A STUDY OF THE PROTEIN-LIPID COMBINATIONS IN BLOOD AND BODY FLUIDS

I. Normal Human and Dog Plasma and Horse Serum

By MARY E. TURNER and R. B. GIBSON

(From the Laboratory of Pathological Chemistry, State University of Iowa, Iowa City)

(Received for publication March 24, 1932)

INTRODUCTION

During the past few years considerable information has been accumulated regarding variations in the blood lipids under normal and pathological conditions. It has been shown that the lipids are associated in some manner with the plasma proteins, since the former are in part precipitated with them under certain conditions, and are only separated with difficulty by means of ordinary fat solvents. The exact nature of the relationship between the proteins and the lipids is unknown.

Previous workers on the subject of the lipid combinations with plasma or serum proteins have studied the problem from various angles. Bang (1) and Handovsky, Lohmann and Bosse (2) found considerable quantities of cholesterol associated with the plasma globulins. Gardner and Gainsborough (3) studied the relative amounts of cholesterol associated with the globulin and albumin fractions and found the former fraction included a larger quantity. Theorell (4) was able to remove 77 per cent of the total cholesterol from horse plasma by complete precipitation of the protein with ammonium sulfate, and when he added cholesterol to serum, he could recover a portion of it from the fibrinogen and globulin fractions after fractionation with ammonium sulfate. Young (5) stated that there was a weak combination between the cholesterol esters and the serum albumin. Hardy (6), Haslam (7), and Chick (8) found that very small amounts of ether-soluble constituents were associated with the serum proteins. Neuschlosz (9) found that variations in the amount of cholesterol carried down with the proteins depended upon the method of precipitation. He showed that, although he obtained a more complete precipitation of the protein when high concentrations of alcohol were used, the protein fraction contained less liquid material, due to the extraction of the lipids by the alcohol. Precipitation by ammonium sulfate resulted in a higher lipid concentration. Frankenthal (10) removed larger amounts of lipids with the globulin fraction than with the albumin by precipitation with ammonium sulfate. By electrodialysis, she was able to precipitate the entire cholesterol content of the serum with the globu-

lins. Troensegaard and Koudahl (11) were unable to bring down the cholesterol and phosphatides with the globulins if they were precipitated before the albumins.

Hardy and Mrs. Gardner (12), Chick (13), and Haslam (7) found phospholipid material associated with the globulin fraction of the plasma. Macheboeuf (14) precipitated a protein fraction from horse serum and plasma with ammonium sulfate which upon reprecipitation was constant in composition. It contained 22.7 per cent phosphamino lipids, 17.9 per cent cholesterol esters, and 59.1 per cent proteins. This is the largest amount of total lipid associated with protein precipitates reported. Macheboeuf, Wahl, and Sandor (15) also reported analyses on normal human plasma in which they found that the free cholesterol exceeded the cholesterol esters associated with the albumins, and that the reverse was true in the case of the globulins.

Lustig and Katz (16) studied both the phospholipid and cholesterol content of beef plasma proteins. They found less cholesterol and phospholipids in the euglobulin fraction than in the pseudoglobulin and albumin fractions. Went and Goreczky (17) showed that when serum proteins underwent an ultrafiltration process the cholesterol and phospholipid in the filtrate varied directly with the amount of protein present. A lecitho-protein present in tissue fibrinogen was described by Mills (18). This combination, however, presents a problem of different character from that found in the case of globulins and albumins.

The possibility of interpreting abnormal lipid-protein values under pathological or experimental conditions, and also of obtaining information upon the type of association present led the authors to a detailed study of the problem. Since abnormal conditions were to be studied on both animal and human blood, it was thought desirable to make a preliminary study of the normal relationships between species. For this purpose three types of blood, namely, human, dog, and horse, were employed, and a study of the lipids associated with the albumin and globulin fractions was made.

