Discussion. The use of tungstate and sulfuric acid to prepare a protein free filtrate, while not removing the carbohydrate is a standard procedure in blood analysis work.[4] The fact that the blood group specific substances are carbohydrate like lends them admirably to this procedure. The method was probably never attempted before because of the nature of the reagents used since there was some doubt in our mind initially as to whether the blood group specific substance would withstand this treatment. It is not claimed that a superior product is obtained by the Folin-Wu technic in comparison to the method of Goebel, but the procedural simplicity of the former makes it a method of choice which should be easily used when animal tissues are the source of blood group specific substances.

Summary. A method for preparing blood group specific substance A from Difco neopeptone by the Folin-Wu technic is described and comparisons with other preparations are made. The method is simple and the product obtained compares favorably in purity and potency with products obtained by other methods.

[4] Andrews, J. C., and Kyker, G. C., 1947, A Laboratory Manual of Biological Chemistry, Edwards Brothers, Inc., Ann Arbor, Mich.

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Influence of Adrenal Cortical Steroids and Related Compounds on Sodium Metabolism.* (17446)

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In a previous paper from this laboratory[1] it was demonstrated that as little as one microgram of desoxycorticosterone produced a significant retention of sodium in the adrenalectomized male rat. This communication is concerned with the study of various adrenal cortical steroids and related compound on sodium metabolism by the same technic. These steroids include 11-dehydrocorticosterone, 17-hydroxycorticosterone, corticosterone, pregnanetrione-3, 11, 20, allopregnanetriol-3 (β), 17 (α), 21-one-20, alloprenanepentol-3 (β), 11(β), 17(α), 20, 21-3(β), 20, 21 triacetate, Δ^4-prenenol-21-trione-3,12,20 acetate, testosterone, estradiol, and pregnanetrione-3,12,20.

Animals, Materials, Methods. The rats were obtained from Carworth Farms. They were bilaterally adrenalectomized in one stage under ether anaesthesia. The animals subsisted exclusively on a chow diet both before and after adrenalectomy. When more than 24 hours elapsed between adrenalectomy and the day the experiment was run the rats were given normal saline in place of ordinary drinking water until the morning of the experiment.

The test material was injected subcutaneously in 0.25 cc of corn oil One hour later the rats received subcutaneously, 2 cc of a solution containing sodium chloride and the radiosodium.† The dose of sodium chloride was 35 μg per gram of body weight. The animals were placed in glass collecting cages and the urine collected for 6 hours. The urine was dried and the concentration of radiosodium in the residue determined as described previously.[1]

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[1] Dorfman, R. I., Potts, A. M., and Feil, M. L., *Endocrinology,* 1947, **41**, 464.

† The radiosodium was supplied by the Monsanto Chemical Company, through the U. S. Atomic Energy Commission.

TABLE I.
Influence of 11-Dehydrocorticosterone Acetate on Excretion of Na in the Adrenalectomized Male Rat.

Control			Experimental			
Mean B.W. g ± S.E. (No.)	Mean Na excretion % ± S.E.	Amt, μg	Mean B.W. g ± S.E. (No.)	Mean Na excretion % ± S.E.	Change, %	t
115 ± 5 (9)	4.57 ± 1.0	25	113 ± 3 (9)	5.04 ± 0.75	+10	
115 ± 5 (9)	4.57 ± 1.0	75	119 ± 3 (9)	3.39 ± 0.45	—26	1.03
115 ± 5 (9)	4.57 ± 1.0	100	122 ± 3 (9)	3.79 ± 0.54	—17	
124 ± 3 (8)	4.30 ± 0.55	100	137 ± 4 (9)	4.88 ± 0.42	+14	0.81

TABLE II.
Influence of 17-Hydroxycorticosterone on Excretion of Na in Adrenalectomized Male Rats.

Control			Experimental			
Mean B.W g ± S.E. (No.)	Mean Na excretion % ± S.E.	Amt, μg	Mean B.W. g ± S.E. (No.)	Mean Na excretion % ± S.E.	Change, %	t
135 ± 3 (9)	4.59 ± 0.48	25	142 ± 5 (9)	4.88 ± 0.93	+ 6	
128 ± 4 (8)	1.48 ± 0.27	50	124 ± 3 (8)	2.44 ± 0.26	+65	2.570
127 ± 5 (9)	1.51 ± 0.25	50	120 ± 4 (9)	2.67 ± 0.52	+77	2.020
127 ± 5 (9)	1.51 ± 0.25	200	121 ± 6 (9)	2.18 ± 0.25	+44	1.88
139 ± 6 (8)	6.69 ± 0.55	200	143 ± 5 (8)	7.06 ± 1.06	+ 6	

The results were expressed as the amount of radiosodium excreted compared to the total amount of radiosodium administered in per cent. The effect was measured by comparing the mean percentage excretion of the experimental animals to the mean percentage excretion of the control animals which were run simultaneously.

