Cholesterol and Lecithin in the Chylous Urine.

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Many investigations have been made about chyluria, and yet much remains to be solved. Regarding the behavior of cholesterol and lecithin in it, for instance, we now know very little.

Cholesterol and lecithin are essential companions of fats in the blood and most tissues where they probably play a very important rôle, physiologic as well as pathologic. They are, therefore, the subject of general interest of recent investigators studying fats in the animal body. To trace the origin of fats in the chylous urine, attempts were made, too, to determine whether they are present in it or not[1]. By reason of that it is relatively well defined and more stable, the observations reported for cholesterol, as a rule, are much more reliable than those for lecithin.

Most of the authors who have given attention to the subject have positively found cholesterol in chylous urine. Eggel[2], Brieger[3], Scheube[4], Erben[5] and Salkowski[6] could all separate it as the

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1) F. Hoppe-Seyler, Medicinisch-chemische Untersuchung, No. 4 (1871), 551; F. Eggel, D. Arch. klin. Med., **6** (1869), 421; R. Waldvogel and A. Bickel, ibid., **74** (1902), 511; S. Gandin, Ergebnisse d. inn. Med. u. Kinderheilk., **12** (1913), 218.

2) F. Eggel, l.c.

3) L. Brieger, Zeitschr. physiol. Chem., **4** (1880), 407.

4) B. Scheube, Die Krankheiten der warmen Länder, Jena 1896, 291.

5) F. Erben, Zeitschr. physiol. Chem., **30** (1900), 436.

6) H. Salkowski, Berl. klin. Woch., **44** (1907), 51.

characteristic crystals from the ethereal extract of chylous urine.

Magnus-Levy[1] presumed that the failure of the early workers to find cholesterol was probably due partly to the imperfect method employed and partly to the insufficient quantity taken for analysis.

It is reported by Sanes and Kahn[2] that in the later course of a case of non-parasitic chyluria the excretion of cholesterol has decreased almost to disappearance, being accompanied by the reduction of the fat content, although earlier it could be easily detected.

Thus, the question about cholesterol in chylous urine seems apparently to be solved; but, in reality, it is far from it.

Cholesterol is found in the animal body in both the free and combined state as esters of fatty acids. Just the knowledge of the relation between these two forms of cholesterol, but not simply of the total amount, as we shall see later on, should be of great value for the study of its significance in the organism. Pribram[3] said, correctly, in this respect: "— geht hervor, dass die Untersuchung auf den Cholesteringehalt des Serums manchen Einblick in die Pathogenese von Krankheiten verspricht, dass aber im Gegensatz zu den meisten einschlägigen Untersuchungen die einfache Untersuchung auf Cholesterin schlechtweg nicht genügt, sondern dass auch der Verteilung des Cholesterins, dem Vorkommen in freiem und gebundenem Zustande grössere Aufmerksamkeit zu schenken wäre." The question naturally arises: how it is in the chylous urine? Few observations, if any, were hitherto made in this respect. I could find only a communication of Slosse[4] relating to cholesterol esters in chylous urine. But he did not go further than the detection of cholesteryloleate in the urine.

While Sanes and Kahn could detect neither lecithin nor choline in the chylous urine, other authors mentioned above claimed to have found also lecithin in it. Nevertheless, in none of their papers is there any direct evidence to substantiate the existence of this phosphatide. They all attested it only on account of the proof of phosphorus in an ethereal extract of urine or of the formation of a small quantity of

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1) A. Magnus-Levy, Zeitschr. klin. Med., **66** (1908), 482.

2) K. I. Sanes and M. Kahn, Archives of Int. Med., **17** (1916), 181.

3) H. Přibram, Centralbl. f. inn. Med., **36** (1915), 325.

4) A. Slosse, Maly's Jahresbericht f. Tierchemie, **31** (1901), 833; **32** (1902), 822.

crystals which resemble in their form the double salt of platinum chloride and "neurine." From such findings only, however, any definite conclusion can hardly be reached.

The recent improvements in the methods of estimating cholesterol and isolating phosphatides rendered more detailed studies desirable in this direction.

During the past two years, through the favour of Professor Sugimura who had in his clinic patients suffering of chyluria, I was given a part of the urine and was thus able to analyse it with special reference to cholesterol and lecithin. The results obtained will be recorded in the following pages.