METHODS

Precipitation of the proteins. A portion of plasma or serum (at least 10 cc.) was diluted with three volumes of water and brought to one-fourth saturation with ammonium sulfate solution at room temperature. If a precipitate of fibrinogen formed, it was removed and discarded. The globulins were then precipitated by half saturation and removed from the solution by centrifugation. Filtering in the usual manner was avoided in order to prevent loss of lipid material on the filter paper. The precipitated protein was washed with half-saturated ammonium sulfate solution and the washings were added to the solution containing the albumins. The globulins were dissolved in water. Both fractions were coagulated by heat and the pH was adjusted to the point of maximum precipitation by the addition of 10 per cent acetic acid. After standing until the precipitates settled, the supernatant liquids were removed by siphoning. Several washings were made with distilled water in order to

remove the salt from the proteins. No attempt was made to remove the last traces of ammonium sulfate as it did not interfere with the extraction of the lipids.

Extraction of the lipids. The lipids were removed from the moist proteins by extracting with hot 95 per cent alcohol three times, hot absolute alcohol once, and ether three times, all in the same tube. After centrifugation, the solvents were decanted while still warm. The amounts of solvents were in such proportion as to give when mixed a solution containing approximately 35 per cent ether and 65 per cent alcohol. After cooling, the extracts were diluted to a definite volume. The albumin fraction required less intensive extraction for removal of the lipid material than did the globulin fraction. This method of extraction was adapted from Cavett (19) who found that the proteins extracted by this method were completely freed from lipids. He also demonstrated the importance of removing the lipids from the proteins while they were yet moist.

A second portion of plasma or serum, usually consisting of 5 cc., was extracted with 100 cc. of hot alcohol-ether solution by the regular Bloor procedure (20). The statement of Bloor that these proteins were free from lipids was also verified by Cavett (19).

The extracted proteins were dried and weighed. When the amount of plasma permitted, the relative amounts of the proteins were also determined colorimetrically by a modification of the Wu method (21).

It was first planned to fractionate and study the euglobulins since some previous workers included this protein in their studies. However, they worked with large amounts of plasma and in human cases this was not practicable. In two cases, however, the euglobulin, pseudoglobulin and albumin were fractionally precipitated from diluted plasma by means of ammonium sulfate. It was obvious that the amount of pseudoglobulin in normal plasma was so small that a volume of blood approximating 50 cc. would have been necessary in order to obtain a sufficient quantity for reliable analysis of the extracted lipids. About one-third of the globulins is composed of pseudoglobulin or less than one gram per 100 cc. of plasma. For this reason, it was decided to deal only with total globulins and albumins. Information obtained subsequently on pathological material (22) strengthened this decision as the euglobulin fraction became very small with a corresponding increase in the pseudoglobulin fraction.

Determinations of lipids. Bloor's oxidative method (23) was used for the determination of fatty acids. This method consists essentially of saponification of the alcohol-ether solution, extraction of the acidified residue with petroleum ether, and oxidation of the ether residue with sulfuric acid-dichromate reagent. Correction was made for the cholesterol content.

The phospholipids were determined as lecithin phosphorus according to the method of Bloor (24) and Benedict and Theis (25). According to this procedure the extracted lipids are oxidized by a mixture of nitric and sulfuric acids, and the phosphoric acid reduced by hydroquinone-sulfite and sodium molybdate to a blue compound which is estimated colorimetrically.

The free and total cholesterol were analyzed by Turner's (26) modification of the Okey method (27) for the determination of cholesterol by oxidation of the digitonide. Iodine numbers were determined by Gibson's micro method (28).

RESULTS

The alcohol-ether extract of the protein fractions and also of the total plasma were examined for their content of fatty acids, lecithin, and free

and total cholesterol. Iodine numbers of the total lipids were determined. Results are expressed as percentage of lipid per hundred cc. of plasma and also as mgm. of lipid per gram of protein since the amounts of globulin and albumin per unit volume are not the same. Typical determinations on human and dog plasma and horse serum are shown in Tables I, II, and III, respectively. The range of results between samples is shown in Tables IV and V, in which the maximum and minimum lipid values obtained from four samples of plasma are given. Vertical results do not necessarily refer to the same sample. The relative amounts of lipids in the globulin and albumin fractions, expressed as ratios, are given in column 4 in each table.

