Gilman and Goodman have recently been reported in abstract by Walker.[7]

Conclusion. An antidiuretic substance can be obtained from the urine of dehydrated rats. This substance is not present in significant quantities in the urine of non-dehydrated rats. Its action upon water diuresis is similar to that of pitressin, though we cannot say that it is pitressin.

9969

Effect of Choline on Atherosclerosis in the Rabbit Aorta.*

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The cholesterol content of rat liver is increased by the feeding of cholesterol,[1, 2] and its accumulation is accompanied by the deposition of large amounts of neutral fat. The increased cholesterol is chiefly in ester form.[3] The fatty livers produced by feeding 1% of cholesterol can be prevented by feeding large amounts of choline.[2] In the rabbit, cholesterol feeding results in the deposition of cholesterol in the aorta in the form of atheromatous plaques.[4-8] Atherosclerosis in rabbits has been prevented by feeding a pancreas extract.[9] The antagonism exhibited between choline and cholesterol deposition in the rat led us to investigate the effect of choline on the formation of plaques in the rabbit aorta.

Twelve female rabbits, 1.3 to 3.0 kg in weight, averaging 2.14 kg were divided into 3 groups. The first group received Purina

[7] Walker, A. M., *Proc. Am. Physiol. Soc.*, April, 1938, p. 210.

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[1] Chanutin, A., and Ludewig, S., *J. Biol. Chem.*, 1932, **102**, 57.

[2] Best, C. H., Channon, H. J., and Ridout, J. H., *J. Physiol.*, 1934, **81**, 409.

[3] Okey, R., PROC. SOC. EXP. BIOL. AND MED., 1933, **30**, 1003.

[4] Cowdry, E. V., *Arteriosclerosis*, Macmillan & Co., 1933.

[5] Rosenthal, S. R., *Arch. Path.*, 1934, **18**, 473.

[6] Menne, F. R., Beeman, J. A. P., and Labby, D. H., *Arch. Path.*, 1937, **24**, 612.

[7] Turner, K. B., and Bidwell, E. H., PROC. SOC. EXP. BIOL. AND MED., 1937, **35**, 656; 1935, **62**, 721.

[8] Aylward, F. X., and Stott, Wm., *Biochem. J.*, 1937, **31**, 2055.

[9] Huber, M. J., Brown, G. O., and Casey, A. E., PROC. SOC. EXP. BIOL. AND MED., 1937, **37**, 441.

rabbit chow *ad libitum;* the second, rabbit chow plus 0.43% cholesterol and 5% hydrogenated cottonseed oil (Primex); the third, rabbit chow plus cholesterol and 715 mg of choline hydrochloride (Eastman) per liter of drinking water. The animals were kept in groups and daily water consumption of each group was recorded. This gave the average intake of choline for each rabbit. The cholesterol was dissolved in heated primex and poured over the rabbit chow and thoroughly mixed. The blood was analyzed periodically for cholesterol. At the end of 4 months, the animals were killed, the aorti examined both grossly and histologically, and analyzed for free and ester cholesterol. Determinations of fat and cholesterol were also made on liver, kidney, spleen, and lung tissue. The cholesterol was determined by a modification of the Schoenheimer-Sperry method.[10] In a second series, the cholesterol content of the diet was reduced to 0.22%, and the choline hydrochloride given in concentrations of 0.5 or 1.0 g per liter of drinking water.

All diets were readily consumed and the animals gained in weight from an average of 2.14 kg to 3.44 kg in Series I; from 1.53 kg to 3.37 kg in Series II. Two deaths from unknown causes occurred during the second experiment, one on the cholesterol diet, the other on cholesterol plus the higher level of choline.

A marked increase in blood cholesterol was observed on all the levels of cholesterol fed, irrespective of the choline intake. In our first series the total blood cholesterol rose from 1.13 to 6.21 mg/cc. In our second series, the increase was from 1.13 to 9.9 mg/cc. The increases occurred in both the free cholesterol and the ester fractions, the greatest increases being in the latter. The maximum value for blood cholesterol was attained in from 6 weeks to 2 months on the higher level, 0.43% of cholesterol, whereas on the lower level (0.22%), the maximum was reached between the third and fourth month. However, the maximum values of blood cholesterol attained were actually higher on the lower level of intake (Table I). In neither series did choline exert any detectable effect on the cholesterol content of the blood.

The results of tissue analyses from the high cholesterol series are given in Table II. Fatty livers resulted from feeding 0.44% cholesterol, the values rising from a fat content of 3.89% of the fresh weight on the control diet to 9.04% on cholesterol and 13.7% on cholesterol plus choline. The level of choline employed, therefore, failed to reduce the deposition of liver fat. The cholesterol content of the control livers was 10.8 mg per gram. Cholesterol

[10] Schoenheimer, R., and Sperry, W., *J. Biol. Chem.*, 1934, **106**, 745.

TABLE I.
Cholesterol in Rabbit Blood, mg/cc whole blood.

	Series I			Series II			
Time	Control	.43% cholesterol	.43% cholesterol + 121 mg choline daily	Control	.22% cholesterol	.22% cholesterol + 108 mg choline	.22% cholesterol + 216 mg choline
0	1.00	1.13	1.09	1.27	1.22	1.13	1.21
2 wk	1.18	1.35	1.87				
1 mo.	1.24	4.65	6.17	1.28	3.98	3.69	3.33
6 wk	1.31	6.21	5.58				
2 mo	1.80	4.92	6.18				
3 ''	.94	4.96	4.99	1.61	6.1	9.9	7.36
4 ''				1.35	8.1	7.24	8.92

TABLE II.
Effect of Cholesterol and Choline on Rabbit Tissues.

