hemin decomposition.

The possible destruction of hematin compounds in the presence of oxidizing lipids *in vivo* (*e.g.*, the decomposition of cytochromes in the presence of peroxidizing mitochondrial lipids) is of interest from the standpoint of the lesions observed in animals deficient in vitamin E or exposed to ionizing radiation. The present results emphasize the strong oxidative properties of preformed peroxides toward such compounds. However, in the colloidal medium and under the pH conditions which prevail *in vivo* the role of fatty acid and peroxide free radicals may assume primary importance.

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Diuretic Effect of Angiotensin in the Chicken.* (31477)

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Pickering and Prinzmetal(1) demonstrated that renin was natriuretic in the rabbit, and Hughes-Jones, Pickering *et al*(2) suggested that this effect was due to a direct tubular effect of angiotensin. Recent studies by Laragh(3) and Langford and Pickering(4) have confirmed the diuretic effect of angiotensin in high doses in man and rabbit and again suggested that a tubular effect was present as glomerular filtration was reduced. Vander(7) in the dog found depression of distal sodium reabsorption when angiotensin was injected into the renal artery.

Nechay(5) used the Sperber chicken preparation to study theophylline diuresis. Substances introduced into the renal portal circulation *via* the leg vein will perfuse first and in the highest concentration the tubules of the ipsilateral kidney. An ipsilateral excess of electrolyte and water excretion was noted, suggesting that at least part of the effect of the xanthine compound was on the tubules. We have used the same approach to study the mechanism of angiotensin diuresis, and also we have studied the effect of angiotensin upon sodium excretion by conventional clearance techniques.

Method. White Rock hens weighing 2 to 3 kg were used. They were allowed to eat freely until the start of the experiment. An initial dose of 25 mg pentobarbital was used to supplement local anesthesia with 1% procaine. Funnels were sutured over each ureter, a catheter inserted into a leg vein, and the chicken suspended in a sling.

In the first group of experiments on 10 chickens, a 30-minute preliminary collection was made while 5% glucose/water was infused into a leg vein. Angiotensin II, Ciba, was then infused at 0.75 μg, 3.0 μg and 6.0 μg/minute for the three 30-minute periods, the dose being increased stepwise in that order. A final 30-minute collection was made without angiotensin. Indigo carmine was infused into the leg vein at the start of most experiments. If it did not appear first and in highest concentration in the urine from the infused side, the chicken was discarded, for

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TABLE I. Excretion of Water and Concentration of Sodium from the Ureter on the Side of the Infusion into the Chicken's Leg Vein and from the Non-Infused Side. Collection periods are 30 min.

Rate of angiotensin infusion		Control period		.75 μg/min		3.0 μg/min		6.0 μg/min		Control	
Animal No.		Na, mEq/l	H_2O, ml	Na, mEq/l	H_2O, ml	Na, mEq/l	H_2O, ml	Na, mEq/l	H_2O, ml	Na, mEq/l	H_2O, ml
1	Inf.	6	6.9	6	7.5	10	7.0	69	32.0	35	7.3
	Non-inf.	4	6.2	8	7.7	8	5.8	38	17.8	18	5.4
2	Inf.	41	1.6	61	42.5	78	39.5	55	15.0	30	3.0
	Non-inf.		None	65	24.0	77	43.5	33	9.3	16	1.0
3	Inf.	48	1.3	118	16.5	124	40.0	122	46.0	115	2.0
	Non-inf.	48	1.3	98	13.8	118	36.0	43	44.5	109.2	2.8
4	Inf.	35	1.0	66	18.0	41	17.0	76	15.5	58	11.0
	Non-inf.	40	1.0	93	17.4	78	19.0	83	13.5	67	18.0
5	Inf.	10	12.5	34	21.5	65	41.0	69	48.5	37	20.0
	Non-inf.	10	9.0	14	21.8	38	30.0	46	19.0	34	13.0
6	Inf.	57	2.1	93	16.8	85	33.0	85	52.3	20	12.2
	Non-inf.	59	2.1	119	4.0	115	19.5	89	35.2	100	2.2
7	Inf.	60	1.7	91	29.0	107	55.2	97	43.5	43	8.3
	Non-inf.	51.7	1.3	97	15.0	100	46.5	85	43.5	51	8.1
8	Inf.	48.5	1.3	98	57.7	110	45.5	106	41.1	38	2.9
	Non-inf.	50	1.0	87	37.5	113	34.2	105	26.0	33	2.2
9	Inf.	91	8.0	99	20.0	83	37.2	99	58.4	47	20.0
	Non-inf.	105	7.0	105	9.5	79	22.2	95	25.2	49	16.0
10	Inf.	23	1.3	109	9.4	89	33.0	85	57.6	53	5.5
	Non-inf.	29	1.1	135	7.4	73	26.0	59	29.0	41	8.2

Difference of Sodium and Water Excretion, Treated as Paired Data After Subtraction of Control Values.