The data on human blood came from four normal healthy individuals, who were bled usually at 11 a.m. The precipitated plasma proteins contained about 50 per cent of the total lipids present in the plasma, although there was great variation when the individual lipids were considered (Table I). Approximately 45 per cent of the total fatty acids, 28 per cent of the phospholipids and 75 per cent of the total cholesterol were found in the protein fractions. A larger percentage of free cholesterol than of cholesterol esters was removed with the proteins; however, since the free cholesterol constituted less than half of the total, the absolute amount of cholesterol esters found with the proteins was larger.

Per unit weight, the globulin fraction contained more lipid material than the albumin fraction. Average figures for the total lipids showed that the globulins contained about one-third more lipid material than the albumins per gram of protein. The ratio of globulin lipid to albumin lipid was greatest for phospholipid and least for cholesterol. However, when the lipids were calculated as mgm. per 100 cc. of plasma, the albumin fraction contained a higher percentage than the globulin fraction since there was more albumin than globulin. Per volume of plasma there was approximately twice as much total cholesterol associated with the albumin fraction as with the globulin fraction. However, the globulins constituted about one-third of the total proteins present, so that there was little difference in the distribution of cholesterol per gram of protein between the two fractions. About 45 per cent of the fatty acids and also of the phospholipids were found with the globulins. The globulin fraction both by weight and volume included a larger percentage of the ester than of the free cholesterol. There was a greater variation between samples in the cholesterol content of the proteins per unit of volume than in the fatty acid or phospholipid content (Table IV).

The iodine numbers of the total lipids brought down with the globulin and albumin fractions were lower than those of the total plasma lipids. A slightly lower iodine number was always found on the lipids from the albumin fraction than from the globulin lipids. These facts suggest that some oxidation of the lipids might have taken place during the preparation of the protein fractions.

TABLE I

Normal human plasma (one sample)

Class of lipid	Globulin lipid	Globulin lipid / Total protein lipid	Albumin lipid	Globulin lipid / Albumin lipid	Total protein lipid (calculated)	Protein lipid / Total lipid	Total plasma lipids
	mgm.	*per cent*	*mgm.*	*ratio*	*mgm.*	*per cent*	*mgm.*
Fatty acids * *per 100 cc. plasma*	100.0	43.1	132.0		232.0	45.5	510.0
per 1 gram respective protein	35.8		24.0	1.49			
Lecithin *per 100 cc. plasma*	30.0	45.5	36.0		66.0	27.8	238.0
per 1 gram respective protein	10.7		6.5	1.63			
Cholesterol							
Free *per 100 cc. plasma*	26.0	34.2	50.0		76.0	78.4	97.0
per 1 gram respective protein	9.3		9.1	1.02			
Ester *per 100 cc. plasma*	32.0	36.4	56.0		88.0	74.5	118.0
per 1 gram respective protein	11.4		10.2	1.12			
Total *per 100 cc. plasma*	58.0	35.4	106.0		164.0	76.3	215.0
per 1 gram respective protein	20.7		19.2	1.08			
Total lipid *per 100 cc. plasma*	158.0	40.0	238.0		396.0	54.6	725.0
per 1 gram respective protein	56.4		43.3	1.30			
Iodine number	64		57				75

Protein content by weight: Albumin 5.5 grams per 100 cc.
Globulin 2.8 grams per 100 cc.
Total 8.3 grams per 100 cc.

* Corrected for cholesterol.