My heartfelt thanks are expressed here to Professor Sugimura for his kindness to give the valuable material and to permit the use of the history of the cases.

Clinical History.

Case 1. Man aged 31. Five years ago the patient in catching a cold, got a swelling of the right testicle. Soon after he noticed a milky appearance of the urine. This condition lasted a few days. After this he sometimes passed turbid milky urine after an intemperate act or hard work. For the last months his urine had always the given character. Besides, he complained of weakness and of a dull pain at the waist. This brought him to the hospital. A cystoscopic examination showed the bladder mucosa dull but smooth, vascularity being distinct. The orifice of the right ureter was of normal appearance. From the left which was very active in motion a turbid urine, mixed often with white slimy masses, flowed out. The daily amount of urine was 1500–2500; the specific gravity varying between 1·010 and 1·021. The reaction was in general slightly acid. The urine was of a milky color, containing sometimes a small quantity of blood clot. When allowed to stand, a yellowish-brown jelly-like mass was settled down. The better part of this was easily dissolved in ether and there remained a little of fibrous substance which gave the xanthoproteic and the Millon's reaction. Filaria could be found neither in blood nor in urine.

Case 2. Man aged 65. Since about 6 years he passed a milky, blood mixed urine for 10–20 days in every spring. Otherwise he has been healthy. During the past six months the attacks returned in

rapid succession. The urethral orifice was swollen and so sensible that it was impossible to carry out the cystoscopy. The urine was milky, containing yellowish or bloody masses. The reaction of the urine was neutral or slightly alkaline; the specific gravity from 1·012 to 1·020. The daily amount was 1200–1400 c.c. In the specimen of blood filaria have been observed.

Extraction with ether.

In order to separate out the fatty matter, the urine free from blood was repeatedly shaken with ether. The ethereal extract was filtered and evaporated; the residue was again taken up in pure ether, filtered and evaporated. The operation was repeated as many times as the new ethereal solulution still was not clear. The weight of the so purified residues when dried at 40° in a current of carbon dioxide was: in case 1, 80 grms. from about 16 liters of urine passed in 10 days, in case 2, 38·5 grms. from 7500 c.c. The average percentage content of the fatty matter in the urine was therefore 0·49 resp. 0·51, the daily fluctuation being from 0·3 to 0·9.

Table I.

Amount of ethereal extract, albumen, urea and total nitrogen in the urine of case 1.

Day of exp.	Volume of urine	Specific gravity	Total nitrogen	Urea	Albumen	Ethereal extract	
	c.c.		per cent	per cent	per cent	Daily amount grms.	per cent
I	1200	1·021	0·72	0·99	0·61	5·537	0·46
II	1500	1·021	0·69	0·71	0·73	8·864	0·59
III	1880	1·021	0·68	0·72	0·84	8·957	0·48
IV	800	1·021	0·63	0·60	0·67	3·534	0·44
V	910	1·021	0·65	0·88	0·97	4·286	0·47
VI	1880	1·021	0·68	0·62	0·94	10·785	0·57
VII	1910	1·020	0·61	0·73	1·01	16·590	0·87
VIII	1940	1·016	0·58	0·64	0·72	9·228	0·48
IX	2530	1·011	0·49	0·57	0·68	7·458	0·29
X	2010	1·015	0·52	0·62	0·68	5·505	0·27

The method used can scarcely be regarded as quantitative. But, judging from the fact that the food the patients took did not contain much of fats[1], the values found for the daily output of the ether solu-

1) The food was in the main rice and greens with a little of fish or meat.

ble substance fairly harmonize with those of the cases reported in the literature, particularly of the case of Carter[1]. This would seem to indicate that there was no considerable loss of the substance based upon the incompleteness of extraction.

Cholesterol.

The dried ethereal extract was treated by pure acetone; thereby a considerable part was dissolved. The resultant acetone solution was evaporated to dryness under diminished pressure; the residue was purified by dissolving in acetone and evaporating again. After being dried at 40° for two hours in carbon dioxide atmosphere the total acetone soluble fraction weighed about 75 grms. (case 1) resp. 37·5 grms. (case 2). Fat as well as cholesterol should be contained in it.

The acetone soluble fraction of case 1.—This was dissolved in boiling alcohol, rapidly filtered and allowed to cool down for some time; 7·52 grms. of a crystalline substance settled out (crude cholesterol).

