C. CHOLESTEROL SECRETION IN THE URINE. PART I.

BY JOHN ADDYMAN GARDNER AND HUGH GAINSBOROUGH.

From the Biochemical Laboratory, St George's Hospital.

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ACCORDING to the view most commonly held at the present time, the liver is the organ chiefly concerned with the excretion of cholesterol (exogenous and endogenous). But, in addition to the excretion in the bile, loss occurs in the secretion of sweat and sebum in man, and in the wool fat in sheep; considerable amounts also are eliminated in milk. The kidney has not generally been considered an organ concerned with the excretion of cholesterol, though for a long time it has been known that normal urine contains traces of cholesterol and that this is increased sometimes in pathological conditions.

Bacmeister and Havers [1914] and Pribram [1906] reported only traces in normal urine. More extended investigations were made by Gérard [1911], who found 11 mg. of cholesterol in 70.9 litres of normal urine, which, reckoning the daily urine output as 1500 cc., would be an excretion of 0.23 mg. per day. In pathological cases, Kaiserling-Orgler [1902] and F. Munk [1913, 1916, 1918] reported the presence of doubly refracting lipoids in the urine in cases of renal disease. These were, however, only microscopical examinations. Gross [1921] by chemical means detected the presence of cholesterol in urine in kidney diseases and Bauman and Hausmann [1920] made quantitative estimations of neutral fat and cholesterol in a case of "lipo-lipoiduria" in a nephritic. Falk and Siebenrock [1911] failed to find anisotropic substances in the urine in diabetes mellitus but found considerable quantities in a case of diabetes complicated with nephritis. Genck [1918] found no doubly refracting substances, that is cholesterol esters, in the urine sediment from five diabetic patients. M. B. Schmidt [1921] reported the presence of cholesterol in the urine in a case of acute yellow atrophy of the liver in which the kidney showed marked fatty change: the blood contained 0.24 % of cholesterol and the post-mortem urine 32.35 mg. per 100 cc. Grunke [1922] using Weston's modification of Autenrieth and Funk's method found in normal urine only traces of cholesterol of the order of 1 mg. per day; out of nine cases of icterus, in only one case did he find more than traces, viz. 10.1 mg. of cholesterol per day with a serum cholesterol content of 0.182 %; of five diabetic urines

one only showed more than traces, viz. 12.9 mg. per day with a serum content of 0.236 %. The urine sediments according to Grunke were too sparing to be regarded as a source of the cholesterol found; on the other hand, the cholesterol blood pictures suggested no relationship with the cholesterol excreted by the kidney.

The discovery by one of us [Gardner, 1924] in the bile of the hippopotamus of a small quantity of a cholesterol compound not hydrolysable by alkalies but hydrolysable by strong acids, which substance it was thought might be an ethereal sulphate, suggested the possible presence of this body in the urine and the possibility of correlating this with the blood picture in cases of marked hypercholesterolaemia.

We therefore examined normal urines taken from healthy students, and also urines obtained from pathological cases.

Method used.

The urine was very carefully filtered to make sure that cell debris was got rid of, and was then made up, sometimes after partial concentration, to 2 % content of caustic soda and thoroughly extracted with ether. To do this the urine was treated with one-half of its bulk of ether, repeatedly shaken during the day, allowed to stand over night, then separated and re-extracted with fresh ether in a similar way during four or five days, until we were quite certain that all the cholesterol was extracted. The ethereal solution was evaporated, the residue hydrolysed by alcoholic solution of sodium ethoxide and the cholesterol estimated by digitonin, using the procedure of Frazer and Gardner [1910]. The extracted urine was next evaporated to dryness and the residue boiled with glacial acetic acid under a reflux for 5 to 6 hours. The fluid was then diluted with water, the acetic acid neutralised with strong caustic soda and then made alkaline. The alkaline fluid was thoroughly extracted as before, the ethereal solution evaporated and the residue boiled with alcoholic sodium ethoxide to hydrolyse any cholesterol acetate, and the cholesterol then determined as before.

The results are summarised in Table I.

It will be seen that normal urine contains a measurable amount of cholesterol, partly in the form of either free or ester cholesterol and partly in the form of some compound hydrolysable by strong acid and not by alkalies. We are unable to say definitely what this compound is, but the following facts—that it is not hydrolysable by alkalies, but is hydrolysable only by strong acid, that it appears to be in a very stable condition as it does not decompose during evaporation and that it remains in solution after coagulation of the proteins in a highly albuminous urine—suggest that it is sodium or potassium cholesteryl sulphate. These salts have been shown by Gardner and Fox [1924] to behave to alkalies and acids in this manner and also to be soluble in water. We have not yet succeeded in isolating such a compound from urine though many

Table I.

