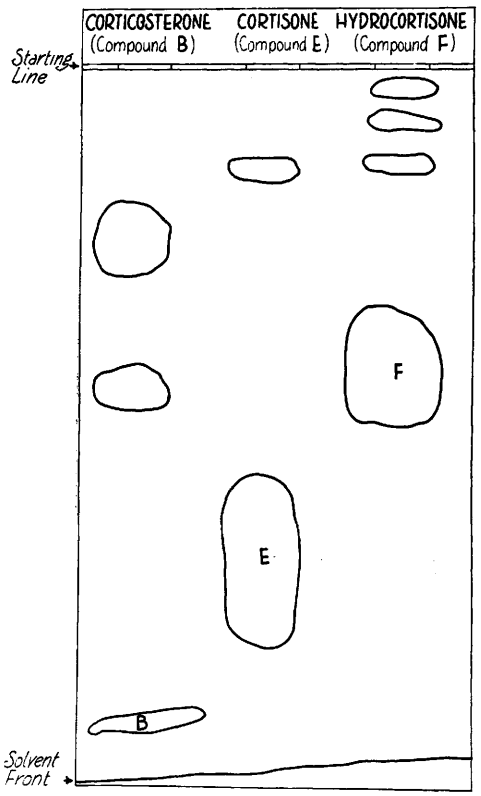

FIG. 1. Paper chromatogram of adrenal slice incubations of corticosterone, cortisone, and hydrocortisone. Compounds were outlined by their U. V. absorption. These substances also reduced blue tetrazolium.

tissue produced two compounds more polar than the starting material. A polar compound was produced when cortisone was incubated with adrenal tissue whose chromatographic mobility was similar to one of the compounds from the hydrocortisone incubation (Fig. 1).

Since the 3 products of hydrocortisone incubations possess the Δ^4-3-ketone and α-ketol groupings and have distinctly lower mobilities than the original substrate, it is likely that additional oxygen functions have been put into the nucleus perhaps at carbons 6, 16, or both.

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Effects of Amphetamine and Its Isomers on Excretion of Sodium and Water in the Rat.* (20193)

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Recent reports(1-3) indicate that certain sympathomimetic drugs have important effects on the renal excretion of electrolytes and water. Although amphetamine is sympathomimetic, it has been reported that its urinary effects are variable(4) or negligible(5). The determination of urinary sodium has not, however, been carried out in animals or men receiving amphetamine; and in the experiments cited, no control of water or sodium intake was attempted. In the present communication, the effects of amphetamine and its isomers on the excretion of sodium and water in rats under controlled conditions of water and salt loading are described. Amphetamine (Benzedrine) and its dextrorotatory isomer (Dexedrine) and to a somewhat lesser extent the levorotatory isomer (Levedrine) appear to be powerful natriuretic and diuretic agents in all conditions studied.

Methods. Rats weighing from 200 to 350 g and of either sex were used. The drug to be investigated was given by intraperitoneal injection. Animals under salt load received 20 ml of isotonic saline by the same route. Other animals were used after an 18-hour fast during

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TABLE I. Sodium and Weight Losses in Rats Receiving Amphetamine and Its Isomers.

Drug	Dosage, mg/rat	State of animal	No. of animals	Duration metabolic period, hr	Sodium loss, mg/rat (range), ± = S.D.	Wt loss, g/rat (range), ± = S.D.
				(a)		
d-a*	1	18 hr fast	6	3	2.1 (1.9 – 2.3)	4.5 (4.0 – 5.0)
C	0	"	6	3	.25(.11– .39)	.5 (.33– .67)
				(b)		
d-a	1	Normal, unfasted	6	3	7.5 (4.3 –10.7)	12.0 (9.3 –14.7)
C	0	"	6	3	.8 (.5 – 1.3)	2.5 (2.0 – 3.0)
				(c)		
dl-a	1	20 ml isotonic NaCl	20	6	41.5 ± 2.2	16.9 ± .9
d-a	1	"	20	6	39.3 ± 4.5	15.7 ± 1.5
l-a	1	"	20	6	33.0 ± 2.2	12.8 ± .8
C	0	"	20	6	20.6 ± 3.5	8.4 ± .8
				(d)		
d-a	1	"	12	6	45.8 (42.1–47.3)	16.0 (13.5–19.5)
C	0	"	12	6	24.0 (22.6–27.1)	10.6 (9.2–11.9)
d-a	1	"	12	9	60.0 (50.0–68.2)	19.0 (15.3–23.1)
C	0	"	12	9	41.9 (38.3–45.1)	18.0 (14.1–22.5)
d-a	1	"	12	12	62.8 (59.2–66.0)	24.3 (22.5–26.5)
C	0	"	12	12	48.3 (47.0–49.5)	26.0 (23.5–28.2)
d-a	1	"	12	24	71.3 (65.8–77.0)	34.0 (29.6–38.3)
C	0	"	12	24	61.9 (57.0–68.2)	33.3 (30.0–36.4)
				(e)		
C	0	"	12	6	24.9 (20.7–29.2)	
d-a	0.1	"	12	6	24.6 (21.0–28.5)	
"	0.2	"	12	6	25.0 (20.5–28.4)	
"	0.5	"	12	6	28.6 (20.1–40.5)	
"	1.0	"	12	6	37.5 (34.0–42.8)	
"	2.0	"	12	6	35.0 (29.0–40.7)	