Following is a list of compounds studied and their source.

Ciba Pharmaceutical Products, Inc.; Desoxycorticosterone, Desoxycorticosterone acetate, Testosterone, Estradiol.

Dr. R. D. H. Heard, McGill University; Δ^4-pregnenol-21-trione-3, 12, 20-21-acetate

The Upjohn Company; Allopregnane-3(β), 17(α) 21-one-20, 17-Hydroxycorticosterone, Corticosterone

Dr. E. C. Kendall, Mayo Clinic; 11-Dehydrocorticosterone acetate, Corticosterone, 17-Hydroxycorticosterone

Dr. T. F. Gallagher, Memorial Hospital; Pregnanetrione-3,11,20

Dr. T. Reichstein, University of Basle; Allopregnanepentrol-3(β), 11(β), 17(α), 20, 21, 3(β), 20, 21-triacetate

Dr. D. Prins, Cleveland Clinic; Pregnanetrione-3,12,20

Results. In a previous publication (Dorfman, Feil, and Potts[1]) it was shown that desoxycorticosterone produces a significant sodium retention in adrenalectomized male rats in amounts as low as one microgram. The desoxycorticosterone acetate produced retention at a concentration of 25 μg but lower concentrations of the ester have not been studied.

Table I lists 4 experiments on the influence of 11-dehydrocorticosterone acetate on sodium metabolism in the adrenalectomized male rats. No significant changes in sodium excretion were found at concentrations of 25 to 100 μg

TABLE III.
Influence of Δ^4-Pregnenol-21-Trione-3,12,20-21-Acetate on Excretion of Na in Adrenalectomized Rats.

Control			Experimental			
Mean B.W. g ± S.E. (No.)	Mean Na excretion % ± S.E.	Amt, μg	Mean B.W. g ± S.E. (No.)	Mean Na excretion % ± S.E.	Change, %	t
132 ± 4 (9)	1.53 ± 0.23	100	127 ± 3 (9)	1.75 ± 0.17	+14	0.76
124 ± 3 (8)	4.30 ± 0.55	400	126 ± 5 (5)	4.21 ± 0.50	— 2	
169 ± 3 (11)	3.52 ± 0.41	1340	177 ± 4 (12)	2.36 ± 0.39	—33	2.03

TABLE IV.
Activities of Various Steroids on Na Excretion in Adrenalectomized Rats.

Compound	Dosage, μg	Type of activity
Desoxycorticosterone	1*	Retention
Desoxycorticosterone acetate	25*	"
Δ^4-Pregnenol-21-trione-3,12,20-21-acetate	100	Negative
	400	"
	1340	Retention
17-Hydroxycorticosterone	25	Negative
	50	Increased excretion
	200	Negative (?)
11-Dehydrocorticosterone acetate	100	Negative
Pregnanetrione-3,11,20	200	"
Corticosterone	400	"
Allopregnanepentol-3(β), 11(β), 17(α), 20,21-3(β),20,21-triacetate	800	"
Allopregnanetriol-3(β), 17(α), 21-one-20	800	"
Testosterone	2000	"
Estradiol	2000	"
Pregnanetrione-3,12,20	2000	"

* Lowest concentration studied.

of this adrenal cortical steroid. The influence of 17-hydroxycorticosterone on the excretion of sodium in the adrenalectomized rat is represented in Table II. At a level of 25 μg a mean excretion of 4.88% ± 0.93% was found as compared to the control value of 4.59% ± 0.48% which represented an insignificant change of +6%. When the dose of 17-hydroxycorticosterone was increased to 50 μg an increase in sodium excretion of + 65 and + 77% respectively in 2 experiments was observed. The t values for these experiments were 2.570 and 2.020. At 200 μg a tendency toward increased excretion of sodium was observed in one experiment (+44%, t = 1.888) while in a second experiment no significant change was observed.