TABLE II

Normal dog plasma (one sample)

Class of lipid	Globulin lipid	Globulin lipid / Total protein lipid	Albumin lipid	Globulin lipid / Albumin lipid	Total protein lipid (calculated)	Protein lipid / Total lipid	Total plasma lipids
	mgm.	*per cent*	*mgm.*	*ratio*	*mgm.*	*per cent*	*mgm.*
Fatty acids * *per 100 cc. plasma*	77.0	57.0	58.0		135.0	42.1	321.0
per 1 gram respective protein	30.0		15.9	1.88			
Lecithin *per 100 cc. plasma*	26.0	61.2	16.5		42.5	32.0	132.0
per 1 gram respective protein	10.1		4.5	2.24			
Cholesterol							
Free *per 100 cc. plasma*	16.1	43.4	21.0		37.1	74.1	50.0
per 1 gram respective protein	6.3		5.7	1.10			
Ester *per 100 cc. plasma*	21.0	46.7	24.0		45.0	67.2	67.0
per 1 gram respective protein	8.2		6.6	1.24			
Total *per 100 cc. plasma*	37.0	44.6	46.0		83.0	71.0	117.0
per 1 gram respective protein	14.5		12.6	1.15			
Total lipid *per 100 cc. plasma*	114.0	52.3	104.0		218.0	49.8	438.0
per 1 gram respective protein	44.5		28.5	1.56			
Iodine number	72		60				81

Protein content by weight: Albumin 3.65 grams per 100 cc.
Globulin 2.56 grams per 100 cc.
Total 6.21 grams per 100 cc.

* Corrected for cholesterol.

TABLE III

Normal horse serum (one sample)

Class of lipid	Globulin lipid	Globulin lipid / Total protein lipid	Albumin lipid	Globulin lipid / Albumin lipid	Total protein lipid (calculated)	Protein lipid / Total lipid	Total serum lipids
	mgm.	*per cent*	*mgm.*	*ratio*	*mgm.*	*per cent*	*mgm.*
Fatty acids * *per 100 cc. serum*	132.0	75.0	44.0		176.0	44.0	400.0
per 1 gram respective protein	28.7		20.0	1.43			
Lecithin *per 100 cc. serum*	68.0	77.4	20.0		88.0	33.8	260.0
per 1 gram respective protein	14.8		9.1	1.63			
Cholesterol							
Free *per 100 cc. serum*	36.8	68.2	17.2		54.0	72.0	75.0
per 1 gram respective protein	8.0		7.8	1.03			
Ester *per 100 cc. serum*	35.8	70.2	15.2		51.0	63.7	80.0
per 1 gram respective protein	7.8		6.9	1.13			
Total *per 100 cc. serum*	72.6	69.2	32.4		105.0	67.7	155.0
per 1 gram respective protein	15.8		14.6	1.08			
Total lipid *per 100 cc. serum*	204.6	73.0	76.4		281.0	50.6	555.0
per 1 gram respective protein	44.6		34.7	1.29			
Iodine number	65		54				77

Protein content by weight: Albumin 2.2 grams per 100 cc.
Globulin 4.6 grams per 100 cc.
Total 6.8 grams per 100 cc.

* Corrected for cholesterol.

TABLE IV

Normal human variations (four samples of plasma)

