placed on a diet of dog biscuit with 1 per cent sodium chloride and

5 per cent dextrose in their drinking water. After two to four days they were considered ready for use. At 9:00 a.m. of the initial day they were divided into cages of from five to ten rats and placed on a sodium-free diet (Table I) with distilled water for drinking. At 1:00 p.m. of this day they were given subcutaneously their first injection of 0.05 cc. of the alcoholic solution of corticoids. This was repeated at 12-hour intervals as long as the animals survived. With each assay at least one cage was given extract of normal urine and one cage was given 10 per cent ethanol, using the same size dose. Each cage of animals was checked every eight hours for deaths and the survival time recorded in hours.

TABLE I
Sodium-free diet (29)

Casein (vitamin free)	450 gm.
Crisco	200 gm.
Starch	1570 gm.
Dextrose	1750 gm.
Salt mixture*	100 gm.
Distilled water	250 cc.
Thiamine hydrochloride	25 mg.
Riboflavine	50 mg.
Calcium pantothenate	50 mg.
Pyridoxine	25 mg.
Choline	500 mg.
Niacinamide	0.5 mg.
Vitamin A†	0.2 cc.
Vitamin K	10 mg.
Vitamin D‡	0.3 cc.
Alpha tocopherol§	0.1 cc.

* Modified Hubbell salt mixture made without sodium salts (30).

† 250,000 units per cc.

‡ 2 mg. per cc. of calciferol.

§ 50 mg. per cc.

Glycogenic activity was assayed by using male rats weighing 140 to 180 gm. (8). These were adrenalectomized and placed on a diet of dog biscuits and a solution of 1 per cent NaCl. Three days following adrenalectomy these rats were taken off food for 24 hours. Starting at 6:00 a.m. on the day following this fast they were each given 0.20 cc. of the pooled urinary extracts (as described in the results) every hour for seven consecutive hours. One to two hours after the last injection they were given 0.2 gm. of sodium evipal intraperitoneally, and approximately 1 gm. of liver was removed and plunged into hot 30 per cent KOH. Using the method of Good, Kramer, and Somogyi (9) the liver glycogen was isolated and hydrolyzed and the resulting glucose determined by the Shaffer-Somogyi method

TABLE II
Effect of urine extracts on rat survival time

Patient	Age	Diagnosis	Clinical data													Experimental data			
			Duration	Edema	Ascites	Cardiomegally	Hepatomegally	Resp. rate	Orthopnea	Pul. edema	Blood pres.	Pulse rate	Urine	ECG	Remarks	Rat survival time experimental urine	Rat survival time control urine	"t"	"p"
C. C.	70	Arteriosclerotic heart disease	2 wk.	+++	0	+	4FB	30	++	+++	130/78	88	Alb. ++	1-3 Heart block, LBBB, 1st degree A-V block	Controlled in 9 days	169 ± 59.9* (7)	hrs.	3.05	<0.01
C. D.	56	Arteriosclerotic heart disease	2 yr.	+++	+	+	4FB	30	++	+++	150/90	100	Neg.		Controlled in 17 days	162 ± 39.3 (9)	95 ± 21.3 (7)	4.28	<0.01
W. N.	26	Rheumatic heart disease	3 mo.	++	0	+++	3FB	18	0	++	105/75	108	Neg.	Auricular fib.	Controlled in 6 days	110 ± 32.5 (5)	0	0	
I. D.	67	Arteriosclerotic heart disease	6 mo.	++	0	+++	2FB	20	0	+	140/90	84	Sug. +++	1st degree A-V block, Old ant. infarct	Controlled in 5 days	116 ± 75.9 (6)	110 ± 50.2 (4)	0.134	>0.9
R. L.	50	Cor pulmonale asthma	5 yr.	+++	0	+	2FB	45	++	+++	120/80	98	Neg.		Controlled in 8 days	148 ± 19.0 (4)	2.97	<0.01	
H. D.	63	Hypertensive heart disease	2 mo.	++	0	+++	0	24	0	++	190/100	108	Alb. +		Controlled 2-4 days	116 ± 75.9 (6)	105 ± 41.2 (6)	0.233	>0.7
E. J.	64	Hypertensive heart disease	6 mo.	+++	0	+++	4FB	40	+++	+++	190/115	100	Alb. ++ Sug. +		Died 5 days after admisin.	143 ± 43.2 (6)	85 ± 22.2 (6)	1.60	0.1
T. B.	69	Hypertensive heart disease	2 mo.	+	0	0	1FB	30	0	++	200/80	90	Neg.		Controlled in 6 days	131 ± 68.4 (5)	103 ± 48.7 (6)	0.627	0.5
F. S.	52	Hypertensive heart disease	4 wk.	+++	0	+	0	26	0	++	140/100	110	Neg.		Controlled in 6 days	136 ± 39.0 (6)	131 ± 30.2 (6)	1.40	0.2
C. Corn.	67	Arteriosclerotic heart disease Auricular fib.	3 wk.	+++	0	+++	4FB	24	0	++	130/90	80	Neg.	Auricular fib.	Digitalis intoxic. Controlled 13 days	167 ± 51.2 (6)	108.8 ± 43.8	2.38	0.02