Δ^4-pregnenol-21-trione-3,12,20 was studied at dose levels of 100, 400, and 1340 μg (Table III). At a dose level of 1340 μg a statistical significant retention of 33% (t = 2.03) was found. This is the first instance of biological activity being found for this compound. At 100 and 400 μg dose levels no significant change in sodium excretion was observed.

All other steroids studied were inactive at the doses tested. These included: pregnanetrione-3,11,20 (200 μg), corticosterone (400 μg), allopregnanepentol-3(β), 17(α), 11(α), 20, 21-3(β), 20,21-triacetate (800 μg), allopregnanetrione-3,12,20 (2000 μg), testosterone (2000 μg), and estradiol (2000 μg).

Discussion. A compilation of the results is presented in Table IV. Desoxycorticosterone is by far the most active substance tested having given positive results at one microgram. The acetate produced significant retention at 25 μg. No lower concentrations have been

tested. Δ^4-pregnenol-21-trione-3,12,20 acetate has been found to cause significant sodium retention at 1340 μg and a negative effect at 400 μg. Thus the introduction of the 12 keto group results in a compound possessing about 2 to 5% of desoxycorticosterone. 17-Hydroxycorticosterone produced an increased sodium excretion at 50 μg but the effect was either minimized or obliterated at 200 μg. Increased urinary excretion of sodium has been observed previously for 17-hydroxycorticosterone and 17-hydroxy-11-dehydrocorticosterone in normal dogs and rats[2] and in partially depancreatized rats.[3] Ingle and coworkers[4] have found that relatively large doses of ether 17-hydroxycorticosterone or 17-hydroxy-11-dehydrocorticosterone caused an immediate increase in sodium excretion during the first 24 hours but that the sodium excretion returned to the control levels in spite of continued treatment.

Summary. Eleven steroid compounds have been studied as to their influence on sodium excretion in adrenalectomized rats. Desoxycorticosterone caused significant retention at one microgram, and the acetate was effective at 25 micrograms, the lowest concentration studied. Δ^4-pregnenol-21-trione-3,12,20-21-acetate gave a significant retention at 1340 micrograms. 17-Hydroxycorticosterone caused a significant excretion of sodium.

The following steroids were found to be negative: 11-dehydrocorticosterone acetate (100 μg), pregnanetrione-3,11,20 (200 μg), corticosterone (400 μg), allopregnanepentol-3 (β), 11(β), 17(α), 20, 21-3(β), 20, 21-triacetate (800 μg), allopregnanetriol-3(β), 17 (α), 21-one-20 (800 μg), testosterone (2000 μg), estradiol (2000 μg), and pregnanetrione-3,12,20 (2000 μg).

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2 Thorn, G. W., Engel, L. L., and Lewis, R. A., *Science*, 1941, **94**, 348.

3 Ingle, D. J., and Thorn, G. W., *Am. J. Physiol.*, 1941, **132**, 670.

4 Ingle, D. J., Sheppard, R., Evans, J. S., and Kuizenga, M. H., *Endocrinology*, 1945, **37**, 341.

Evaluation of Autolyzed Mouse Brain Tissue Method for Isolation and Adaptation of Poliomyelitis Virus. (17447)

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A method for isolation and adaptation of poliomyelitis virus by direct passage into mice was described by Milzer and Byrd.[1] The method consists in mixing suspensions of infected feces or central nervous system (CNS) material with an equal amount of a 10% suspension of autolyzed normal mouse brain tissue (ABT) and injecting the mixture intracerebrally into white mice. It is obvious that a usable method for primary isolation of poliomyelitis virus without the use of expensive monkeys would present a great advantage. The authors undertook, therefore, to verify this method and carried out a total of 19 experiments with mice and 4 with cotton rats.

Materials and Methods. Feces. Five specimens were taken from poliomyelitis patients, but not tested for virus content. The presence of poliomyelitis virus in 7 other stool specimens was demonstrated by monkey inoculation prior to adaptation experiments. (The stool specimens were obtained through courtesy of Dr. Franklin H. Top, Herman Kiefer Hospital, Detroit, and Dr. Joseph L. Melnick, Yale University).

Monkey adapted strains. Spinal cords of monkeys infected with poliomyelitis virus strains Buffalo, Mahoney and Tennessee were obtained through courtesy of Dr. Thomas Francis, Jr., University of Michigan. The

1 Milzer, A., and Byrd, C. L., *Science*, 1947, **105**, 70.