

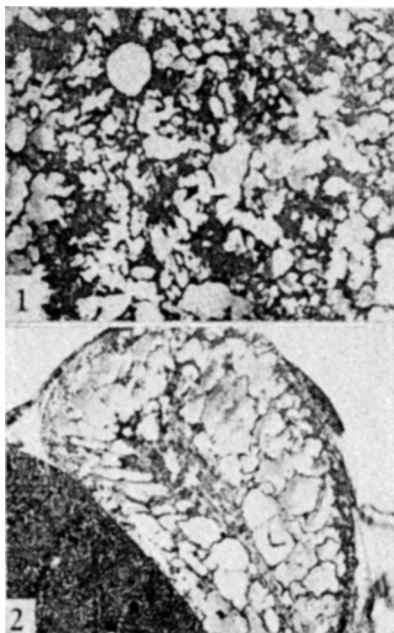


FIG. 1.

Section of liver of tumor bearing animal No. 15 ♂ AC₁, showing ++++ sinusoidal dilatation. Mag. 32 mm objective and 8x ocular.

FIG. 2.

Section of adrenal gland of tumor bearing animal No. 15 ♂ AC₁, showing ++++ sinusoidal dilatation. A small section of the kidney is included in the lower left corner. Mag. 32 mm objective and 8x ocular.

dilatation occurred in mice bearing intra-mesenteric transplants exceeding 5 g at about 4-6 weeks after transplantation.

Discussion. The pathogenesis of these changes remains to be described. Their occurrence with tumors that have no apparent hormonal activity tends to eliminate the possibility of the steroid hormones as the causative agent. They may be due to a hormonal imbalance as a hepatic perilobular sinusoidal dilatation has been described as occurring in the livers of hyperthyroid rats(7); however, histological examination of the thyroids of the animals in this experiment revealed normal histology. This tumor may be producing a specific protein factor or inducing a deficiency state yet to be recognized.

Summary. 1. Mice having a transplanted interstitial cell testicular tumor developed sinusoidal dilatation in the liver, spleen and adrenal glands. 2. These changes were more marked in animals with intra-mesenteric transplants than in animals with subcutaneous transplants. 3. This testicular tumor did not have any hormonal activity in castrate animals.

7. Eker, K., and Efskind, L., *Acta Path. et Microbiol. Scand.* 1944, v21, 609.

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Development of Hypertension in the Adrenalectomized Nephritic Rat Maintained on NaCl.* (18008)

A. I. KNOWLTON, E. N. LOEB, B. C. SEEGAL, H. C. STOERK AND J. L. BERG

From the Departments of Medicine and Bacteriology, College of Physicians and Surgeons, Columbia University, and the Merck Institute for Therapeutic Research, Rahway, N. J.

From reports in the literature, it is difficult to determine whether adrenal cortical hormones are essential for the development or maintenance of experimental renal hypertension.

In the dog, Goldblatt(1) has stated that

"bilateral adrenalectomy eliminates completely the response to constriction of the renal arteries." Page's(2) and Blalock's observations(3) are in accord with this statement. On the other hand, Rogoff *et al.*

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1. Goldblatt, H., *Ann. Int. Med.*, 1937, vII, 69.

2. Page, I. H., *Am. J. Physiol.*, 1938, v122, 352.

3. Blalock, A., and Levy, S. E., *Ann. Surg.*, 1937, v106, 826.

4. Rogoff, J. M., Nixon, E. N., and Stewart, G. N., *Proc. Soc. Exp. Biol. and Med.*, 1939, v41, 57.

TABLE I.
Allocation of Animals.

Group	% NaCl in diet	% NaCl in drinking water	Operation	Rabbit anti-rat kidney serum	No. of rats	
					Allocated	Incl. in data
1	1.0	0	0	0	10	10
2	1.7	.9	0	0	10	10
3	1.7	.9	Bilateral adrenalectomy	0	22	20*
4	1.0	0	0	.8 cc	10	10
5	1.7	.9	Bilateral sham adrenal- ectomy (9 rats) None (26 rats)	.8 or .9 cc	35	34†
6	1.7	.9	Bilateral adrenalectomy	.7, .8, or .9 cc	58	21‡

* One rat died during operation.

† 1 rat omitted because of gross adrenal tissue.

‡ 1 rat omitted from data on blood pressure due to gangrene of tail tip.

§ See Table II.