A part of 5 grms. of this crystalline substance was saponified by heating with a 15 % alcoholic potash and then the saponified product was extracted with ether. From this ethereal extract ether was evaporated off. The residue was recrystallized out of hot alcohol and dried in a vacuum desiccator over sulfuric acid. In this way 0·15 grm. of characteristic white shining thin plates, having one corner broken, were obtained. These crystals melted at 149° and gave the cholesterol reaction of Salkowski and that of Liebermann-Burchard.

For the purpose now to estimate cholesterol, both forms separately, 0·9914 grm. of the crude cholesterol was subjected to Windaus' procedure, modified by Mueller[2].

	Weight of digitonin compound grm.	Cholesterol grm.
Free	0·0052	0·0013
Combined	0·1423	0·0346

Accordingly, the crude cholesterol fraction must have contained 0·009 grm. of free and 0·26 grm. of combined cholesterol, the total sum being 0·269 grm.

Still more cholesterol might have remained in solution in the

1) D. W. Carter, Archives of Int. Med., **18** (1916), 541.
2) H. Mueller, Jl. Biol. Chem., **21** (1915), 24.

mother liquor resulting from crystallization of crude cholesterol on cooling. This alcoholic mother liquor was then freed from alcohol by evaporation under diminished pressure. The dried residue was taken up in ether, making up the whole volume to 200 c.c. Portions of 10 c.c. each were used to the estimation of cholesterol by the same method and found to contain as the mean 0·0148 grm. of free and 0·0532 grms. of combined cholesterol.

Therefore, the total quantity of cholesterol originally contained in 0bout 16 liters of chylous urine, amounted to 1·63 grms.; i.e., nearly two per cent of the entire ether soluble substances excreted. Among them about 81 per cent was present in the combined form as esters.

The acetone soluble fraction of case 2.—This fraction was directly dissolved in a small quantity of ether, transfered quantitatively to a measuring flask and filled up with ether to 100 c.c. Samples of 10 c.c. each of this ether solution were used for the estimation of cholesterol according to Windaus-Mueller as before. The results were as follows:

	Weight of digitonin compound	Cholesterol
Sample 1	grm.	grm.
Free	0·2073	0·0518
Combined	0·3894	0·0974
Sample 2		
Free	0·2084	0·0521
Combined	0·3899	0·0975

Hence, there were 0·510 grm. of free and 0·974 grm. of combined cholesterol; accordingly 1·494 grms. in all, corresponding to 3·98 per cent of the total ether soluble substances. The percentage ratio of the combined cholesterol to the total is about 65.

It may be interesting now to compare the results of the foregoing determinations with the corresponding figures recorded for blood and lymph.

Table II and III contain some of the values given in the literature, besides the data obtained in the present examination.

Most of the figures shown in Table III were calculated .by the author from the results of estimations of the early workers.

TABLE II.

Cholesterol in mg. per 100 c.c normal human blood and lymph.

Whole blood.

140–160	Autenrieth & Funk (Münch. med. Woch., **60**, i, p. 1243)
197	Iscovesco (Société de Biologie, **72**, p. 257)
100–143	Henes (Deutsch. Arch. klin. Med., **111**, p. 122)
79–150	Csonka (Jl. Biol. Chem., **24**, p. 431)
167–252	Denis (ibid, **29**, p. 93)
219 (mother)	Hymanson & Kahn (Am. Jl. Obst., **73**, p. 1041)
210 (new-born child)	Hymanson & Kahn (Am. Jl. Obst., **73**, p. 1041)
144 (women)	Herrmann & Neumann (Biochem. Z., **43**, p. 47)
180 (pregnant women)	Herrmann & Neumann (Biochem. Z., **43**, p. 47)
92 (new-born child)	Herrmann & Neumann (Biochem. Z., **43**, p. 47)
210 (men)	Bloor & MacPherson (Jl. Biol. Chem., **31**, p. 79)
230 (women)	Bloor & MacPherson (Jl. Biol. Chem., **31**, p. 79)
133–135	Myers & Wardell (ibid, **36**, p. 147)

Plasma.

220 (men)	Bloor & MacPherson (Jl. Biol. Chem., **31**, p. 79)
240 (women)	Bloor & MacPherson (Jl. Biol. Chem., **31**, p. 79)

Serum.

