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Research Article

THE PRODUCTION OF CARDIAC LESIONS BY REPEATED INJECTIONS OF DESOXYCORTICOSTERONE ACETATE [1, 2, 3]

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Desoxycorticosterone acetate prolongs the lives and restores many of the functions of patients suffering from Addison's disease and of adrenalectomized animals (1 to 5). The beneficial effects are related to the action on the kidneys which leads to excretion of potassium and retention of sodium (1, 5). Clinical observations have suggested that injurious results due to the steroid may appear along with the beneficial effects. While receiving desoxycorticosterone acetate, certain patients develop edema which has been attributed to excessive nephrogenic retention of sodium chloride and water (3, 5). Furthermore, a number of these patients have developed cardiac symptoms, including advanced congestive failure and enlargement of the heart by roentgenogram (2 to 8). In some cases the cardiac decompensation was regarded as a manifestation of previously existing disease of the heart which was aggravated by a return of the blood pressure to normal or high values (3). Certain authors have attributed cardiac failure to the added burden brought about by the increased volume of circulating fluids which retention of sodium chloride by the kidneys has caused (5). McGavack (6) pointed out that continued treatment leads to low concentration of potassium in serum and that this abnormality may account for the heart failure. Since it is recognized that desoxycorticosterone acetate exerts little or none of the glycogenic function of the adrenal cortex, disturbances in carbohydrate metabolism might be anticipated in Addisonian patients treated with the synthetic hormone (9). Hypoglycemia has been observed but does not seem to be an important factor except during prolonged fasting (9). Cardiac failure remains unexplained and seems to be the chief untoward effect that accompanies the therapeutic use of desoxycorticosterone acetate in Addison's disease.

In experimental animals, repeated injections of the synthetic compound lead to gross deficits of potassium and abnormal retentions of sodium in skeletal muscle (10, 11). These changes are probably a reflection of a tendency to develop low concentrations of potassium in serum (10, 11, 6). Increased urinary volume which is apparently brought about by increased thirst and drinking is a part of the picture (11 to 13). With prolonged treatment with large doses in dogs, a peculiar muscular paralysis develops which is relieved by administration of potassium salts (11). The injection of desoxycorticosterone acetate has not been shown to produce cardiac symptoms in experimental animals (14).

On the other hand, diets low in potassium produce the same changes in skeletal muscle as have been demonstrated after repeated injections of desoxycorticosterone acetate (15, 16) and cardiac lesions are strikingly developed in rats (16 to 19), mice (20), and pigs (19), fed diets deficient in potassium. Certain observations (19) lead to the conclusion that a dietary deficiency in pyridoxin played a rôle in the development of the cardiac lesions accompanying diets low in potassium. The present paper reports work which demonstrates that cardiac lesions are produced by prolonged injections of desoxycorticosterone acetate in rats. The lesions are not prevented by a relative excess of thiamin or pyridoxin in synthetic diets, nor are the lesions aggravated by suboptimal amounts of these vitamins in the diets. Analyses of muscle and serum are reported to show the effect of desoxycorticosterone acetate in rats. In cats and dogs, analyses of other tissues are given, with especial attention to the variations of potassium in the heart.

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[2] Aided by a grant from the Fluid Research Fund of the Yale University School of Medicine.

[3] We are indebted to Ciba Products, Inc., for the desoxycorticosterone acetate and to the Upjohn Company for the adrenocortical extract used in the experiments.

METHODS

White male rats weighing 200 to 350 grams were used. The desoxycorticosterone acetate was dissolved in warm alcohol and then precipitated by adding enough 5 per cent glucose or saline to make a suspension in 5 per cent alcohol containing 2 mgm. of the synthetic hormone per ml. This mixture was injected subcutaneously in 1, 2, or 4 mgm. doses daily except Sundays. The rats were killed by withdrawing as much blood as possible from the abdominal aorta while the rat was under ether anesthesia. Muscle or other tissue were removed immediately and analyzed as in previous studies (21). Serum analyses were obtained from blood kept under mineral oil until separated from the erythrocytes.

The stock rats were fed Purina Fox Chow and this diet was continued during many of the experiments. By analysis this food contains 15 millimoles of potassium per 100 grams. Two basic synthetic diets were used for rats. Diet A contained the following: vitamin free casein (Labco) 180 grams, sucrose 630, Crisco 100, cod liver oil 10, liver B fraction 20, soya bean oil 20, salt mixture 40. To a kilogram of the diet the following vitamins were added: thiamin 20 mgm., riboflavin 20 mgm., nicotinic acid 100 mgm., calcium pantothenate 20 mgm., and cholin 1 gram. Three variations of the above were made: (*1*) no added pyridoxin (OB6), (*2*) 2 mgm. pyridoxin (NB6), and (*3*) 20 mgm. pyridoxin (HB6). The salt mixture gave 15 millimoles of potassium per 100 grains of diet. Diet A was intended to give liberal amounts of the vitamins and study the effect of variations in pyridoxin. None of the rats, even on the diet free of B6, showed acrodynia. This was probably due to the presence of soya bean oil (22). Diet B contained the following: Labco casein 180 grams, sucrose 630, Crisco 100, cod liver oil 10, liver B fraction 20, soya bean oil 20, salt mixture 40. The following vitamins were added to 1 kgm.: riboflavin 20 mgm., nicotinic acid 100 mgm., calcium pantothenate 20 mgm., pyridoxin 20 mgm., cholin 1 gram. To one lot, 2 mgm. of thiamin were added (NB1), and to another, 0.5 mgm. of thiamin (LB1). Diet B was intended to study the effects of low intakes of thiamin. The potassium content of this diet was 15 millimoles per 100 grams.