	No. of animals	Cholest. in diet %	Avg cholest. consumed daily mg	Choline conc. g/L	Avg daily consump. of added choline mg	Avg daily consump. of "Choline" (ration) mg	Avg total choline consumed daily mg	Liver fat %	Liver cholest. range mg/g	Liver cholest. avg mg/g	Aorta cholest. range mg/g	Aorta cholest. avg mg/g
Series I												
Controls	4	—	—	—	—	82	82	3.89	7.0-18.5	10.8	5.7-13.7	8.6
Cholesterol	4	.43	460	—	—	82	82	9.04	12-25	19.2	23-41	30.7
Cholesterol + choline	3	.43	460	.715	121	82	203	13.7	17-29	24.4	14-54	31.9
Series II												
Controls	2	—	—	—	—	82	82	4.0	11.8-13	12.4	5.3-6.2	5.8
Cholesterol	3	.22	240	—	—	82	82	6.9	10.7-11.8	11.2	21-48	31.6
Cholesterol + low choline	4	.22	240	.50	108	82	190	7.0	8.4-16.6	11.6	26-37	30.7
Cholesterol + high choline	3	.22	240	1.00	216	82	298	6.4	9.2-14.8	12.0	11.8-30	22.6

feeding raised this level to 19.2 mg per gram, and cholesterol plus choline, to 24.4 mg per gram. Dietary choline was therefore without effect in reducing liver cholesterol.

No fatty livers were observed on the lower levels of cholesterol fed (Table II). The liver cholesterol was remarkably constant on all these diets; it varied from 11.2 to 12.4 mg per gram; the highest value, incidentally, was obtained on the control diet. Choline, therefore, was without effect on either liver fat or liver cholesterol.

The aorta, on the contrary, like the blood, was profoundly affected by the ingestion of 0.22% cholesterol (Table II). Characteristic atheromatous plaques were observed in the aorti of all animals receiving cholesterol, regardless of the amount of choline ingested. The lesions were similar to those described in detail by others,[4-8] and while considerable variation was observed between individuals, there were no obvious differences between the various groups. This conclusion is supported by the results of chemical analyses. The cholesterol content of the normal aorta was 5.8 mg per gram. The aortas of cholesterol-fed rabbits contained 31.6 mg per gram. The administration of choline failed to affect the cholesterol content of the aorta in 6 out of 7 rabbits. The aorta of the seventh rabbit contained only 11.8 mg cholesterol per gram, and both gross and histological examination showed a decreased number of lesions. The blood of this rabbit, however, like that of the others in the group, contained large amounts of cholesterol. It is doubtful, therefore, whether the decreased number of atheromatous lesions should be attributed to the action of choline. The animal may have come from a different strain than the others of the series, since this was the only one with yellow body fat.†

On the higher level of cholesterol fed, (0.43%) atheromatous lesions, accompanied by a high cholesterol content, 14.6 to 54.6 mg cholesterol per gram of aorta, were observed in all animals (Table II) irrespective of choline intake. The cholesterol content of rabbit kidney was relatively constant on all diets, and the variations in the cholesterol content of the spleen had little relation to the diets employed.

Summary. Fatty livers, with some increase in liver cholesterol, were produced by feeding rabbits a diet containing 0.43% cholesterol; 0.22% cholesterol produced no changes in liver fat. Both levels of cholesterol produced marked atheromatous lesions, and a

† A recent report (Steiner, A., PROC. SOC. EXP. BIOL. AND MED., 1938, **38**, 231) states that 500 or 750 mg choline hydrochloride daily failed to prevent atheromatous lesions in rabbits induced by a weekly intake of 3 g of cholesterol.

striking increase in the cholesterol content of the aorta and blood. Three hundred mg of choline hydrochloride daily failed to counteract the effect of cholesterol in the blood, liver or aorta.

9970 P

Use of Ascitic Fluid in the Treatment of Primary Shock.

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It has been abundantly demonstrated that the symptoms of primary shock can be relieved by prompt administration of fluid to replace that diverted from the general circulation. Blood is the ideal fluid for this purpose since its protein content prevents too rapid elimination by the kidneys. Of the blood, the serum alone is vital to this action. However, transfusion cannot be performed without time-consuming preliminaries. The ideal fluid for emergency use would, therefore, be some stable, universally compatible protein solution which might be kept on hand at all times. A sterile body transudate would present several advantageous features, in particular the nature of its protein content which is identical with that in blood serum. Ascitic fluid, among the transudates, would be especially desirable since it is elaborated in non-infectious conditions and is available in most clinics in large amounts.

A study of ascitic fluid has been carried out in this laboratory to determine its suitability for this use. Nineteen specimens of ascitic fluid were used, the majority of which were obtained from cases of advanced portal cirrhosis of the liver. All specimens were filtered under aseptic conditions, cultured for sterility and their respective agglutinins determined. Protein and pH determinations were carried out in the usual manner. All fluids were found to be not only sterile but strongly bacteriostatic. The pH was uniformly in excess of 8.65. This was reduced to 7.5 with N 10 hydrochloric acid prior to administration. The protein content ranged from 2.1 to 4.2 mg %. It was found impractical to remove the agglutinins by the classic globulin precipitation method, as dialysis to remove the ammonium sulphate produced too great a dilution. Many of the agglutinins were amenable to destruction by heat—especially the