(4), have reported that hypertension does develop in the dog following the combined operation of clamping the renal arteries and bilateral adrenalectomy, and persists to death of the animal in adrenal insufficiency. Collins and Wood(5) agree with Rogoff that constriction of the renal arteries of adrenalectomized dogs results in hypertension, but they noted further that adrenalectomy in dogs with previously established hypertension results in a gradual reduction in blood pressure. In the rat Gaudino(6) reported that bilateral adrenalectomy usually resulted in a fall in the blood pressure of previously hypertensive animals, and in the few animals in which hypertension reappeared he has been able to demonstrate residual adrenal tissue in all but one.

The present studies were undertaken to determine whether bilateral adrenalectomy alters the blood pressure response of the rat to the type of renal damage induced by cytotoxic serum. The data comprise the accumulated findings from 3 separate series of experiments carried out over the past 15 months.

Methods. 145 rats of the Long-Evans

strain were employed, all but 13 of which were males. The 13 females were divided evenly between Groups 3, 5 and 6. The ages of all rats ranged from 53-76 days at the beginning of the experimental period. The allocation of the animals is presented in Table I. Group 1 served as a control and received the laboratory stock diet(7); Group 2 received a modification of this diet to insure a high NaCl intake, as did Group 3, which in addition were bilaterally adrenalectomized. Of the remaining Groups 4, 5 and 6, all received rabbit anti-rat kidney serum, Group 4 being maintained on stock diet and Groups 5 and 6 on this diet modified to contain a high salt content. In addition, bilateral sham adrenalectomy was performed in 9 of the 35 rats of Group 5, while all the rats of Group 6 were bilaterally adrenalectomized.

Bilateral adrenalectomy was performed on the rats of Groups 3 and 6 at the start of the experiments by one of the authors (H.C.S.), under ether anesthesia without aseptic precautions. The adrenals were removed with forceps through bilateral lumbar incisions, care being taken to remove the entire peri-adrenal fat intact without manipulating the gland itself. The operation was followed im-

5. Collins, D. A., and Wood, E. H., *Am. J. Physiol.*, 1938, v123, 224.

6. Gaudino, N. M., *Rev. Argent. de Biol.*, 1944, v20, 470.

7. Loeb, E. N., Knowlton, A. I., Stoerk, H. C., and Seegal, B. C., *J. Exp. Med.*, 1949, v89, 287.

mediately by a clysis of 5 cc of normal saline and the animals were then maintained on a high NaCl intake as indicated in Table I. Sham adrenalectomy consisted of similar bilateral lumbar incisions through which the perirenal fat was manipulated with forceps but not removed.

Blood pressure determinations were made on all animals prior to the beginning of the experiment and thereafter at intervals of one to 2 weeks. Two methods of determining blood pressure were employed: (1) the indirect plethysmographic method described by Williams, Harrison and Grollman(8) and modified by Sobin(9), in the first 2 series of experiments; and (2) the indirect microphonic manometer method described by Friedman and Freed(10), in the third experiment. Since preliminary determinations made with both methods on a series of 19 rats having blood pressures ranging from 100-212 mm/Hg showed no greater discrepancy than the limitation of error in either individual method, the results with the two methods have been considered comparable and reported together. With both methods, each individual blood pressure reading recorded is the average of ten successive determinations having no greater range than 20 mm/Hg.

Renal damage was induced by the injection of rabbit anti-rat kidney serum. Two different pools of sera, of comparable potency,

were employed. The injections were given intravenously in divided doses over a 3-day period. In the adrenalectomized rats of Group 6, injections were begun 18 days post-operatively; in the intact Group 5, injections were begun 18 days after the institution of the high NaCl intake; and in the intact Group 4, at a comparable date.

During the experimental period, the animals were weighed at frequent intervals. The serious difficulties encountered in working with adrenalectomized animals are apparent in Table II. Quantitative urinary albumin was determined(11) on all but 3 animals on one or more occasions. At the end of the experimental period, *i.e.*, after 7 to 9 weeks, the animals in the unoperated Groups 1, 2 and 4, as well as 25 rats from Group 5 were sacrificed. The remaining 10 rats of Group 5 were subjected to a 21-day period of NaCl withdrawal before sacrifice. In the control adrenalectomized Group 3, 4 died during the

TABLE II.
Fate of 58 Rats Allocated to Group 6.

Included in final data:	21 rats
Discarded, due to:	
Death from anesthesia during adrenalectomy	6
Death following adrenalectomy and prior to administration of serum	9
Death within 3 wks after serum administration	16
Presence of adrenal tissue	6

TABLE III.