120–140	Grigaut (Société de Biologie, **68**, p. 827)
177–232	Iscovesco (ibid, **73**, p. 318)
158–182	Henes (Deutsch. Arch. klin. Med., **111**, p. 122)
140–230	Klein & Dinkin (Zeitschr. physiol. Chem., **92**, p. 302)
185	Weston (Jl. Biol. Chem., **38**, p. 383)
130–190	Gorham & Myers (Archives of Int. Med., **20**, p. 599)

Lymph and chyle.

20–100	(chyle) Hensen (Arch. f. ges. Physiol., **10**, p. 94)
70	(chyle) Hamill (Jl. of Physiol., **35**, p. 151)
96–255	(blister fluid) Ferré, Maurice & Defaye (Société Biologie, **73**, p. 141)

Chylous urine.

3·4	Brieger (Zeitschr. physiol. Chem., **4**, p. 407)
10	Grimm (Virchow's Archiv, **111**, p. 341)
40–85	Sanes & Kahn (Archives of Int. Med., **17**, p. 181)
19	Pecker (Jl. Pharm. et Chim., **16**, p. 139)
10	Sano
20	Sano

TABLE III.

The ratio of the combined cholesterol to the total.

	Whole blood.
39·2–46·7	(man) Schultz (Biochem. Zeitschr. **42**, p. 255)
43·3–73·7	(man) Csonka (Jl. Biol. Chem., **24**, p. 431)
32·3–35·7	(men) } Bloor & Knudson (ibid, **29**, p. 7)
29·7–41·8	(women) }
24·6	(rabbit) } Wacker & Hueck (Arch. f. exp. Pathol. u. Pharm., **74**,
38·9	(horse) } p. 416)
25·3–28·7	(dog) Knudson (Jl. Biol. Chem., **32**, p. 337)
	Plasma.
46–68	(men) } Bloor & Knudson (Jl. Biol. Chem., **29**, p. 7)
51–70	(women) }
59	(man) Bloor & MacPherson (ibid, 31, p. 79)
48·5–57·0	(dog) Knudson (Jl. Biol. Chem., **32**, p. 337)
	Serum.
82·2–87·6	(man) Schultz (Biochem. Zeitschr., **42**, p. 255)
68·7–75·4	(man) Kauders (Biochem. Zeitschr., **55**, p. 96)
70·4–72·4	(man) Klein & Dinkin (Zeitschr. physiol. Chem., **92**, p. 302)
62·6	(rabbit) } Wacker & Hueck (Arch. f. exp. Pathol. u. Pharm., **74**,
77·5	(horse) } p. 416)
67·7	(ox) Thaysen (Biochem. Zeitschr., **62**, p. 115)
	Chyle.
57·6–69·7	(dog) Mueller (Jl. Biol. Chem., **22**, p. 1)
	Chylous urine.
65·3	Sano
81·3	Sano

It is most probable that the amount of cholesterol, fat and allied substances in blood is remarkably constant under normal conditions, although some authors as Terroine[1] will admit a variation to a wide extent. As a consequence, it is certain that one of these substances is in a fixed proportion to another. The same has been found true also of the proportion between the free and the combined cholesterol in blood.

Wacker and Hueck[2], Gardner and his collaborators[3], Muel-

1) E. F. Terroine, Jl. de Physiol. et de Pathol. Gen., **16** (1914), 212, 386.

2) L. Wacker and W. Hueck, Arch. exp. Pathol. u. Pharm., **74** (1913), 416.

3) M. T. Fraser and J. A. Gardner, Proc. Roy. Soc. Lond. (B), **81** (1909), 230; ibid, **82** (1910), 559; G. W. Ellis and J. A. Gardner, ibid, **86** (1912), 13; J. A Gardner and P. E. Lander, Biochem. Jl., **7** (1913), 576.

ler[1] and Knudson[2] stated that, if an animal was administered with cholesterol, whichsoever free or combined, the increase in both forms does occur in the blood. Free cholesterol might be partially esterified during absorption from the intestine and appear so in the chyle, and accordingly in the blood, and vice versa during absorption of cholesterol esters there might take place a partial hydrolysis. The constant relation between them would be probably born in this way.