Angiotensin infuse rate	.75 μg/min	3.0 μg/min	6.0 μg/min
Water			
Mean difference	7.31 ml	5.81 ml	13.92 ml
S.E.M.	2.62	1.94	4.26
t	2.343	2.99	3.27
p	.05	.02	.01
Total sodium			
Mean difference	.66 Eq	.88 Eq	1.78 Eq
S.E.M.	.266	.237	.35
t	2.48	3.7	5.09
p	.05	.01	.001

it was felt that the tubules were not being perfused by portal blood.

In another series of experiments, 0.156 g inulin was infused into a wing vein over 10 minutes, then 2.9 mg inulin/minute was infused thereafter. All collection periods were 30 minutes. After two collection periods, angiotensin infusion was begun through a leg vein. The amount infused was less than in the previous studies, to determine the effect of other doses than those previously used. Fifteen chickens were studied successfully, of which 6 representative examples are presented. The studies were done with varying rates of 5% glucose/water or at times 0.9% saline infusion, as indicated in the tables. These chickens were lightly anaesthetized with pentobarbital. The usual initial amount of pentobarbital was 25 mg, followed by 25 mg/hour, as required. Indigo carmine was not infused, as it interfered with inulin determination. Blood samples for Na and inulin analysis were obtained from a vein of the other wing.

TABLE II. Inulin Clearance, Sodium and Water Excretion, and Filtration Fraction of Sodium of Chickens Infused in Leg Vein with Angiotensin. Collection periods 30 min.

Exp No.	Angiotensin l. leg vein, μg/min	Period	GFR		V		$U_{Na}V$		Na exer. / Na filt. × 100	
			R	L	R	L	R	L	R	L
I-14	.08 μg/min/kg	1	.66	.54	.34	.30	.00784	.0084	7.8	10
Initial water load	for Periods 3-5	2	1.4	1.4	.24	.23	.00383	.0056	1.7	2.5
of 40 ml/kg 5%		3	2.1	1.6	.40	.46	.0100	.0322	2.9	12
dextrose H_2O at		4	1.8	1.7	.47	.55	.0192	.0507	6.2	17
1 ml/min I.V.		5	1.7	1.6	.47	.60	.0477	.0427	15	15
		6	1.4	1.2	.43	.60	.0238	.0469	9.1	21
		7	1.6	1.5	.32	.55	.0166	.043	5.4	15
I-15	.08 μg/min/kg	1	2.0	1.8	.58	.59	.023	.018	8.1	7.1
Initial water load	for Periods 3-5	2	3.4	5.3	.56	.56	.032	.030	6.2	3.7
of 40 ml/kg 5%		3	4.3	3.0	.85	.87	.089	.077	13	17
dextrose H_2O at		4	2.0	1.6	.68	.70	.075	.062	24	25
1 ml/min I.V.		5	1.6	1.1	.58	.57	.061	.043	24	25
		6	.88	.85	.32	.36	.020	.021	14	15
I-17	.38 μg/min/kg	1	.83	.58	.13	.13	.0041	.0044	3.1	4.7
Initial water load	for Periods 3-5	2	1.8	1.7	.27	.26	.0090	.0088	3.0	3.1
of 40 ml/kg 5%		3	1.7	2.1	.40	.80	.029	.067	10	19
dextrose H_2O at		4	1.1	2.2	.45	1.02	.050	.098	27	26
1 ml/min I.V.		5	.42	2.3	.27	1.20	.067	.036	51	35
		6	.42	1.9	.22	.83	.030	.074	43	23
		7	1.5	1.8	.57	.56	.059	.051	24	17
I-18	.38 μg/min/kg	1	1.4	.76	.26	.20	.004	.0044	1.9	3.8
Initial water load	for Periods 3-5	2	3.6	4.1	.33	.15	.0057	.0097	1.0	1.5
of 40 ml/kg 5%		3	2.9	3.1	.92	.98	.3772	.4118	18	16
dextrose H_2O at		4	1.3	1.7	.86	1.23	.1266	.1346	40	49
1 ml/min I.V.		5	.98	1.2	.78	1.0	.0587	.0580	63	69
		6	1.4	1.3	.53	.52	.045	.045	19	21
		7	1.5	1.4	.44	.42	.023	.021	8.8	8.7
I-19	.38 μg/min/kg	1	1.5	1.6	.077	.077	.0086	.0081	4.0	3.6
Initial water load	for Periods 3-4;	2	3.8	3.2	.18	.18	.0217	.0189	4.0	4.1
of 40 ml/kg 5%	.08 μg/min/kg	3	1.8	1.6	.66	.60	.0697	.0637	26	27
dextrose H_2O at	for Period 5	4	.71	.75	.42	.47	.0497	.0563	46	49
1 ml/min I.V.		5	.59	.64	.37	.37	.0453	.0437	49	43
		6	.65	.70	.32	.32	.0370	.0373	36	33
		7	.94	.88	.45	.43	.0473	.0453	31	32
I-20	.08 μg/min/kg	1	1.0	.98	.26	.16	.0319	.0198	2.1	12.7
Initial water load	for Periods 3-5	2	1.2	3.0	.28	.14	.0335	.01905	17	4.0
of 40 ml/kg 5%		3	.97	.72	.50	.32	.055	.0374	35	32
dextrose H_2O at		4	.55	.58	.50	.32	.024	.050	32	46
1 ml/min I.V.		5	.87	1.0	.62	.29	.053	.0322	38	20
		6	.70	.94	.44	.08	.0285	.0108	25	71
		7	4.0	6.0	.28	.08	.0180	.01196	2.7	1.2