Group	Regimen	No. of rats	Body wt, g	Kidney wt, g	Urine albumin, g/l	Instance of renal lesions
1	—	10	251	1.87	2.5	—
2	NaCl	10	246	2.03	2.5	—
3	ADX + NaCl	20	191	1.70	1.6	None of 8 examined
4	AKS	10	263	2.08	29.0	8 of 10 "
5	NaCl + AKS	35	267	2.49	20.2	33 of 33 "
6	ADX + NaCl + AKS	{ 15* 6†	{ 186* 252†	{ 1.91* 2.44†	{ 18.3* 18.2† }	6 of 8 " ‡

* Non-hypertensives.

† Hypertensives.

‡ In remaining 13 animals in this group which died (spontaneously or on salt withdrawal) autopsies were performed at varying intervals after death and in these autolysis prevented distinction of mild renal damage. In the 9 of these 13 studied histologically no advanced renal lesions were seen.

8. Williams, J. R., Harrison, T. R., and Grollman, A., *J. Clin. Invest.*, 1939, v18, 373.

9. Sobin, S. S., *Am. J. Physiol.*, 1946, v146, 179.

10. Friedman, M., and Freed, S. C., *Proc. Soc.*

EXP. BIOL. AND MED., 1949, v70, 670.

11. Shevky, M. C. and Stafford, D. D., in *Laboratory Methods of the U. S. Army*, (J. S. Simmons, Ed.), Phila., Lea and Febiger, 4th edition, 1935, 290.

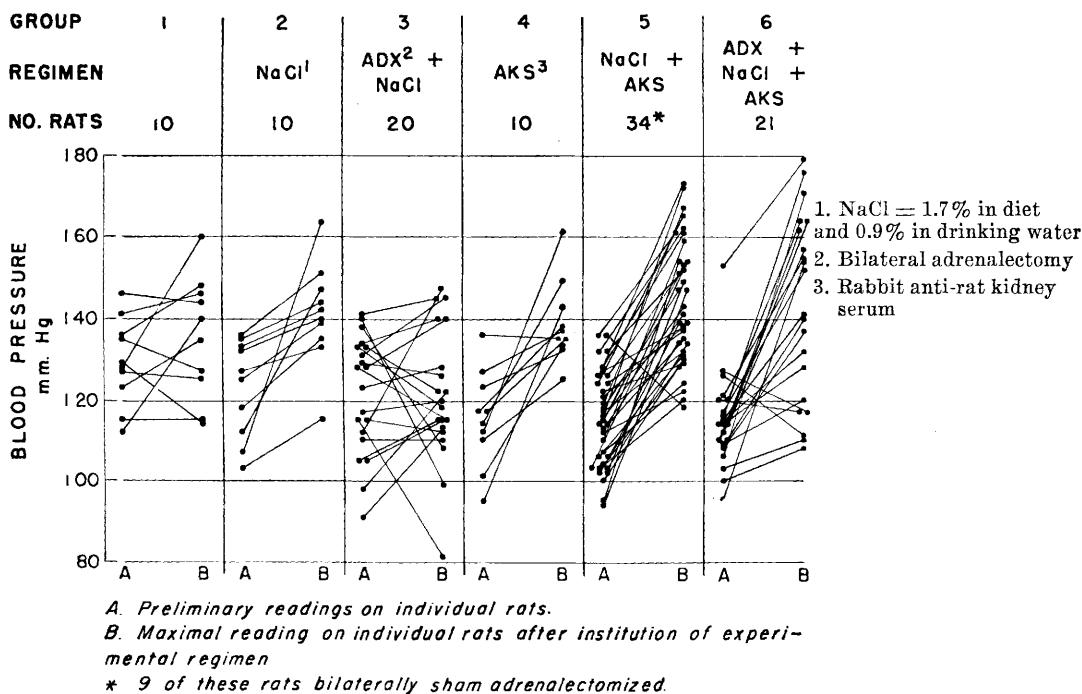


FIG. 1.

Control and maximal blood pressure readings.

period of observation, 8 were sacrificed and the remaining 8 were subjected to NaCl withdrawal. In the nephritic adrenalectomized Group 6, 5 rats died, 8 were sacrificed and the remaining 8 animals were subjected to NaCl withdrawal. At death or sacrifice, the animals were weighed and heart and kidney weights recorded. (In the adrenalectomized rats in which sodium was restricted terminally, the weight prior to the institution of this regimen has been recorded as the final body weight).