The cats were fed canned salmon and milk. From analyses of the salmon, it was calculated that the intake of potassium was rather high,—about 11 millimoles per kilogram of cat. The dogs were given a synthetic diet low in potassium made up of casein, sugar, fats, vitamins, and a salt mixture free of potassium. The dog injected with desoxycorticosterone acetate received the regular kennel diet.

RESULTS

The photomicrographs illustrate the cardiac lesions produced by injections of desoxycorticosterone acetate (Figures 1, 2, 3). For comparison, lesions produced by a diet low in potassium

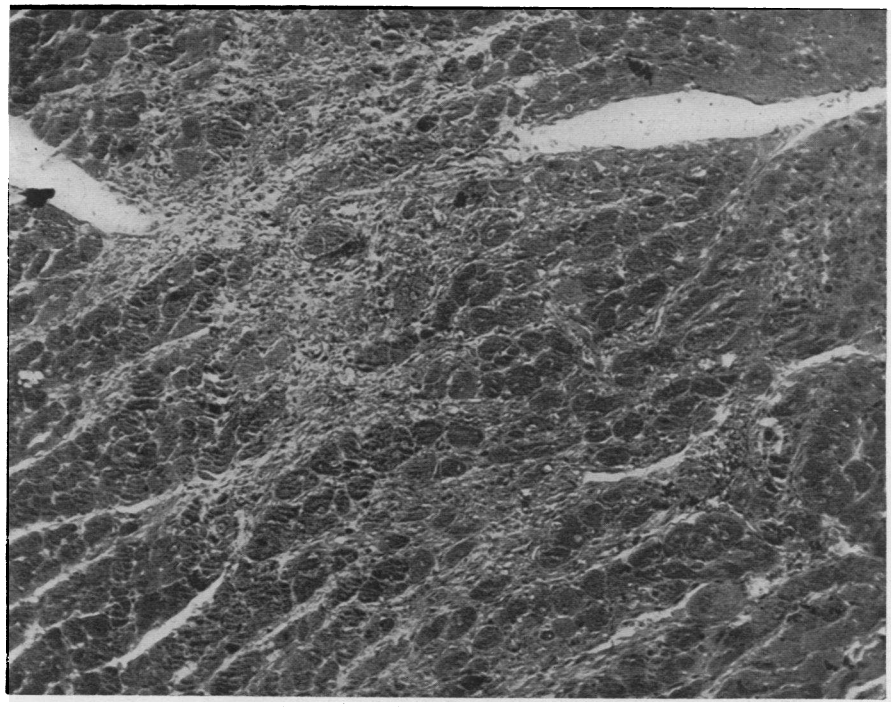

FIG. 1. CARDIAC LESIONS PRODUCED BY INJECTIONS OF DESOXYCORTICOSTERONE ACETATE

Rat. 151. Fed Purina Fox Chow. Injected with 4 mgm. of desoxycorticosterone acetate for 30 days. Diet contains 24 mM. soduim and 15 mM. potassium per 100 grams.

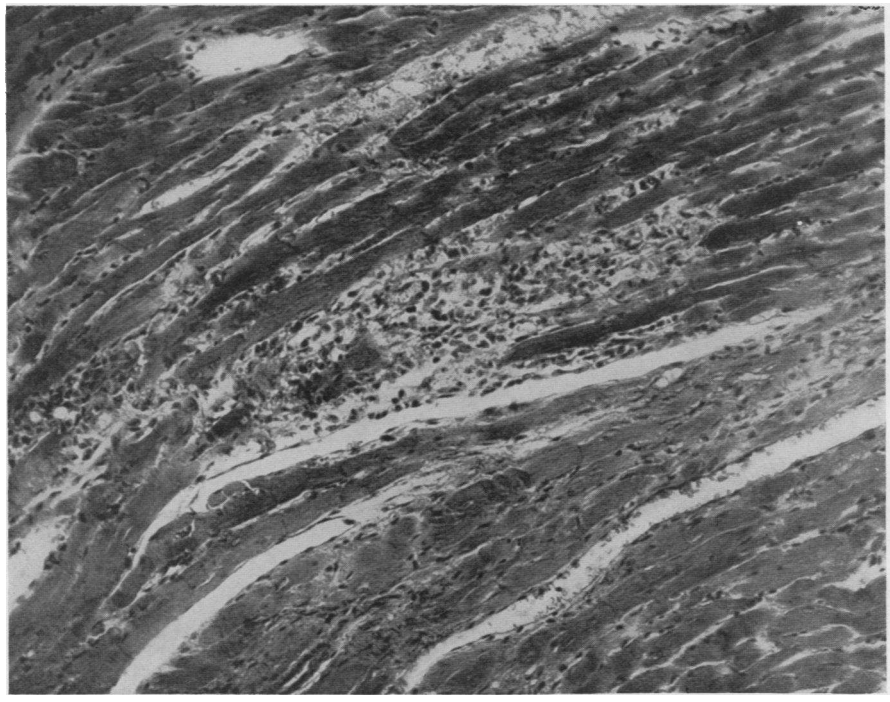

FIG. 2. CARDIAC LESIONS PRODUCED BY INJECTIONS OF DESOXYCORTICOSTERONE ACETATE

Rat. 155. Fed Purina Fox Chow. Injected with 4 mgm. of desoxycorticosterone for 30 days.