Inulin was assayed by the resorcinol method (6). Sodium was determined by the autoanalyzer.

Results. Initial group (only water and sodium excretion determined). Diuresis occurred promptly. The increase was usually bilateral though greater on the side infused. For analysis, the excretion of sodium and water during the initial period without angiotensin was substracted from the values at each of the 3 dose levels. Student's t test was then done on the difference between the two values, for the infused and non-infused kidneys. The results for water and sodium excretion are shown in Table I. Significantly more water and sodium were excreted from the ureter of the infused side. The increased amounts of sodium excreted on the experimental side were associated with the increased volume, for the sodium concentration increase was approximately equal on the two sides.

A clear pattern of lateralization was not seen in the chickens studied with inulin. In the majority of cases, a marked diuresis and natriuresis was associated with decreased G.F.R. In numerous collection periods more than 40% of the filtered sodium was excreted reaching 69% in one collection period (I-18).

The absolute amount of filtered sodium reabsorbed by each kidney was markedly reduced in some experiments (I-15, I-18, I-19, and I-20), reaching 10-fold in collection period 4, I-19. Na and H_2O excretion was approximately tripled at that time. In I-18, collection period 5, a similar reduction in Na reabsorption was associated with a 15-fold increase in sodium excretion.

One experiment suggesting predominance of angiotensin effect was I-14, where the per cent of filtered Na excreted increased from 2.5% to 12% in the perfused side. A similar change was present in I-17. At the same time there was a slight increase in per cent of the filtered Na excreted on the contralateral side. In this animal, however, there was a slight increase in G.F.R. and Na reabsorption did not decrease.

These figures are in Table II.

Discussion. In the first group studied without anesthesia, except for a small initial dose of pentobarbital, there was significantly more sodium and water excreted from the ureter on the experimental side at each of the 3 infusion rates of angiotensin. This finding is compatible with the initial assumption that angiotensin does have a depressing effect on sodium and water reabsorption by the renal tubules. It does not explain the mechanism of the bilateral diuresis which occurred.

The second set of experiments was done to clarify the bilateral increased excretion. In this set of experiments, the glomerular filtration rate was estimated. There was considerable variability in G.F.R. from period to period, and between the animals. While there were a few periods where increased sodium and water excretion was associated with the increased G.F.R., the usual situation was for the marked increase in excretion to be associated with a marked bilateral decrease in G.R.R. This, therefore, produced a dramatic increase in the fraction of filtered sodium which was excreted; as high as 69% in one period.