Pathological condition	Cholesterol in plasma*		Urine		Cholesterol, free and in ester forms, extracted from alkaline solution by ether		Cholesterol set free after acid hydrolysis		Total cholesterol all forms mg. per 100 cc.	Cholesterol per day		Total cholesterol all forms per day mg.	
	Free %	Ester %	Vol. used in cc.	No. of days	Digitonide weighed g.	Cholesterol mg. per 100 cc.	Digitonide weighed g.	Cholesterol mg. per 100 cc.					
Mixed normal	—	—	2000	—	—	—	—	—	0.172	—	—	1.7-2.6 †	
Mixed normal	—	—	13250	—	0.0487	0.1787	0.0279	0.1024	0.2811	0.203	1.8-2.7	1.0-1.5	2.8-4.2 †
Carcinoma stomach	—	—	1000	1.5	0.0049	0.119	Nil	Nil	0.119	0.119	0.8	Nil	0.8
Grave's disease	0.0476	0.0529	1500	2	0.0051	0.083	0.0447	0.725	0.808	0.808	0.62	5.4	6.05
Polycystic kidneys	0.0367	0.065	2540	2	0.0061	0.058	0.0021	0.0201	0.0781	0.0781	0.74	0.26	1.00
Chronic nephritis	0.056	0.0912	1545	2	0.0079	0.124	0.0106	0.167	0.291	0.291	0.96	1.29	2.25
Subacute par-enchymatous nephritis	0.2032	0.4288	3130	2	0.2329	1.81	0.1032	0.801	2.611	2.611	28.3	12.5	40.8
Subacute par-enchymatous nephritis	0.212	0.2809	2000	1.83	0.2273	2.76	0.0832	1.01	3.77	3.77	30.14	11.03	42.4
Subacute par-enchymatous nephritis	0.0993	0.6942	1400 1850 4104	2 2 5	0.0086 0.0891 —	0.149 1.17 1.128	0.1237 0.1882 0.1746	2.15 2.47 1.03	2.30 3.64 2.16	2.30 3.64 2.16	1.05 10.8 8.924	15.03 22.9 8.24	16.08 33.7 17.26 §

* These figures do not necessarily correspond exactly in date to the time of collection of the urine.

† Weight of digitonide 0.0142 g. Total all forms determined together.

‡ Assuming daily output 1.0-1.5 litres of urine.

§ Full analysis in Table II.

experiments have already been made, but this is not to be wondered at, considering the small quantities present.

It will be noticed that as far as the cases examined are concerned, the high content of ethereal sulphate, if such it be, goes hand in hand with a high plasma content of cholesterol.

Five cases of advanced renal disease are tabulated but only in the three cases of typical subacute parenchymatous nephritis which were characterised by a high plasma cholesterol content were excessively large quantities of the ethereal sulphate found. These three cases were of the type of nephritis which frequently shows the presence of large quantities of cholesterol esters in the kidney. This would suggest that in cases of marked hypercholesterolaemia, the organism tries to get rid of the excess blood cholesterol as a conjugated sulphate in the urine in the same manner as some poisonous bodies, phenols, etc.

In order to throw more light on the distribution of the cholesterol in highly albuminous urine the last-mentioned case was further examined. Four litres of urine, the output of 5 days, after careful filtration were made faintly acid with acetic acid and boiled. The urine from a previous examination had been found to contain approximately 2.6 % of protein. The precipitated protein was filtered and washed and dissolved in 2 % caustic soda. This was then thoroughly extracted with ether and the free and ester cholesterol separately determined. The free cholesterol yielded 0.0706 g. of cholesterol digitonide and the ester 0.1051 g. cholesterol digitonide. The filtrate from the proteins was then made up to 2 % content of caustic soda and repeatedly extracted with ether until no further cholesterol could be obtained. This ethereal solution by analysis in a similar way yielded 0.0112 g. digitonide from the free cholesterol and 0.0037 g. digitonide from the ester cholesterol. The thoroughly extracted urine was then evaporated to dryness, and the residue boiled with glacial acetic acid as already described and then after extraction and saponification gave 0.1746 g. cholesterol digitonide.

The percentage and daily output of cholesterol in the different fractions are set out in Table II.

Table II. *Detailed analysis showing distribution of cholesterol in an albuminous urine.*

	Protein fraction		Protein free filtrate		Protein fraction mg. per day	Protein free filtrate mg. per day
	Digitonide in g.	mg. per 100 cc. whole urine	Digitonide in g.	mg. per 100 cc. whole urine		
Free cholesterol	0.0706	0.418	0.0112	0.066	3.26	0.528
Ester cholesterol	0.0105	0.623	0.0037	0.022	4.96	0.176
Cholesterol obtained after acid hydrolysis	—	—	0.1746	1.034	—	8.24
Total, all forms	—	1.041	—	1.122	8.22	8.944

It will be noticed that 60 % of the total cholesterol, precipitated with the proteins, is in the form of ester, which is very similar to that found in plasma.

This result suggests that the bulk of the free and ester cholesterol in this nephritic urine was in combination with the proteins in much the same way as it is held in solution in plasma. It will be noticed that 91 % of the free and ester cholesterol apart from the ethereal sulphate was precipitated along with the proteins. We shall, however, have to make more experiments before we can be sure of this relationship. The free and ester cholesterol not precipitated with the proteins is of much the same order of magnitude as in normal urine but the bulk of it is in the form of free cholesterol.

The cholesterol as so-called ethereal sulphate, set free after acid hydrolysis, is present in quantities approximately equal to that found in other forms. The excretion by the kidney is obviously very minute relatively to that dealt with by the liver and scarcely affects the figures given in previous papers concerning the cholesterol balance.

Nevertheless, in considering the general cholesterol problem, this function of the kidney does not appear to be without significance.

The explanations advanced in this paper must be regarded as quite of a preliminary nature and we hope to give the results of further work in future.

SUMMARY.

Cholesterol could be determined by gravimetric estimation in all the urines examined. It exists in the urine in free and ester forms and also as a compound which is only hydrolysable by strong acids. This substance we suggest is cholesterol ethereal sulphate.

In cases of parenchymatous nephritis which were accompanied by hypercholesterolaemia there were found in the urine large increases in the content of all forms of cholesterol.

In a highly albuminous urine, the cholesterol in free and ester forms was almost wholly precipitated with the proteins on boiling with very dilute acetic acid. The precipitated cholesterol showed the same ratio of ester to total cholesterol as is found in plasma. The so-called ethereal sulphate form was still present in solution in the precipitated urine in approximately the same quantity as the total other forms in the whole urine.

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