* a = amphetamine; C = control.

which no water was supplied. In some experiments animals were used without preliminary fasting or dehydration. The animals were placed in metabolic cages in groups of 3 to 5 and followed for metabolic periods of up to 24 hours. No food or water were allowed during the metabolic period. Sodium losses were determined by extracting the combined excreta with water and analyzing the extract with a flame photometer. Weight losses were used as a rough index of water losses. So far as possible, each rat was made to serve as its own control in consecutive experiments. Simultaneous control groups were also run in each experiment.

Results. Effect of d-amphetamine on sodium and weight losses in starving rats. Rats were starved for 18 hours and then received 1.0 mg of d-amphetamine in 1.0 ml of saline. Control animals received 1.0 ml of saline. The animals receiving d-amphetamine showed a considerable increase over the controls in both sodium excretion and weight loss during a 3-hour metabolic period. The results of a typical experiment are shown in Table I (a).

Effect of d-amphetamine on sodium and weight losses in unstarved rats. Results similar to those given above were obtained when there was no preliminary fast. The sodium and weight losses provoked by d-amphetamine were somewhat greater in these animals than in the starved animals. The results of a typical experiment are given in Table I (b).

Effects of amphetamine, d-amphetamine, and l-amphetamine on sodium and weight losses in salt loaded rats. In these experiments the control animals received isotonic saline intraperitoneally in 20 ml doses. The experimental animals received 1 ml of the drug alone with the saline. At the 6-hour period the results obtained were those presented in Table I (c). Similar results were obtained at 9, 12, and 24 hours, so far as sodium losses were concerned; weight losses tended to become equal in the drug treated and the control groups with time (Table I (d).)

Effect of dosage. The relationship between

dosage of d-amphetamine and sodium and weight loss was followed in salt loaded rats at periods of 3 to 24 hours. The results at 6 hours are typical of the findings at all periods. Doses of .1 and .2 mg per rat were completely ineffective so far as sodium and water losses were concerned; a very slight effect was observed at the .5 mg level; maximum effectiveness was attained at a dose level of 1.0 mg per rat and increasing the dose to 2.0 mg was without further effect. The results of an experiment with varying doses of d-amphetamine at the 6-hour period are given in Table I (e).

Effect of d-amphetamine on the glomerular filtration rate of fasting rats. The glomerular filtration rate was measured in 9 normal rats and 9 d-amphetamine treated rats by the "undisturbed" method of Pultz, Herrold, and Sapirstein(6). The animals were not under saline load and filtration rates were measured after an 18-hour fast. In preliminary experiments on nephrectomized rats, the "effective delay time" was found to be unchanged by the administration of 1.0 mg of d-amphetamine. The volume of distribution of mannitol was likewise unchanged from the normal. (Effective delay time normals, 3 minutes; d-amphetamine treatment, 3 minutes; mannitol space. nephrectomized normal, 28.6% body weight, d-amphetamine treatment, 28.5% body weight). The mannitol clearance in the normal animals was found to be .62 ml/100 g/min. (S.D. .08) and in the d-amphetamine treated animals .85 ml/100 g/min. (S.D. .06).

Discussion. In all experiments animals receiving amphetamine or either of its isomers showed evidence of central nervous system stimulation and increased activity. This occurred even at the lower dosage levels used in the experiments described in Table I (e), which were without effect on sodium or weight losses. From these findings, it appears improbable that the effect on sodium and weight losses can be attributed to increase somatic activity.