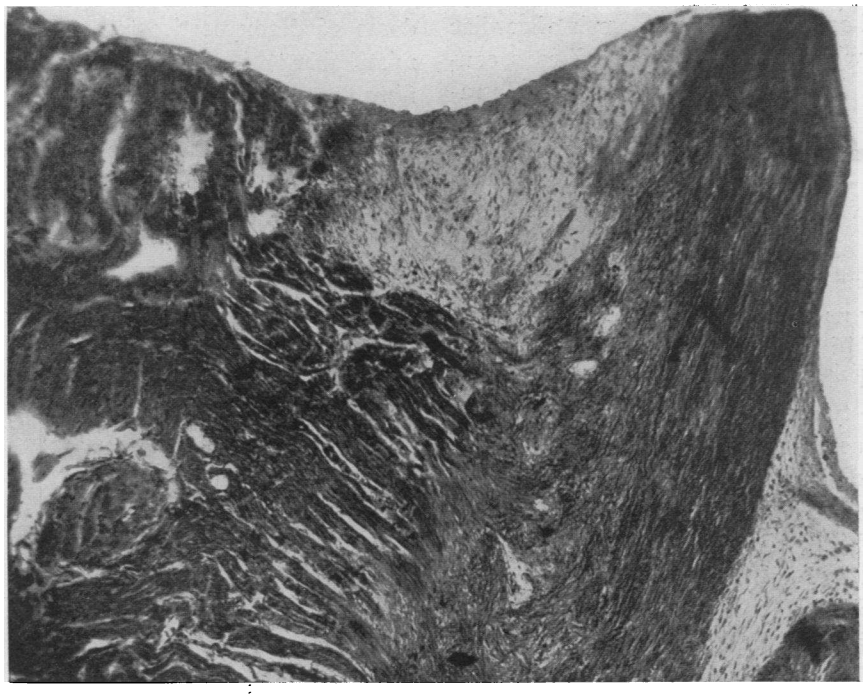

FIG. 3. CARDIAC LESIONS PRODUCED BY INJECTIONS OF DESOXYCORTICOSTERONE ACETATE

Cat 2. Received 8 mg. desoxycorticosterone acetate daily for 25 days.

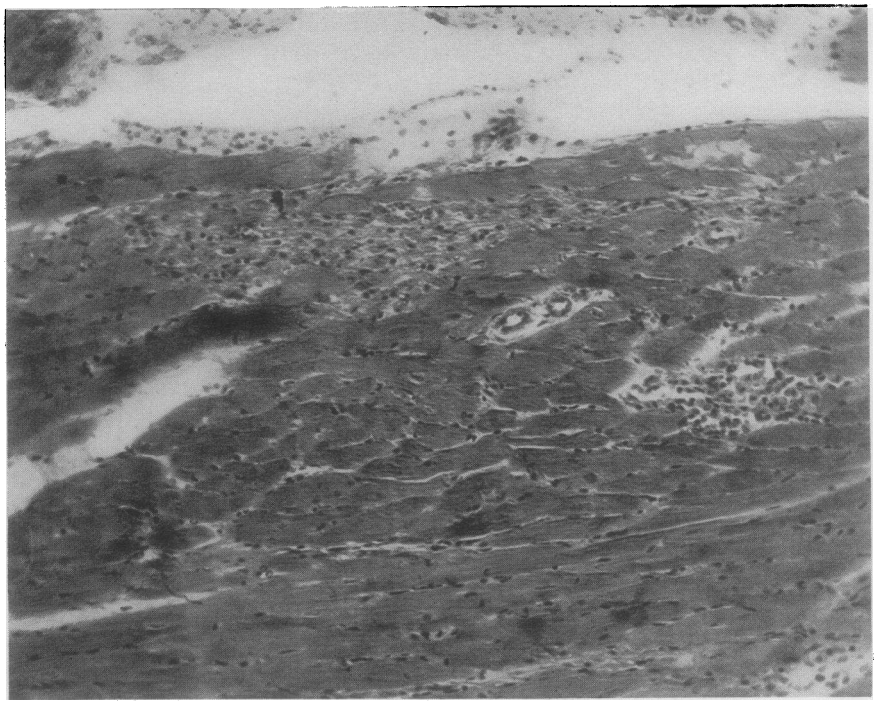

FIG. 4. CARDIAC LESIONS PRODUCED BY A DIET LOW IN POTASSIUM

Rat M 42. Fed diet low in potassium for 69 days. Diet contains 1.6 mM. potassium and 18 mM. sodium per 100 grams.

are also reproduced (Figure 4). Essentially, there are small or large areas of necrosis of cardiac muscle fibres which are replaced by fibrous connective tissue. There is no evidence of infiltration by polymorphonuclear leukocytes. Skeletal muscle, diaphragm, and liver have been examined but do not show lesions. The kidney weights are increased (23) and the tubules enlarged, with multiplication of tubular cells. Both the heart and renal lesions cannot be distinguished from the lesions seen by us and others in rats fed a diet low in potassium (16 to 20).