Excepting in morbid conditions, this constant relation between two forms of cholesterol will be temporarily disturbed after a fatty meal. As it is well known, during absorption of fat free from cholesterol there may be seen a steady increase in fat, fatty acid and lecithin of the blood. At the same time, according to Bloor[3] and Knudson, also the percentage of the combined form of cholesterol to the total rises noticeably, while the latter remains nearly constant. Thus, a somewhat general constant relation will be maintained among all these fatty substances but total cholesterol, even after a fatty meal. Knudson observed that the feeding of dogs on olive oil produced an average increase of 47·4 per cent of the original content of cholesterol esters in the whole blood, the total cholesterol being almost unaffected. The greatest value noted for the increase of esters was 57·8 per cent, rising from 57 mgrms. to 90 mgrms. per 100 c.c. blood. The increase in esters of the corpuscles was most strikingly; he calculated it from 300 to 2,000 per cent. Consequently, the ratio of the cholesterol esters to the total cholesterol had been raised from 25·3–28·7 to 35·5–41·8 in the whole blood and from 48·5–57·0 to 63·0–71·1 in the plasma.

Returning to our results, the cholesterol content in the urine was 0·01–0·02 per 100 c.c or 2·04–3·98 per cent of the total ether soluble substance. The percentage of cholesterol in the total ether soluble substance is recorded in the literature as, 2·02[4], 1·17[5], 1·35[6], 0·39[7] in cases with ordinary diet, or 1–1·34 for the fat added and 2·7 for the diet poor in fat[8]. The somewhat higher results of the present observa-

1) J. H. Mueller, Jl. Biol. Chem., **22** (1915), 1.
2) A. Knudson, ibid, **32** (1917), 337.
3) W. R. Bloor, Jl. Biol. Chem., 24 (1916), 447.
4) L. Brieger, Zeitschr. physiol. Chem., **4** (1880), 407.
5) F. Erben, ibid, **30** (1900), 436.
6) K. I. Sanes and M. Kahn, Archives. Int. Med., **17** (1916), 181.
7) L. Feuerstein & K. Panek, Maly's Jahresber. f. Tierchemie, **33** (1903) 989.
8) F. Grimm, Virchow's Archiv, **111** (1888), 341.

tion is perhaps owing partly to the application of the improved method.

In comparing the cholesterol content in our chylous urine with the average amount in human chyle or lymph, it is about one-fourth to one-fifth of the latter. Therefore, if we compute it on the basis of the view that the chyluria is brought forth by the mixture of chyle escaping directly into urine, our chylous urine must have contained roughly one-fourth—one-fifth of chyle.

The amount of chyle mixed in urine may be calculated also on comparing the protein content of urine and chyle, as Magnus-Levy[1] has done. The average protein content in chyle used by him as the standard of the calculation was that reported by Munk and Rosenstein[2], i.e. 3·5 per cent, which is in harmony with the newer observations[3]. Calculated in this way, the proportion of the volume of chyle in our chylous urine is 1:4·4, the average protein content of the latter being 7·58 per thousand. So, the results of calculations in different ways do agree sufficiently. This would indicate also that the cholesterol in the chylous effusions may be an index of the amount of chyle and similary also of blood under certain conditions.

In addition to this the high value for the proportion between the combined and the total cholesterol in our cases, too, supports the view mentioned above.

Finally, the statement of Slosse[4] that he found cholesterol wholly in form as esters in his cases, too, shows that the greater part of cholesterol in the chyous urine is combined with fatty acids, though his conclusion cannot be considered as strictly correct.

Lecithin.

As has been described above, the bulk of the fatty matter was dissolved by the treatment with acetone. The remaining acetone insoluble part was used for the preparation of lecithin. Inasmuch as we have at present no reliable method for the estimation of lecithin I was confined to obtain a trustworthy evidence of its presence in chylous urine.

The acetone insoluble fraction of case 1.—This was taken up in

1) A. Magnus-Levy, Zeitschr. f. klin. Med., **66** (1908), 482.

2) J. Munk and A. Rosenstein, Virchow's Archiv, **123** (1891), 230.

3) T. Panzer, Zeitschr. f. physiol. Chem., **30** (1900), 113; T. Sollmann, Am. Jl. Physiol., **17** (1903), 487; J. M. Hamill, Jl. of Physiol., **35** (1906), 151.