* Standard deviation.
Numbers in parentheses represent number of animals assayed.

(10). The results were then expressed as mg. of glucose per 100 gm. of body weight.

The statistical significance of the data was determined by obtaining the value of "P" corresponding to the calculated value of "t" for the difference between the means of the results obtained in the experimental and the control animals (11).

RESULTS

The effect of urine extracts on the survival time of adrenalectomized rats on a sodium-free diet is summarized in Table II. Four urine extracts of patients with congestive heart failure produced a significant prolongation of the survival time of adrenalectomized rats over that of normal subjects when these were assayed simultaneously. The remaining six did not. Correlating this with the clinical findings, it can be seen that these former four patients were sicker than the remainder with one exception, E. J. Three of the four had orthopnea, all had three to four plus peripheral edema, and three to four plus pulmonary edema. Clinically, E. J. would also fall into this group; but his urine did not prolong the lives of the experimental animals significantly. Of the former group, three had arteriosclerotic heart disease and one had chronic cor pulmonale. In the latter group there were four cases of hypertensive heart disease, one of arteriosclerotic heart disease, and one of rheumatic heart disease.

In order to show that adrenalectomized animals would live under the conditions of the experiment if given one of the cortical hormones, 12 adrenalectomized rats were given subcutaneously 0.5 mg. of desoxycorticosterone acetate in propylene glycol twice daily. The procedures used were the same as those used in animals receiving injections of urine extracts. There were no deaths during the desoxycorticosterone injections which were continued for 216 hours. After these injections were stopped the animals lived an average of 194 ± 109.1 hours. The long survival time of these rats subsequent to discontinuing the desoxycorticosterone was possibly due to the slow absorption of the hormone in some animals.

In order to show that the lives of adrenalectomized animals would not be prolonged by the solvent (10 per cent alcohol) alone, 25 animals were given 10 per cent alcohol using the same size dose and procedures as with those animals receiving urine extracts. These animals lived an aver-

age of 108 ± 38.0 hours. This value is approximately the same as the average for the survival time of animals receiving normal urine extracts (95.3 ± 44.3 hours).

In practically all animals receiving the urinary extracts there were observed toxic reactions. Animals became sluggish within two to four days after beginning the injections. Rats weighing less than 70 gm. were not used since it was found that they died very early in a trial run (50.5 hours). Room temperature could not be controlled satisfactorily, and some animals died on cold nights. These factors are probably responsible for the large standard deviations in some instances.

Based on the clinical and experimental findings, the urine extracts were pooled into two groups. Group A consisted of those patients who were the sickest and whose extracts resulted in a significant

TABLE III

Comparison of glycogenic activity of urine extracts from normal subjects and from heart failure patients

Extract	Average liver glycogen (mg./100 gm. of rat)	"P"
Control urine	$0.985 \pm 1.56^* (\delta) \dagger$	
Experimental urine:		
Group A	$27.18 \pm 8.27^* (5)$	<0.01
Group B	$24.13 \pm 9.04^* (5)$	<0.01

* Standard deviation of the group.