The second group of experiments, therefore, provided further proof that the diuresis was due to decreased tubular reabsorption of sodium and water. The bilateral effect was considered to be due to angiotensin which escaped past the renal tubules and entered the general circulation. The second group did not show the increase in water and sodium excretion of the experimental compared to the controlled side that was seen in the first group. The reason for this difference is not clearly apparent. The second group was handled, anaesthetized and manipulated much more because of the necessity of infusion into one wing, vein, and withdrawal of blood from the other wing. In addition, because the indigo carnot possible to check in advance for adequacy of tubular perfusion by portal blood. Wiener, Burnett and Remmick noted that the tubular secretion of para-amino hippuric acid infused into the portal vein of the chicken varied from 95% to 48% (8). Therefore, the tubules in the second group may have been bypassed to a considerable extent by the blood from the leg.

In a small further series, studied under similar conditions to the first group, a lateralizing effect was noted, so we are willing to ascribe the loss of lateralization to the increased manipulation of the inulin infused animals.

Summary. One group of chickens infused into the renal portal vein showed more water and sodium excreted from the experimental side, though bilateral diuresis occurred. In another group G.F.R. (inulin clearance), was determined and marked bilateral diuresis was noted even though G.F.R. was often markedly reduced. Both of these demonstrations suggested a tubular site of action for angiotensin's diuretic effect.

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Hypocholesterolemic Activity of Mucilaginous Polysaccharides in White Leghorn Cockerels.* (31478)

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Previous studies in our laboratory with White Leghorn cockerels fed various semi-purified diets demonstrated that the response of plasma cholesterol levels to supplemental dietary cholesterol was markedly affected by the nature of the carbohydrate component of the diet(1). With sucrose as the sole source of carbohydrate, resultant plasma cholesterol levels were approximately double those obtained when glucose replaced sucrose. In the course of subsequent investigations, it was observed that certain mucilaginous polysaccharides elicited significant hypocholesterolemic activity when added to the basal cholesterol supplemented diet at levels as low as one per cent. Data are presented here on 16 mucilaginous polysaccharides which were found to have hypocholesterolemic activity in our test systems with the chick.

Methods. Three basal diets were employed. The composition of 2 of these diets has been previously reported(1); they were prepared from purified ingredients containing either 20% casein, 8% gelatin and 61.3% sucrose (Diet 1), or 25% purified soybean protein and 64% glucose (cerelose) (Diet 2). Another diet (Diet 3) was formulated from a variety of natural foodstuffs from vegetable and animal sources to simulate a commercial-type diet (Table I). In Diets 1 and 2, test substances (including 3 g cholesterol dissolved in 3 g corn oil/100 g diet) were added at the expense of carbohydrate. In Diet 3, all supplements were added directly to the complete diet. Day-old single-comb White Leghorn cockerels‡ were divided into groups of 12, and

TABLE I. Diet 3 (Commercial-Type Diet).

Ingredient	g/kg diet
Corn, yellow, fine ground[a]	614
Soybean meal (44%), fine ground[a]	200
Corn gluten meal (41%)[a]	50
Fish meal[a]	50
Alfalfa meal (20%)[a]	20
Distillers solubles (30%)[a]	25
Delamix[b]	1
Limestone, pulverized	20
Bone meal (steamed)[a]	12
Sodium chloride	5
Choline chloride (25%)	1
Fortafeed 2-49C[c]	1
Vitamins A + D[d]	1
Merck B_{12} (40)[e]	0.5

[a] Obtained from A. J. Mowerson and Co., Wyckoff, N. J.

[b] A trace mineral concentrate with calcium carbonate as carrier (obtained from Limestone Products Corp., Newton, N. J.); 1 g contains (ppm): Mn 60, I 1.2, Fe 20, Cu 2, Zn 0.1 and Co 0.2.

[c] A vitamin feed supplement (obtained from American Cyanamid Co., Wayne, N. J.); 1 g contains the following vitamins (mg): riboflavin 4.4, niacin 8.8, pantothenic acid 19.8, choline chloride 22 and folic acid 0.1.

[d] Approximately 8800 units vit A and 1770 units vit D_3 are added together to the diet by mixing appropriate quantities of Nopcay "10" and Super Nopdex "15" (obtained from Nopco Chemical Co., Harrison, N. J.) which contain 10,000 units vit A /g and 15,000 units vit D_3/g, respectively.

[e] A vit B_{12} concentrate (obtained from Merck & Co., Rahway, N. J.) containing 40 mg vit B_{12}/lb; $0.5\ g \equiv 5\ \gamma\ B_{12}$.

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‡ Three different strains were imployed in the course of the studies: Mount Hope, Darby and Babcock, obtained from Kerr Chickeries, Frenchtown, N. J., Hall Brothers Hatcheries, Wallingford, Conn., and Spring Lake Farm, Wyckoff, N. J., respectively.