Cats 2 and 4 were examined histologically. Cat 2 showed typical lesions in the heart which are regarded as produced by the desoxycorticosterone, in spite of the fact that this cat was suffering from an infection and was very sick and refused to eat during the last 4 days of her life. Dogs 586 and 802 were examined histologically and showed no lesions in the heart. The kidneys, however, of both the cats and dogs show lesions in the tubules like those seen in the rats, but much less marked.

Table I shows the incidence of lesions in rats fed the various diets and injected with various doses of desoxycorticosterone acetate. One unmistakable area of replacement of musculature by fibroblasts was considered sufficient to indicate

TABLE I

Incidence of cardiac lesions

Diet		DOCA		Number of rats	Number with lesions
Kind[1]	Vitamin[2]	mgm.	days		
P		2	14	7	1
P		2	30	12	9
P		4	30	10	9
A	OB6	1	30	4	2
A	OB6	2	30	2	0
A	OB6	4	30	2	2
A	NB6	2	30	4	2
A	NB6	4	30	4	2
A	HB6	2	30	1	1
A	HB6	4	30	3	3
B	LB1	4	30	4	2
B	NB1	4	30	4	2

[1] P indicates Purina Fox Chow; A and B, the synthetic diets described under methods.

[2] The following vitamin supplements were added to a kilogram of diet: OB6, no pyridoxin; NB6, 2 mgm. pyridoxin; HB6, 20 mgm. pyridoxin; LB1, 0.5 mgm. thiamin; HB1, 2 mgm. thiamin. See text for composition of A and B.

that lesions were produced. In most instances, only one section was examined but experience with two separate imbeddings indicates that striking lesions might be found in one section when absent in the other. Hence positive findings are more significant than negative findings. Although some hearts are obviously more injured than others, we were unable to satisfy ourselves that we were justified in laying emphasis on quantitative interpretation. The data will, therefore, be considered merely on the basis of lesions found or not found.

Of sections examined from 7 rats given 2 mgm. of desoxycorticosterone acetate daily for 14 days, one showed lesions. In most experiments, the injections were continued for 30 or 40 days. Since no obvious increase in the incidence of lesions developed between the thirtieth and fortieth day and most of the experiments were run for 30 days, all the longer experiments are listed as injected for 30 days. For the prolonged period of injections, lesions were found in 34 of 50 rats. Lesions were present when the daily dose was 1, 2, or 4 mgm. There was a suggestion that the lesions were more marked and more frequent in the rats given the larger doses.

Because it has been suggested that the cardiac lesions produced by diets low in potassium are aggravated by a deficiency in pyridoxin, experiments were set up to test this possibility for the injury produced by injections of desoxycorticosterone acetate (A diets). The diets contained rather liberal amounts of added thiamin, riboflavin, nicotinic acid, calcium pantothenate, cholin and liver B fraction. Diets were tested containing no pyridoxin, an adequate, and a high level. Pyridoxin intake did not apparently affect the production of lesions.

In the experiments of Thomas *et al.* (19) smaller doses of thiamin were given than the amount obtained from our A diets. These authors found evidence that the cardiac lesions produced by diets low in potassium were aggravated by a deficiency in pyridoxin. The B diets were, therefore, made up to test whether minimal and suboptimal amounts of thiamin brought out more extensive lesions than those previously obtained on the A diets. As may be seen from the table the level of intake of thiamin did not influence the cardiac lesions produced by prolonged injections of desoxycorticosterone acetate.

Purina Fox Chow was supplemented in three ways by adding (*1*) 20 grams of soya bean oil, (*2*) 20 mgm. of pyridoxin, and (*3*) 20 mgm. of thiamin per kilogram of ground material. These results are not included in the tables. In each

TABLE II

Serum and muscle of rats

	Number of rats	Serum				Muscle per 100 grams fat free solids					
		per 100 ml.	per L.	per L. filtrate							
		H_2O	K	Cl	Na	H_2O	Cl	Na	K	P	N
		grams	*mM.*	*mM.*	*mM.*	*grams*	*mM.*	*mM.*	*mM.*	*mM.*	*grams*
Normal	13	92.5 ±0.6	4.0 ±0.8	113.0 ±3.3	146.9 ±3.0	341 ±6.6	7.2 0.5	10.0 0.6	48.9 ±0.6	33.4 ±1.5	14.8 ±0.3
DOCA 10	21	94.1 ±0.6	3.39 ±0.8	109.9 ±3.1	150.0 ±6.2	332 ±9.0	6.27 ±0.7	14.4 ±1.5	41.6 ±2.4	32.0 ±1.1	15.4 ±0.1
DOCA 30	21	94.1 ±0.8	4.23 ±1.1	104.7 ±4.9	149.7 ±4.7	332 ±8.7	6.48 ±0.7	16.6 ±2.0	38.4 ±2.5	32.1 ±0.9	15.3 ±0.3
LK 14	15	93.2 ±1.2	4.5 ±0.8	113.4 ±2.5	146.3 ±5.4	327 ±5.5	6.3 ±0.7	11.8 ±0.8	40.3 ±2.1	31.3 ±0.7	15.2 ±0.2
C.E. 31	1	93.9		111	149	329	6.0	10.9	43.0		15.3

In this and subsequent tables, average ± standard deviations are given.
DOCA 10—rats getting 2 mgm. desoxycorticosterone for 10 to 14 days.
DOCA 30—rats getting 2 or 4 mgm. desoxycorticosterone for 30 days.
LK 14—rats getting low potassium diet for 14 days.
C.E. 31—rat getting 4 cc. Upjohn's adrenocortical extract for 31 days.