The effects of amphetamine and d-amphetamine appear to be quantitatively comparable. l-amphetamine appears to be somewhat less active than d-amphetamine, but nevertheless possesses considerable activity. The fact that the racemic mixture of d- and l-amphetamine, which would be expected to be less active than d-amphetamine, was actually as active as d-amphetamine can probably be attributed to the use of a supramaximal dose of the mixture.

The increased elimination of sodium and water (measured as weight loss) which are observed in the drug treated animals can be accounted for readily by the increase in the glomerular filtration rate. In the fasted animal this increase in filtration rates amounts to .23 ml/100 g/min. or to 35.5 ml/rat/hr. In terms of sodium this is equivalent to 128 mg/rat/hr. In the 3-hour metabolic period, it is necessary to account for only 4.0 ml of water and 1.85 mg of sodium excess in the drug treated fasting animals. Clearly, there is no need to postulate a decrease in tubular reabsorption of sodium and water after drug treatment. In view of the sympathomimetic properties of amphetamine, it is of interest that similar changes in filtration rate have been reported in rats treated with noradrenaline by Eversole *et al.*(1); these authors, however, interpreted the fact that creatinine U/P ratios were altered more than filtration rates to indicate that there was decreased tubular reabsorption of sodium and water with noradrenaline; this argument does not appear to be valid.

Insufficient information is available from the present experiments to indicate definitely whether the increased filtration rate which we believe to be responsible for the increased elimination of water and sodium is due to intrarenal hemodynamic alteration or is consequent on a general rise in the arterial pressure. However, in preliminary experiments in human subjects, we have noted increased sodium and water excretion without blood pressure rise when d-amphetamine is taken. It appears probable that the effect of amphetamine on the renal circulation is a direct one, consisting of afferent dilatation, efferent constriction, or both.

The early rapid weight loss sometimes observed in human subjects on controlled diets who are receiving d-amphetamine may be a consequence of a sodium and water wasting effect similar to what has been reported here in rats. In prolonged therapy, however, there

seems to be no doubt that the weight loss observed is due to diminished food intake.

Although the salt wasting effects of amphetamine and d-amphetamine in the animal loaded with isotonic saline requires dosage levels in the rat far above those which would be considered therapeutic in man, we have found in the preliminary experiments quoted above that fasting human subjects show considerable natriuresis and diuresis with doses of 5.0-10.0 mg of d-amphetamine. The possibility that amphetamine and other sympathomimetic agents may be of value as adjuncts in the treatment of edema is being investigated.

Summary and conclusions. 1. Amphetamine and its isomers are diuretic and natriuretic in starved, normal and salt loaded rats. 2. The glomerular filtration rate of starved rats is elevated by d-amphetamine. The elevation of filtration rate is sufficient to account for the diuresis and natriuresis observed without postulating a decrease in tubular absorption of water or sodium.

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In vitro Inactivation of Newcastle Disease Virus by Penicillin Preparations. (20194)

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In the course of experiments in which it was attempted to estimate the concentration of Newcastle disease virus (NDV) by titration in embryonated eggs, a delay of the death of infected embryos was observed when the infecting virus suspension contained penicillin. Incubation of suspensions containing up to 10000 ELD_{50} (lethal for embryos) of the virus with 5000 units of penicillin for a few hours before injection into chick embryos resulted in an apparent 100-fold decrease in the infectivity of the virus. Streptomycin at concentrations up to 5 mg/ml had no detectable influence on the virus.

The present communication reports on the action of penicillin on NDV.

Materials and methods. Virus. The Newcastle disease virus used in these experiments was isolated from an epidemic in 1949† and stored in the form of infected allantoic fluid at −25°C for approximately 2 years. The virus killed susceptible chickens within 6 days after inoculation, and embryonated eggs within 72-96 hrs. In some experiments the live virus vaccine of Komarov and Goldsmit(9) was used. Titrations were carried out in 9-12 days embryonated eggs. Serial 10-fold dilutions of virus were prepared and 0.1 ml of each dilution was inoculated in the allantoic sacs of each of 4 to 6 eggs. Infected eggs were candled daily. Embryos dying within 24 hours of incubation were discarded. All eggs showing death of embryos after 24 hours were chilled for a few hours, opened, and their allantoic fluid tested for the presence of virus by means of a plate hemagglutination test (13). Virus titers were estimated according to the method of Reed and Muench(18). *Penicillin.* Crystalline penicillin G was used throughout (sodium salt—Merck, or potas-

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