The criterion of the completeness of adrenalectomy was the absence of macroscopic or microscopic evidence of adrenal tissue at sacrifice or death. In the rats subjected to sodium withdrawal, physiological evidence of absence of the adrenals, *i.e.* the inability to survive more than 15 days without added salt was an additional criterion. One rat in Group 3 did not fulfill this last criterion, but is included because at sacrifice after 15 days of NaCl withdrawal, its serum sodium was 112 mEq/l and no adrenal tissue was found.

Results. The rats in the intact groups

gained weight and remained well during the period of observation. In the adrenalectomized Groups 3 and 6, 16 animals died during the operative procedure or shortly thereafter. The average growth of the adrenalectomized animals was less than that of the intact groups although individual animals grew satisfactorily (compare final body weights of Group 3 and non-hypertensive animals of Group 6 with intact groups, Table III). In addition, 16 of the Group 6 animals died too early in the experimental period after serum administration to allow for an adequate number of blood pressure determinations (Table II). Hence the following results and the subsequent discussion concerns the 21 rats described in Table II as included in final data.

Blood pressure. Fig. 1 presents both the initial blood pressure readings on individual rats in each group and the corresponding individual maximal blood pressure readings obtained thereafter during the course of the experiment. No significant change can be seen in Groups 1 or 2 and the lowering of the blood pressure in animals of Group 3 is of

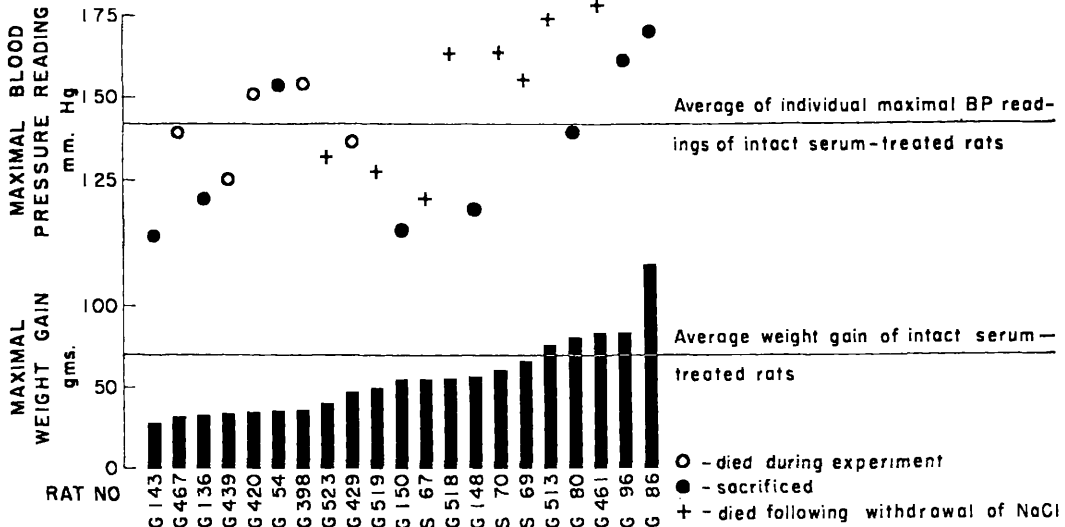


FIG. 2.

Chart of individual adrenalectomized nephritic rats in Group 6 showing maximal blood pressures and maximal weight gains during experimental period.

questionable significance. In the nephritic Group 4 on stock diet the figures are comparable to Groups 1 and 2, only one animal developed a blood pressure greater than 160 mm/Hg. Seven of the 34 nephritic rats on a high NaCl regimen, Group 5, had peak readings above 160, while 6 of the 21 similarly treated adrenalectomized rats, Group 6, had an equal degree of elevation.

In Fig. 2, the maximal blood pressures recorded for the individual adrenalectomized nephritic rats of Group 6 are plotted to show their relation to individual maximal weight gain. In addition, the average maximal blood pressure and the average maximal weight gain of the intact nephritic rats of Group 5 are indicated. From this chart, it will be seen that the highest blood pressures occurred in those rats which grew satisfactorily. The mode of death of the individual rats is likewise shown in this chart. Rats which died during the experimental period (depicted as open circles) tended, as might be expected, to be those which gained less than the average. The closed circles indicate those animals which were sacrificed, while the crosses denote those rats which were subjected to NaCl withdrawal and died following this. It will be seen that these latter animals included a

number of the most hypertensive.