TABLE III

Liver of rats

	Number of rats	Liver weight per cent of body	Per 100 grams fat free solids					
			H_2O	Cl	Na	K	P	N
			grams	*mM.*	*mM.*	*mM.*	*mM.*	*grams*
Normal	10	3.41 ±0.25	290 ±7.5	11.0 ±0.7	10.4 ±1.3	35.9 ±2.3	40.5 ±2.7	12.7 ±0.6
DOCA 10	13	2.66 ±0.25	281 ±17	11.7 ±0.7	12.9 ±1.5	35.6 ±2.2	39.7 ±3.7	13.6 ±1.1
DOCA 30	7	3.47 ±0.28	334 ±18	13.4 ±0.8	14.7 ±1.3	39.9 ±3.2	44.8 ±3.1	14.2 ±0.5
LK 14	6	2.66 ±0.24	292 ±9.5	12.8 ±1.0	11.1 ±0.2	32.8 ±4.3	41.0 ±4.3	12.9 ±0.1

See explanation of Table II.

of the trials, 3 out of 4 rats showed lesions. It was felt, therefore, that the production of lesions by desoxycorticosterone acetate was not dependent on any relative deficiency of the Purina Fox Chow in these substances.

Finally 8 rats were fed Purina Fox Chow and given 2 mgm. of desoxycorticosterone acetate daily for 30 days. Four were given tap water to drink and 4 a solution of potassium chloride (1.5 per cent). These experiments are not included in the tables. Two out of 4 of those given tap water showed lesions, while none of those given potassium chloride in their drinking water showed lesions. The presence of the potassium chloride abolished both the kidney hypertrophy and histological changes in the renal tubules. These experiments seem conclusive that deficit of potassium is the central feature of the cardiac lesions produced by prolonged injections of desoxycorticosterone acetate.

THE EFFECT OF INJECTIONS OF DESOXYCORTICOSTERONE ON MUSCLE AND HEART COMPOSITION

Table II shows the effect of injections of desoxycorticosterone acetate on the electrolytes of serum and muscle of rats. Average figures are given for normals, for rats given injections of desoxycorticosterone, and for rats receiving a diet low in potassium. The table confirms previous work (10, 11) showing that injections of desoxycorticosterone acetate led to a somewhat high concentration of sodium in serum, and a low concentration of potassium and chloride. In the muscle, potassium is reduced and sodium increased. Since muscle chloride remains essentially unchanged, the increase in muscle sodium is not extracellular but replaces potassium lost from the fibers. This change in muscle potassium and sodium is somewhat greater in rats receiving injections for 30 days than in those receiving injections for 14 days. As shown in the table, injections of desoxycorticosterone acetate produce essentially the same change in skeletal muscle as diets low in potassium. The diets low in potassium do not bring about a rise in serum sodium, but frequently develop low concentrations of potassium in serum, particularly after exercise (10, 16). However, resting rats on a diet low in potassium may show little change in the concentration of potassium in serum despite the fact that muscle potassium is considerably reduced. A single rat was analysed after receiving 4 cc. of Upjohn's cortical extract daily for 31 days. The changes are similar but less marked than those of rats receiving desoxycorticosterone acetate.

Table III shows analyses of some of the livers of the rats tabulated in Table II. Liver weights are given as per cent of total weight of the rats. The figures show a distinct loss of weight of the liver during the first 2 weeks on desoxycorticosterone acetate, but after 30 days, the livers assume the same relative weights as the controls. The figures are based on the body weights when the rats were killed but changes in body weights during the experiment do not explain the change in

TABLE IV

Serum and heart of rats on low potassium diets or receiving desoxycorticosterone

	Number	Serum				Heart			
		per 100 ml.	per L.	per L. ultrafiltrate		per 100 grams of solids			
		H_2O	K	Cl	Na	H_2O	Na	K	P
		grams	*mM.*	*mM.*	*mM.*	*grams*	*mM.*	*mM.*	*mM.*
Control	8	92.5	4.2	106	148	334	16.7	36.9	31.1
DOCA	8	94.4	3.8	105	148	355	18.5	34.6	29.4
LK + KCl	8	93.6	4.4	114	146	321	15.7	37.9	29.8
LK	8	93.6	3.4	107	147	359	15.4	35.0	30.8

DOCA—2 mgm. desoxycorticosterone for 30 days.
LK + KCl—Low potassium diet with 0.5 per cent KCl in drinking water for 30 days.
LK—Low potassium diet with distilled drinking water for 30 days.

relative weights of the liver. While the calculation of the extracellular water of liver is dubious, since liver chloride usually is considerably larger than can be contained in the same volume of extracellular fluid as would contain liver sodium, it seems likely that most of the return to normal liver size is accounted for by increase in extracellular water. This conclusion is indicated by the high content of water, sodium, and chloride in the livers of the rats which have received the desoxycorticosterone acetate for 30 days. The amounts of liver potassium, phosphorus, and nitrogen are probably not significantly divergent in the various experimental groups. The livers of the rats fed the diet low in potassium are essentially like those of the rats given desoxycorticosterone acetate for 10 days; *i.e.*, the only significant change is decrease in size. The liver does not apparently lose potassium and gain sodium under conditions leading to such a change in skeletal muscle.