Class of lipid	Globulin lipid	Globulin lipid / Total protein lipid	Albumin lipid	Globulin lipid / Albumin lipid	Total protein lipid (calculated)	Protein lipid / Total lipid	Total plasma lipids
	mgm.	*per cent*	*mgm.*	*ratio*	*mgm.*	*per cent*	*mgm.*
Fatty acids * *per 100 cc. plasma*	100.0–125.0	42.0–46.0	125.0–140.0		225.0–265.0	41.0–48.0	450.0–555.0
per 1 gram protein	35.8– 38.1		23.0– 27.1	1.38–1.49			
Lecithin *per 100 cc. plasma*	30.0– 36.5	44.0–49.0	36.0– 44.5		66.0– 81.0	28.0–35.0	210.0–238.0
per 1 gram protein	9.2– 11.3		6.5– 8.7	1.63–1.85			
Cholesterol							
Free *per 100 cc. plasma*	14.1– 31.2	30.0–41.0	25.9– 50.0		40.0– 76.0	70.0–78.4	67.5– 97.0
per 1 gram protein	5.0– 9.5		4.7– 9.1	1.02–1.15			
Ester *per 100 cc. plasma*	25.0– 36.0	32.0–40.0	48.0– 56.0		73.0– 90.0	65.0–74.5	112.0–123.0
per 1 gram protein	9.2– 11.4		8.7– 10.3	1.12–1.21			
Total *per 100 cc. plasma*	39.1– 67.2	34.0–42.0	73.9–106.8		115.0–164.0	64.0–76.3	179.5–220.0
per 1 gram protein	14.0– 20.7		13.4– 19.2	1.04–1.09			
Total lipid *per 100 cc. plasma*	139.1–192.2	40.0–45.0	198.9–246.0		338.0–438.0	48.0–54.6	629.5–775.0
per 1 gram protein	49.5– 59.0		36.2– 46.0	1.26–1.40			
Iodine number	51 – 66		44 – 60				61 – 77

Protein content by weight: Albumin 4.10–5.50 grams per 100 cc.
Globulin 2.10–3.28 grams per 100 cc.
Total 6.20–8.30 grams per 100 cc.

* Corrected for cholesterol.

TABLE V

Normal dog variations (four samples of plasma)

Class of lipid	Gobulin lipid	Globulin lipid / Total protein lipid	Albumin lipid	Globulin lipid / Albumin lipid	Total protein lipid (calculated)	Protein lipid / Total lipid	Total plasma lipids
	mgm.	*per cent*	*mgm*	*ratio*	*mgm.*	*per cent*	*mgm.*
Fatty acids * *per 100 cc. plasma*	67.2– 77.0	51.0–57.0	58.0– 68.0		132.0–142.0	42.1–47.0	280.0–340.0
per 1 gram protein	29.6– 32.0		15.9– 17.0	1.74–1.88			
Lecithin *per 100 cc. plasma*	21.0– 26.0	57.0–61.0	16.0– 18.0		37.0– 44.0	30.0–37.0	100.0–140.0
per 1 gram protein	9.6– 10.1		4.2– 4.5	2.10–2.40			
Cholesterol							
Total *per 100 cc. plasma*	27.0– 40.0	39.0–46.5	42.0– 55.0		69.0– 95.0	61.0–74.0	103.0–128.0
per 1 gram protein	12.7– 16.0		11.1– 13.8	1.15–1.26			
Total lipid *per 100 cc. plasma*	94.2–117.0	50.5–52.3	100.0–123.0		201.0–237.0	48.0–52.5	283.0–468.0
per 1 gram protein	44.8– 47.0		29.0– 30.8	1.52–1.70			
Iodine number	60 – 72		52 – 60				68 – 81

Protein content by weight: Albumin 3.65–4.00 grams per 100 cc.
Globulin 2.10–2.56 grams per 100 cc.
Total 5.80–6.56 grams per 100 cc.

* Corrected for cholesterol.

Four samples of fasting dog plasma were obtained from three animals weighing between 10 and 13 kilograms, all on the same diet. The lipid content was lower than that of human plasma. Because of the small amount of cholesterol present, it was difficult to obtain both free and total cholesterol, but in one case enough blood was drawn for the complete analysis (Table II). The plasma proteins carried down about half the lipid material, a finding corresponding to the results obtained on human plasma. The percentages of individual lipids included with the protein fractions were also similar. However, the globulin fraction per gram of protein as well as per unit of volume contained more lipid material than was found in the human plasma. The increase per unit volume was about 10 per cent for each lipid. The ratio between the amounts of phospholipid per gram of globulin and of albumin was above two. Fatty acids showed a corresponding increase and cholesterol increased to a lesser degree. The relationships between free and total cholesterol and the protein fractions were not essentially altered. The variations between samples (Table V) were greater in the cholesterol content of the proteins than in the fatty acid or phospholipid contents, as was true in the human plasma. The iodine numbers also maintained the same relations to the total plasma as in human blood.