† Numbers in parentheses represent number of animals assayed.

increase in survival time in adrenalectomized rats (urine extracts of patients C. C., C. D., R. L., and C. Corn.). E. J. was also placed in this group because clinically he belonged there and it was felt the survival time produced by his extracts was similar to that of this group although it was not statistically significant. Group B consisted of those patients who were not so sick and whose extracts resulted in no increase in survival time (urine extracts of patients W. N., I. D., H. D., T. B., and F. S.). These two pooled groups were used in the assay of the glycogenic activity of the urine extracts. The results are given in Table III. Both groups show a marked increase of glycogenic activity over that of control urine extract. The slight difference between the two experimental values was not evaluated because of the small number of animals used.

DISCUSSION

The results of this study indicate that there is an increase in the urinary excretion of corticoids in patients with congestive heart failure. Of the ten patients studied, four who appeared clinically to be the sickest, excreted substances which prolonged the life of adrenalectomized rats while the remaining six did not. There was one patient who clinically belongs in this group whose urine extracts did not prolong the life of adrenalectomized rats. Both of these groups of patients, however, excreted an increased quantity of glyco-genic corticoids. An increase of corticoid substances in the urine has been considered as an indication of increased adrenal cortical activity (4-7). There is, however, in these patients another factor which must be considered. All but two of the patients had liver enlargement; and since the liver plays an important role in the inactivation of steroid hormones (12), the question arises that in these cases there might have been enough liver damage to cause only an apparent increase in corticoid secretion rather than a true increase in corticoid production. Some authors (13) report liver dysfunction in a large percentage of patients with heart failure, but this cannot be correlated with the degree of failure (14).

Increased adrenal cortical activity in other instances is believed to be the result of a reaction to various "non-specific stimuli" (15). Of these anoxia has been shown to produce increased adrenal cortical activity (15-18) and Langley and Clarke (16) believe that disturbances in acid-base balance resulting from hyperventilation can stimulate the adrenal cortex. Both anoxia and hyperventilation are present in patients with heart failure and the increase in corticoids found in this study possibly represents adaptation to these non-specific stimuli.

With an increase in adrenal cortical activity sodium retention would be expected, since some of the cortical hormones, especially desoxycorticosterone, act on the renal tubules to promote the reabsorption of sodium from the glomerular filtrate (19, 20).

Other investigations (21-23) indicate that the primary cause of sodium retention in heart failure is a decrease in renal blood flow and glomerular filtration which results from a shunting of blood

away from the glomeruli secondary to renal vascular changes. Merrill (21) and Mokotoff, Ross, and Leiter (22) have concluded that there is no increase in the reabsorption of sodium in heart failure but that its reabsorption is proportional to the glomerular filtration rate. This suggests either that the adrenal corticoid production is not increased sufficiently to affect sodium reabsorption in heart failure or that the adrenal corticoids act on the renal vascular system. Experimental evidence to support the latter is inconclusive. Talbott, Pecora, Melville, and Consolazio (24) studied renal function in patients with Addison's disease and believe that the decreased renal function observed was due to a decreased tone of the efferent arterioles of the kidney and that this was corrected following the administration of desoxycorticosterone. No changes in renal function were observed in normal individuals following the administration of desoxycorticosterone acetate. Waterhouse and Keutmann (25) believe that in Addison's disease desoxycorticosterone produces a vasoconstrictor effect on the vascular system of the kidney. Other investigators have produced changes in the kidney with desoxycorticosterone (15, 20, 26, 27). Swingle (28) believes that desoxycorticosterone may affect the tone of arterioles. At the present time it seems more likely that the increase in corticoid excretion represents a result rather than a cause of the symptoms seen in heart failure. However, because of the close relation between clinical symptoms and the increase in corticoid excretion, further investigation is to be undertaken.

SUMMARY

1. The urine of ten patients in congestive heart failure was studied for the presence of adrenal corticoids and compared with normal control urine.

2. Of these ten patients, four showed an increase of corticoids over that found in normal urine which prolonged the life of adrenalectomized rats.

3. An increase in corticoids with glyco-genic activity was observed in urine obtained from heart failure patients over that of urine obtained from normal control subjects.

4. The possible place of increased adrenal cortical activity in heart failure is discussed.

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