4) A. Slosse, Maly's Jahresbericht f. Tierchemie, **31** (1901), 833; **32** (1902), 822.

ether; a minimal quantity of a substance of the protein nature, as indicated by its solubility and its reactions, was left undissolved. This insoluble precipitate was separated off by centrifugalization. The residue resulted by evaporation of the ethereal solution was purified by the successive treatment with alcohol, ether and acetone in the usual way. 2 grms. of phosphatide were obtained in this manner.

1 grm. of this phosphatide was saponified with barium hydroxide and the reaction mixture was treated with absolute alcohol. By the addition of platinum chloride to the concentrated alcoholic solution about 0·3 grm. of a yellow crystalline precipitate settled out. Its melting point was 236° after recrystallization out of water. The analysis of it gave the following results.

0·0632 grm. substance gave on combustion 2·48 c.c. (moist) N at 752 mm. and 18°C.
0·1608 grm. substance gave in fusion 0·0507 grm. Pt.

	Calculated for $(C_5H_{14}NOCL)_2$ Pt Cl_4	Found
N	4·55	4·46
Pt	31·66	31·56

The composition of phosphatide was as follows:

0·1044	grm. substance	neutralized	2·4 c.c.	0·1 n H_2SO_4
0·1414	„	„	3·15 c.c.	„
0·1416	„	gave	0·0192 grm.	$Mg_2P_2O_7$
0·1004	„	„	0·0138 „	„

N=3·17 per cent; P=3·80 per cent

Hence N : P = 1·85 : 1

The acetone insoluble fraction of case 2.—This was treated according to a new method of purifying phosphatide which will be publicated in details in other place. The outline of this procedure was as follows:

The acetone insoluble fraction of case 2 was taken up in chloroform, the chloroform solution was washed with a 1 % watery solution of sodium chloride in the nitrogen atmosphere, as many times as the new sodium chloride solution still took up coloring matter. Thereby the nitrogenous impurities were more completely removed than by any other method, while phosphatide itself remained unaltered. The washed chloroform solution was then separated from the salt solution and evaporated under diminished pressure. The residue obtained was extracted again with ether. From the ethereal solution phosphatide was precipitated by means of acetone and dried in a vacuum desiccator over sulfuric acid. The amount of the dried substance was 0·82 grm.

By the treatment of this with absolute alcohol there remained a minimal quantity of a substance insoluble in alcohol. This might be cephalin. After removal of alcohol the residue was dissolved in 25 c.c. of ether. Dried residues from samples of 5 c.c. each of this ether solution were employed for the estimation of nitrogen and phosphorus.

0·1640 grm. substance neutralized 10 c.c. 0·02 n H_2SO_4
0·1640 „ „ gave 0·0214 grm. $Mg_2P_2O_7$
N=1·71 per cent; P=3·64 per cent
Hence N : P = 1·04 : 1

From the remaining part of the ether solution, ether was evaporated, the residue was heated with 1 % sulfuric acid for four hours on a water bath. After this sulfuric acid was removed by barium hydroxide and evaporated to dryness and extracted again with absolute alcohol. Platinum chloride produced a colored precipitate in this alcoholic extract. This precipitate was collected on a filter, washed with alcohol and recrystallized out of water. Orange colored prisms of the melting point of 234°C. The crystals gave the periodide test of choline.

From above observations it is evident that lecithin was actually present in urine of the present cases of chyluria.

The ratio of the elements nitrogen and phosphorus in case 1 was too great for lecithin. But, at that time I had yet no available method of purification. The method proposed by MacLean[1] appeared so be a good one. Unfortunately, however, this method is followed by a great loss of the substance, as has been ascertained by a series of the preliminary experiments. On account of this inconvenience I did not make use of it; on the contrary, I stayed on the ordinary one. It is most probable, therefore, that this too great nitrogen value was caused by the insufficient purification of lecithin.

Summary.

1. Chylous urine contains two forms of cholesterol. In the present cases, 18·7–14·7 per cent of cholesterol was free and 65·3–81·3 per cent in the combined state as esters.

2. Lecithin was isolated from the urine and identified by the composition and the split products.

1) H. MacLean, Biochem. Jl., **6** (1912), 355; **9** (1915), 351; Biochem. Zeitschr., **57** (1913), 132.