ACID-BASE EQUIVALENCE OF THE BLOOD IN DISEASES ASSOCIATED WITH HYPERGLOBULINEMIA; WITH SPECIAL REFERENCE TO LYMPHOGRANULOMA INGUINALE AND MULTIPLE MYELOMA

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Attention was called recently (1) to the occurrence of hyperproteinemia in patients with lymphogranuloma inguinale. Thirty-five cases have now been examined, in 26 of whom the protein content of the serum exceeded 8.0 per cent (Table I). Williams and Gutman (1, 2) pointed out that the hyperglobulinemia observed in lymphogranuloma inguinale would account, in part, for two interesting phenomena associated with the disease: the increased erythrocytic sedimentation rate, often greatly in excess of that consistent with the degree of obviously active infection; and anticomplementary properties of the serum, demonstrable in a significant proportion of these patients.

It is desired here to call attention to another peculiarity of the blood in lymphogranuloma inguinale, thought also to be related to hyperglobulinemia. Electrolyte partitions of the blood in this disease (Table II) revealed that in every instance in which the serum protein was 9.0 per cent or over, there was an associated discrepancy in acid-base equivalence: the sum of the determined acid equivalents exceeded the total base, B — A appeared to be negative.[1] Further investigation showed that a similar discrepancy in acid-base balance of the blood obtained in diseases other than lymphogranuloma inguinale in which there was marked hyperglobulinemia. It would appear from our data that an apparent excess of total determined acid equivalents over total base is, in fact, a peculiarity common to most bloods with definite hyperglobulinemia, irrespective of etiology.

[1] B — A means total base (m.eq. per liter) minus total determined acids (m.eq. per liter). As organic acids and sulfate are not determined, B — A should be positive.

METHODS

Fifty to 60 cc. of blood, collected under mineral oil by venipuncture as rapidly as possible and with minimal stasis, were delivered under oil into 50 cc. narrow neck centrifuge tubes. After sealing with rubber caps, the blood was allowed to clot, then centrifuged under oil. Duplicate samples of serum were withdrawn into 1 cc. stopcock pipettes for determination of CO_2 content by the method of Van Slyke and Neill (3). A pH of 7.35 was assumed in the calculation of bicarbonate from the observed CO_2 content of the serum.

The remainder of the serum was then removed and analysed for the following constituents, all determinations being made in duplicate except in a few instances where the amount of serum available was insufficient. Chloride was determined on 1 cc. samples by the method of Van Slyke and Sendroy as modified by Wilson and Ball (4). Phosphate was determined by the method of Kuttner and Lichtenstein (5) on duplicate samples of the trichloroacetic acid filtrate obtained from 1 cc. of serum. Determination of protein was made on 1 cc. samples by difference, using the macro-Kjeldahl technique for estimation of the total nitrogen and Folin's method with Nesslerization for estimation of nonprotein nitrogen. Albumin, globulin, euglobulin, and pseudoglobulin I and II were estimated in duplicate on 0.5 cc. samples by Howe's method (6), nitrogen being determined by the micro-Kjeldahl technique and titration. Base bound to protein was calculated as the sum of base bound to albumin plus base bound to globulin, using the factors of Van Slyke, Hastings, Hiller and Sendroy (7). Since a pH of 7.35 was assumed throughout,

B protein (in m.eq. per liter) = Albumin (grams per liter) × .273 + Globulin (grams per liter) × .189.

Total base was estimated by determining sodium, potassium and calcium and adding to the sum of the milli-equivalents of these cations 2 milli-equivalents (assumed) for magnesium. Recent modifications in the method for determination of sodium permit of more accurate results by this procedure than by the direct estimation of the total base. Sodium was determined by the modification of Butler and Tuthill (8) of Barber and Kolthoff's method, with certain minor changes in procedure by Baxter, Leland and Kurepkat (9). Potassium was esti-