Table IV shows the analyses of hearts of rats receiving 4 mgm. of desoxycorticosterone acetate, or diets low in potassium, for 30 days. The hearts were pooled and analysed from 8 rats in each group. Equal parts of serum from each rat were mixed and analysed for each group. No chloride or fat analyses could be carried out on the hearts. The diets were synthetic, with ade-

TABLE V

Serum, muscle, and heart in dogs on low potassium diet or receiving desoxycorticosterone

	DOCA or diet	Days	Serum				Tissue					
			per 100 ml.	per L.	mM. per L. ultrafiltrate		per 100 grams fat free solids					
			H_2O	K	Cl	Na	H_2O	Cl	Na	K	P	N
			grams	*mM.*	*mM.*	*mM.*	*grams*	*mM.*	*mM.*	*mM.*	*mM.*	*grams*
							MUSCLE					
Control 1			94.4	3.5	125	151	356	7	9	45	33	14.8
Control 2			94.0	4.4	121	148	352	7	10	45	32	14.7
586	LK	35	93.5	3.8	94	151	315	8	11	34	29	15.7
652	LK	32					335	7	13	39	32	15.6
644	LK	124	91.2	5.6	98	152	348	10		39	27	15.9
651	LK	150	93.3	2.8	121	150	363	10	19	32	29	15.5
802	D	10	93.7	2.3	118	152	372	9	14	40	29	15.2
							HEART					
Control 1							400	14	17	42	37	14.1
Control 2							426	16	20	45	36	14.6
Control 3							418	19	21	42	37	15.4
586	LK	35					433	16	24	29	33	14.4
652	LK	32					391	13	20	43	33	15.2
644	LK	124					440	16	27	49	35	15.8
651	LK	150					405	21	21	29	33	14.9
802	D	10					429	16	24	38	34	14.8

TABLE VI

Serum, muscle, and heart in cats receiving desoxycorticosterone

	DOCA	Days	Serum				Tissue					
			per 100 ml.	per L.	per L. ultrafiltrate		per 100 grams fat free solids					
			H_2O	K	Cl	Na	H_2O	Cl	Na	K	P	N
	mgm.		*grams*	*mM.*	*mM.*	*mM.*	*grams*	*mM.*	*mM.*	*mM.*	*mM.*	*grams*
							MUSCLE					
Controls			93.2 ±0.6	5.7 ±0.3	132.6 ±3.4	155.8 ±3.4	345 ±18	5.9 ±1.1	8.0 ±1.1	47.4 ±2.1	33.5 ±1.5	15.4 0.2
Cat 2	8	25	93.5	4.4	124		339	14	16	34	35	16.2
Cat 3	4	11	93.3	5.3	132	157	329	5.8	9.0	45	31	14.7
Cat 4	4	35	91.5	4.0	131	163	320	7.8	11.3	48	31	15.4
Cat 5	4	11	93.9	4.8	136	154	327	5.6	8.1	46	32	15.3
							HEART					
Controls							410 ±27	19.1 ±1.9	24.4 ±4.4	40.1 ±3.4	34.0 ±2.9	14.1 ±0.9
Cat 2	8	25					429	27	36	48	35	16.0
Cat 3	4	11					395	16	22	39	35	14.0
Cat 4	4	35					381	25	28	40	32	14.8
Cat 5	4	11					426	21	23	44	37	14.9

quate amounts of the known vitamins and 15 mM. of potassium per 100 grams. The low potassium diet was controlled by adding 0.5 per cent potassium chloride to the drinking water of a group on the diet low in potassium. The table shows that the cardiac potassium is slightly lower in the rats receiving desoxycorticosterone acetate than in the control group, and lower in the ones receiving a diet low in potassium than in the rats receiving the same diet plus 0.5 per cent potassium chloride in drinking water. The rats receiving desoxycorticosterone acetate also have slightly high cardiac sodium. The figures suggest that heart muscle reacts somewhat like skeletal muscle, but the changes are certainly not as large proportionately as those found in skeletal muscle.