The horse serum [1] contained larger amounts of globulins than of albumins in the two samples studied. The analysis obtained on one sample is given in Table III. The variations between the two samples were slight. Since the cholesterol content of horse serum is low, sufficiently large quantities were used to determine both free and total cholesterol. The amounts of fatty acids and of phospholipid were greater than those of dog plasma. Approximately the same percentage of lipid material was found with the total protein fractions as well as the same proportions of the individual lipids, as in the human and dog samples. The greater percentage of free cholesterol was also found with the proteins as in the previous determinations. However, since the free cholesterol constituted nearly 50 per cent of the total cholesterol, the total proteins contained nearly equivalent amounts of the two forms of the sterol. This is not a significant variation since the normal proportion of free to total cholesterol is not a constant figure. In the globulin fraction the amount of lipid material per unit volume was increased by an average of 30 per cent over that found in the other two types of blood. However, there was no marked increase in the globulin-albumin lipid ratio per gram of protein as compared to the results obtained from human plasma, because of the greater globulin content. Per unit weight, the globulins contained about one-third more lipids than the albumins. The globulin-albumin phospholipid ratio exceeded the fatty acid or cholesterol

[1] We are indebted to Parke Davis and Company for a generous supply of normal horse serum.

ratios. Slightly less cholesterol ester than free cholesterol was found on the globulin fraction, a difference which, however, is not a significant variation from the condition present in the human or dog plasma. The iodine numbers were lower for the protein lipids than for those of the whole plasma, and also lower for the globulin lipids than for the albumin lipids.

SUMMARY AND CONCLUSIONS

1. The albumins and globulins have been fractionated by means of salt precipitation from human and dog plasma and horse serum. Fatty acids, phospholipids, and cholesterol have been determined in these protein fractions.

2. In general, the total proteins carried down about one-half of the lipid content of the plasma or serum. The globulins showed a definite tendency to contain more lipids than did the albumins. They also contained varying amounts of the individual lipids, the phospholipids being present in a greater percentage than either fatty acids or cholesterol.

3. In horse serum, in which the amount of globulins was larger than of albumins there was no marked change in the amount of lipid per gram of protein, although such was the case per volume of serum.

4. The globulins of the dog plasma contained significantly more of all three lipids than did those of horse serum or human plasma, the increase being the greatest in the case of the phospholipids.

BIBLIOGRAPHY

1. Bang, I., Biochem. Ztschr., 1918, xc, 383. Über Lipämie I.
2. Handovsky, H., Lohmann, K., and Bosse, P., Arch. f. d. ges. Physiol., 1925, ccx, 63. Untersuchungen über die Zusammensetzung des Blutserums und ihre Bedeutung für Giftwirkungen. V. Untersuchungen über den Zustand des Cholesterins im Blutserum.
3. Gardner, J. A., and Gainsborough, H., Biochem. J., 1927, xxi, 141. Studies on the Cholesterol Content of Normal Human Plasma. Part II. The Attraction of the Proteins of Plasma for Sterols.
4. Theorell, A. H. T., Biochem. Ztschr., 1926, clxxv, 297. Über quantitative Bestimmung der Lipoide der aus Pferdeplasma ausgesalzten Eiweisskörper.
5. Young, E. G., Proc. Roy. Soc., London, B, 1922, xciii, 15. On the Optical Rotatory Power of Crystalline Ovalbumin and Serum Albumin.
6. Hardy, W. B., J. Physiol., 1905, xxxiii, 251. Colloidal Solution. The Globulins.
7. Haslam, H. C., Biochem. J., 1913, vii, 492. Separation of Proteins. Part III. Globulins.
8. Chick, H., Biochem. J., 1914, viii, 404. XLV. The Apparent Formation of Euglobulin from Pseudo-Globulin and a Suggestion as to the Relationship Between These Two Proteins in Serum.
9. Neuschlosz, S. M., Biochem. Ztschr., 1930, ccxxv, 130. Über den Lösungszustand des Cholesterins im Blutserum. III. Über die Beziehungen zwischen Cholesterin und Eiweisskörpern.