One rat (No. S 70) from the adrenalectomized nephritic Group 6 has been selected to show in detail the relation of satisfactory growth to the development of hypertension (Fig. 3). While this animal was gaining adequately, a progressive hypertension developed. During the ninth experimental week, the rat lost weight and following this, a significant fall in blood pressure occurred. Subsequently, following sodium withdrawal, a further 65 g weight loss ensued in the seven days prior to death.

NaCl withdrawal. The 8 adrenalectomized nephritic rats of Group 6 lost an average of 47 g (range 30-65 g) and died in 3-15 days when placed on a NaCl free diet and given tap water in place of saline to drink. The 10 intact nephritic rats of Group 5 subjected to a similar regimen of NaCl restriction, gained an average of 32 g (range 10-59 g) over 21 days and none died.

Urinary albumin. In Table III, the urinary albumin excretion in all 6 groups is shown. In all groups given the rabbit anti-rat kidney serum(4,5,6), significant albuminuria developed, indicating the presence of some degree of renal damage in these rats. **Body and kidney weights** at sacrifice also are shown in

Rat no. S70

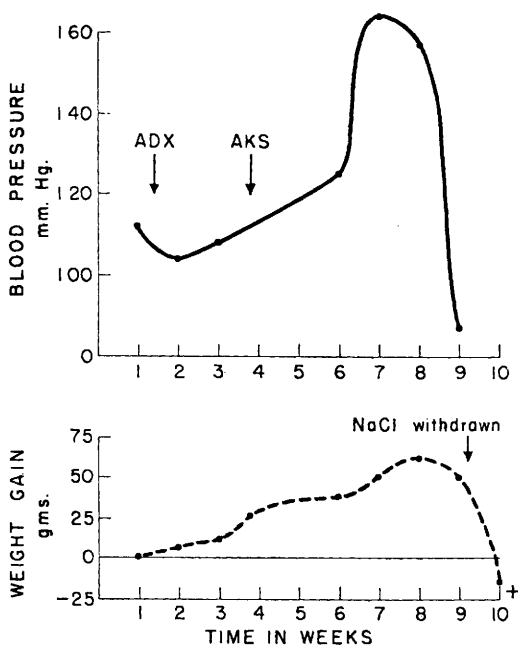


FIG. 3.

Chart of Rat No. S70—blood pressure readings and weights during experimental period.

ADX = Bilateral adrenalectomy

AKS = Rabbit anti-rat kidney serum.

Table III. From this it may be seen that the body weights of the adrenalectomized animals averaged less than that of the intact groups with the exception of the hypertensive adrenalectomized animals of Group 6. The

combination of high NaCl intake and anti-kidney serum resulted in renal enlargement in the intact rats of Group 5. A similar degree of renal enlargement occurred among the hypertensive animals of Group 6. The non-hypertensive animals in this group had smaller kidneys, but since their body weights are so much less than that of Group 5, it is difficult to interpret these findings. Also included in this table is the number of rats showing histological evidence of renal damage. Renal lesions were, if anything, less frequent in the adrenalectomized rats of Group 6 than in the similarly treated intact Group 5 animals.

Summary. In response to cytotoxic serum nephritis, bilaterally adrenalectomized rats develop hypertension as frequently as do animals with intact adrenals. In order for hypertension to appear among such adrenalectomized rats, it is necessary that a satisfactory state of nutrition be maintained over a period of weeks. In our laboratory, the difficulties encountered in fulfilling this provision have been considerable in spite of the employment of a high NaCl regimen. This experience has led us to wonder whether the conflicting results in the literature relating to the question of hypertension in adrenalectomized animals may not be due, in part, to similar difficulties.

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Conjugated Linoleic Acid in Rat Tissue Lipids after Ingestion as Free Acid and as Triglyceride.*† (18009)

RAYMOND REISER (Introduced by L. R. Richardson)

From the Department of Biochemistry and Nutrition, Texas Agricultural Experiment Station, Texas Agricultural and Mechanical College System, College Station, Texas.

The present study was conducted to test the use of conjugated linoleic acid for proposed investigations of the metabolism of glycerides by the use of carbon 14 labeled

glycerol. The progression of the conjugated acid through the various tissues at successive times after its ingestion is intended as a guide for later studies with the labeled glycerol esterified with tagged acids. Advantage was taken of the experiment to test the observations of Frazer that fatty acids after ingestion go to the liver but that unhydro-

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