Because of difficulty in analysing small amounts of tissue, procedures that reduce body potassium were studied in dogs and cats. Table V shows the results in heart and muscle of 4 dogs receiving a diet low in potassium, and 1 receiving daily injections of 10 mgm. of desoxycorticosterone acetate for 14 days. Analogous changes to those found in rat muscle also develop in dog muscle. In 2 of the 4 hearts of dogs receiving the diet low in potassium, distinctly low potassium developed (see dogs 586 and 651). The decrease equals one-fourth of the usual cardiac potassium. These are also the dogs showing the lowest potassium in skeletal muscle. The other 2 hearts of the dogs on the diet low in potassium were essentially normal. Following injections of desoxycorticosterone acetate for 14 days, the cardiac potassium was slightly low but not certainly below the normal range. The results show that procedures leading to loss of body potassium may lead to considerable reduction of cardiac potassium in the dog. However, the same procedure does not lead to this change in all animals.

Table VI shows the results following daily injections of desoxycorticosterone in cats. None of the hearts show significant loss of cardiac potassium. Cat 2 which was very sick, in part owing to an infection, shows high water, chloride, and sodium in the heart. This cat also is the only one showing the characteristic loss of potassium in skeletal muscle. The positive result may be due to the larger dose in this cat. However, the relative refractoriness of the cat to desoxycorticosterone may be more apparent than real. Cat 4 apparently shows the effect of desoxycorticosterone acetate in raising the concentration of sodium in serum. The diet of the cats consisted largely of rather salty canned salmon and by analysis of the diet it was calculated that a 2 kilogram cat got about 11 mM. of potassium and 20 mM. of sodium per day. Per 100 grams, the cat's diet contained 30 and 54 mM. of potassium and sodium respectively, which is about twice as concentrated in potassium as the rats' diets.

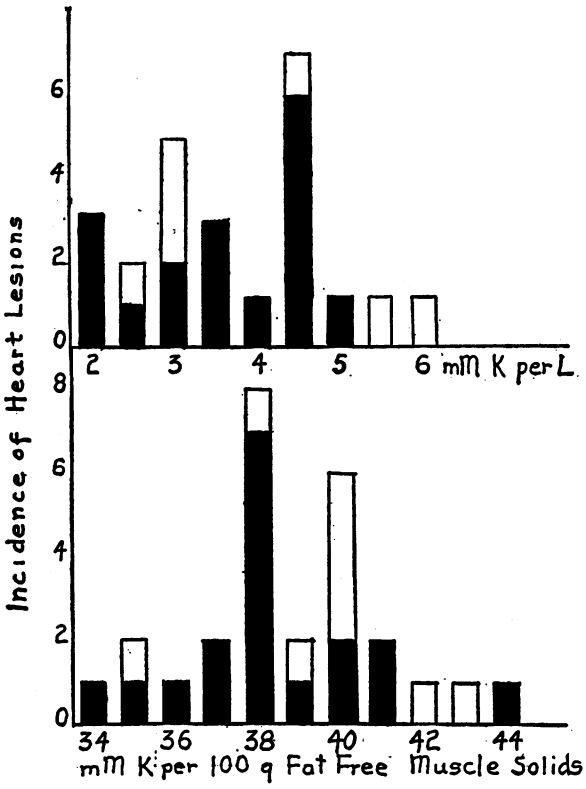

FIG. 5. THE INCIDENCE OF CARDIAC LESIONS WITH RESPECT TO POTASSIUM CONCENTRATION IN SERUM AND IN MUSCLE

The height of the columns indicates the number of rats examined and the black areas the number with cardiac lesions. In the upper part the rats are classified with respect to serum potassium, while in the lower part they are classified with respect to muscle potassium.

Since cats are so particular about their diet, they are probably not suitable for demonstrating deficits of potassium. However, the experiments support the evidence that deficits of potassium are necessary for the production of lesions in the heart.

In Figure 5, the incidence of cardiac lesions is charted against the concentration of potassium per 100 grams of fat free solids of skeletal muscle, and against the concentration of potassium in serum. The chart includes all data on rats obtained from experiments involving diets low in potassium and the injection of desoxycorticosterone acetate, in which serum and muscle analyses were made in conjunction with histological examination of the heart. It should be recalled that the authors have pointed out that muscle potassium in normal rats may vary from 44 to 49 mM. per 100 grams of fat-free solids. When muscle potassium is above 50 mM., serum potassium is high, and when it is below 44 mM., serum potassium has a tendency to be low. Since the concentration of potassium in serum is subject to many sudden and transient variations, it is not surprising that the values in the chart are usually in the normal range but with a tendency for a considerable number to be abnormally low. There is no consistent correlation between the level of serum potassium and the incidence of cardiac lesions. On the other hand, all of the muscle potassiums but one lie below the normal range. The lesions in the heart were found at all levels of muscle potassium from 34 to 44 mM. per 100 grams of fat free solids. Lesions occurred, with few exceptions, when muscle potassium was below 40. Thus the degree of depletion of body potassium, as indicated by the diminution of muscle potassium, is an index of the incidence of cardiac lesions.