10. Frankenthal, K., Ztschr. f. Immunitatsforsch. u. exp. Therap., 1925, xlii, 501. Über die Beziehungen der Serumlipoide zu den Eiweissfraktionen.
11. Troensegaard, N., and Koudahl, B., Ztschr. f. Physiol. Chem., 1926, cliii, 111. Cholesterin als prostethische Gruppe im Serum-Globulin. VIII.
12. Hardy, W. B., and Gardner, S. (Mrs.), J. Physiol. (Proc. Physiol. Soc.), 1910, xl, lxviii. Proteins of Blood Plasma.
13. Chick, H., Biochem. J., 1914, viii, 404. XLV. The Apparent Formation of Euglobulin from Pseudo-Globulin and a Suggestion as to the Relationship Between These Two Proteins in Serum.
14. Macheboeuf, M. A., Compt. rend. Acad. d. sc., 1929, clxxxviii, 109. Recherches sur les phosphoaminolipides et les stérides du plasma et du sérum sanguins.
15. Macheboeuf, M. A., Wahl, R., and Sandor, G., Bull. Soc. chim. biol., 1931, xiii, 504. Recherches sur la néphrose Lipidique, Etude qualitative des lipides et des diverses fractions lipidiques du sérum sanguin.
16. Lustig, B., and Katz, R., Biochem. Ztschr., 1931, ccxxxi, 39. Zur Kenntnis der Unterfraktionen der Globuline und Albumine im Serum. II. Die Verteilung der Lipoide, des Präzipitinogens und der Bakterienagglutinine auf die einzelnen Unterfraktionen des Rinderserums.
17. Went, S., and Goreczky, L., Biochem. Ztschr., 1931, ccxxxix, 441. Die Verteilung der Serumphosphatide und des Cholesterins in durch Filtration durch grossporige Kollodiumfilter gewonnenen Serumultrafiltraten.
18. Mills, C. A., J. Biol. Chem., 1921, xlvi, 135. Chemical Nature of Tissue Coagulins.
19. Cavett, J., Thesis, Univ. of Iowa, 1930. Urinary Proteins in Nephritis: The Identity of the Urinary Proteins with Those of Edema Fluid and Blood Plasma Proteins.
20. Bloor, W. R., J. Biol. Chem., 1928, lxxvii, 53. The Determination of Small Amounts of Lipid in Blood Plasma.
21. Wu, H., J. Biol. Chem., 1922, li, 33. A New Colorimetric Method for the Determination of Plasma Proteins.
22. Turner, M. E., Thesis, Univ. of Iowa, 1931. A Study of the Protein-Lipid Combinations in Blood and Body Fluids.
23. Bloor, W. R., J. Biol. Chem., 1928, lxxvii, 53. The Determination of Small Amounts of Lipid in Blood Plasma.
24. Bloor, W. R., J. Biol. Chem., 1918, xxxvi, 33. Methods for the Determination of Phosphoric Acid in Small Amounts of Blood.
25. Benedict, S. R., and Theis, R. C., J. Biol. Chem., 1924, lxi, 63. A Modification of the Molybdic Method for the Determination of Inorganic Phosphorus in Serum.
26. Turner, M. E., J. Biol. Chem., 1931, xcii, 495. A Simplification of the Okey Method for the Determination of Cholesterol by Oxidation of the Digitonide.
27. Okey, R., J. Biol. Chem., 1930, lxxxviii, 367. A Micro Method for the Estimation of Cholesterol by Oxidation of the Digitonide.
28. Gibson, R. B., and Howard, C. P., Arch. Int. Med., 1923, xxxii, 1. Metabolic Studies in Pernicious Anemia.