DISCUSSION

The lesions in the hearts of the rats are of sufficient severity to explain cardiac failure. Although circulatory insufficiency and cardiac dilatation are the chief untoward results of inadequately controlled treatment with desoxycorticosterone acetate, reports of autopsies in patients so treated have not described lesions like those under consideration. In the first clinical trials when the most cardiac symptoms were encountered, administration of desoxycorticosterone acetate was often combined with diets low in potassium and high in sodium chloride. Undoubtedly this practice aggravates the tendency to lose potassium and the consequent untoward cardiac symptoms. In a few rats, sodium chloride was added to the drinking water. While extensive lesions were produced in this way, the rats run at the same time but receiving tap water showed apparently equally severe lesions. As was pointed out previously, the diet of the cats was high in both potassium and sodium, containing 30 and 50 mM. respectively per 100 grams of diet. These figures may be compared to the rat diet which contained per 100 grams, 15 and 8.5 mM. of potassium and sodium. This peculiarity of the cat diet probably

explains the normal concentration of potassium in serum and muscle as well as the high concentration of sodium in serum. That the dietary factor may be decisive is suggested by the fact that cat 2, which ate poorly, developed deficit of potassium in muscle. Furthermore, addition of 0.5 per cent potassium chloride to the drinking water of rats receiving desoxycorticosterone acetate prevented renal hypertrophy, hyperplasia of renal tubules, loss of potassium from the muscle, and cardiac lesions. The use of desoxycorticosterone acetate in Addison's disease must be controlled so that deficits of potassium are not produced.

In evaluating the present experiments, it must be kept in mind that all animals had intact adrenals. The dose of desoxycorticosterone acetate was 1, 2, and 4 mgm. daily, which is only 2 to 8 times the dose for maintenance of an adrenalectomized rat. If patients are as susceptible to cardiac injury as the rat, therapeutic doses are probably not far below the amount that might be injurious. Probably the therapeutic dose will be found to depend in some way on the intake of both potassium and sodium and by striking a correct balance, the margin of safety can be increased. The diet of the rats contained about 4.4 mM. per 100 calories; a human dietary of 2000 calories and 4 grams of potassium contains about 5.0 mM. of potassium per 100 calories. With loss of appetite and the use of intravenous therapy, potassium intake may seriously decrease and administration of desoxycorticosterone acetate lead to deficits of potassium. In any case, the synthetic hormone should not be used with diets low in potassium or with those containing excessive amounts of sodium chloride.

While only one rat was analysed after receiving adrenal cortical extract, the muscles in this case showed low potassium. Loss of body potassium cannot, therefore, be regarded as a peculiar effect of a synthetic compound. Indeed, one of us (H. C. M.) has unpublished data which show that injections of estradiol benzoate and testosterone propionate produce low muscle potassium. Furthermore, the effects of diets low in potassium show that the kidneys are unable entirely to reabsorb potassium from the glomerular filtrate. Renal hypertrophy enables the tubules to be more successful (18), and the renal hypertrophy of rats receiving desoxycorticosterone acetate is probably entirely analogous to that of rats receiving diets low in potassium (23), despite the fact that the ones receiving the hormone have high urinary volumes and those on the diets low in potassium have low urinary volumes. Apparently under the influence of certain steroids, the tendency not to reabsorb potassium from the glomerular filtrate is so great as to lead to body deficits, even on diets adequate in potassium.

At present no simple laboratory method will surely indicate when a deficit of potassium has developed. In the cats, the abnormally high concentration of sodium in serum was accompanied by normal potassium in muscle and serum; serious deficits of potassium may occur with normal concentrations of this ion in serum in rats. Probably concentration of potassium in serum below 3.5 mM. per liter should be regarded as dangerous. Therapy should not be pushed with only the concentration of sodium or chloride in serum as the criterion of adequate administration; nor does a normal concentration of potassium in serum give assurance that deficit of body potassium has not developed.

Apparently treatment of Addison's disease has met a dilemma. During a crisis, the Addisonian patient is extremely susceptible to the toxic effects of potassium, yet with treatment with desoxycorticosterone acetate he may develop a harmful deficit of potassium. Although renal excretion of sodium and potassium may be related to one another in a reciprocal way, the balance in the body depends on the intake of each independently. Without the hormone, the Addisonian patient tends to lose sodium and retain potassium; a patient receiving desoxycorticosterone acetate tends to lose potassium and retain sodium. Doubtless future work can show what balance between intake of sodium and potassium is the most favorable in the treatment of adrenal insufficiency with desoxycorticosterone acetate.

SUMMARY

Necrosis of the myocardial fibres and replacement by fibroblasts is produced by repeated injections of desoxycorticosterone acetate in rats. The lesions are neither aggravated by absence of pyridoxin nor prevented by liberal additions of

pyridoxin to the diets. Low intake of thiamin does not aggravate the lesion. The lesions cannot be distinguished from those produced by diets low in potassium. The livers decrease in size after injections of desoxycorticosterone acetate for 10 days, but are normal in size after 4 weeks of injections.

The injection of desoxycorticosterone acetate lowers muscle potassium and raises muscle sodium. Analogous changes are not found in the liver. Low cardiac potassium was found in the heart in 2 of 4 dogs fed a diet low in potassium. Injection of desoxycorticosterone produced only suggestive lowering of cardiac potassium in a group of rats, no certain change in any of 4 cats, and no change in 1 dog. Although the heart may lose potassium under conditions leading to loss from skeletal muscle, diminution of cardiac potassium is not a regular occurrence.

The cardiac lesions produced by injections of desoxycorticosterone acetate or diets low in potassium can be prevented by addition of potassium chloride to the drinking water. Deficit of body potassium is apparently essential for the production of these lesions.

Cortical extract produced analogous changes in the muscle of 1 rat.

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