TABLE I

Lymphogranuloma inguinale: Serum protein fractions in 35 cases

Case	Sex	Age	Race	Clinical findings	Frei test	Wassermann reaction*	Dmelcos reaction	Total protein	Albumin	Globulin	Euglobulin	Pseudoglobulin		Plasma fibrinogen
												I	II	
		years						*grams per 100 cc.*	*grams per 100 cc.*	*grams per 100 cc.*	*grams per 100 cc.*	*grams per 100 cc.*	*grams per 100 cc.*	*grams per 100 cc.*
E. A.	♀	43	Negro	Rectal stricture	+	−	−	11.2	2.9	8.3				
								11.2						
								10.7	2.9	7.8	4.0			
								10.1	3.3	6.8				
C. K.	♀	34	Negro	Chronic glomerulonephritis. Urethral stricture	+	Anti-comp.		10.3	3.1	7.2	3.0	3.3	0.9	
								8.4	2.3	6.1	2.7			
								8.3	2.6	5.7				
P. S.	♀	50	Negro	Rectal stricture	+	−	−	9.5	3.1	6.4	3.0	2.5	0.9	
								9.2	3.4	5.8				0.3
E. J.	♀	43	Negro	Rectal stricture	+	Anti-comp.		9.5	3.4	6.1	2.3	3.0	0.8	
								9.3	3.7	5.6				
E. F.	♀	36	Negro	Rectal stricture	+	+	+	9.4	3.6	5.8	2.1	3.0	0.7	
A. Br.	♀	32	Negro	Rectal stricture	+	−	−	9.4	3.4	6.0	2.3	3.0	0.7	
K. W.	♀	24	Negro	Elephantiasis vulvae and rectal stricture	+	+		9.4	3.3	6.1				
								9.3	3.3	6.0	2.6	2.8	0.6	
F. H.	♀	35	Negro	Rectal stricture	+	−		9.2	3.5	5.7				
D. P.	♀	48	Negro	Rectal stricture	+	−	−	9.1	4.0	5.1				
								8.5	3.7	4.8	0.9	3.0	0.9	0.4
A. Bo.	♀	34	Negro	Rectal stricture	+	+	−	9.0	3.6	5.4	1.7	3.0	0.7	
C. B.	♂	38	Negro	Inguinal buboes	+	+	−	8.9	3.8	5.1	2.6			
E. G.	♂	50	Negro	Proctitis	±	−	−	8.8	3.8	5.0	1.4			
C. W.	♀	49	Negro	Rectal stricture	+	−		8.7	3.3	5.4	1.4			0.5
G. J.	♂	40	Negro	Proctitis	±	+		8.7	3.5	5.2	1.6			
								8.7	3.6	5.1	1.5			
G. W.	♀	31	Negro	Rectal stricture	+	−	−	8.7	3.7	5.0				0.4
T. W.	♂	25	Negro	Inguinal buboes	+	−	−	8.6	3.5	5.1	1.6			
C. D.	♂	55	White	Proctitis	+	−	−	8.6	4.0	4.6				
								8.0	3.6	4.4	0.8			
E. D.	♀	37	Negro	Rectal stricture	+	+		8.5	3.9	4.6	1.1			0.6
								8.1	3.9	4.2	1.1			
G. M.	♀	55	Negro	Rectal stricture	+	−		8.5						
J. B.	♂	40	Negro	Rectal stricture	+	+	−	8.4	4.2	4.2	1.2			
C. H.	♂	46	Negro	Inguinal buboes	+	−	+	8.4	4.3	4.1				
M. P.	♀	30	Negro	Rectal stricture	+	+		8.4†	3.8	4.3				0.3
E. R.	♀	40	Negro	Rectal stricture; aneurysm (luetic)	+	+		8.3	4.1	4.2	1.3			
S. S.	♂	44	White	Inguinal buboes	+	−	−	8.2	3.9	4.3				
L. D.	♂	28	Negro	Inguinal buboes	+	−	−	8.1	3.7	4.4				
F. T.	♀	31	Negro	Rectal stricture	+	−	−	8.1	4.0	4.1	0.7			
F. N.	♂	35	White	Inguinal buboes	+	−	−	7.9	4.3	3.6				
A. N.	♀	46	White	Rectal stricture	+	−		7.8	3.5	4.3	0.6			
M. G.	♀	39	White	Rectal stricture	+	+	−	7.8	4.7	3.1	0.8			
C. H.	♀	27	Negro	Rectal stricture	+	−	+	7.6	4.3	3.3	0.8			
A. L.	♂	31	White	Inguinal buboes	+	−	+	7.6	4.4	3.2				
R. L.	♂	54	White	Rectal stricture	+	+		7.5	4.2	3.3	0.9			
J. P.	♂	44	Negro	Inguinal buboes	+	+	+	7.4	4.6	2.8	0.6			
I. T.	♀	55	Negro	Inguinal buboes	+	−	−	7.3	4.2	3.1	0.7			
I. Th.	♂	42	Negro	Proctitis	±	−	±	6.8	4.6	2.2				

* The Wassermann reaction was reported anticomplementary on at least one occasion in 7 cases in this series; in the 2 cases indicated, every Wassermann test done was reported anticomplementary.

† Determinations made on plasma in this case.

mated by the method of Tisdall and Kramer (10) with the exception that the serum was ashed (11), and calcium by the method of Kramer and Tisdall as modified by Clark and Collip (12). For the estimation of magnesium an average normal value of 2 milli-equivalents per liter (13) was assumed.

In addition to the above analyses, the following determinations were made in occasional cases, as described in the text. Sulfate was determined by the method of Loeb and Benedict (14) and fibrinogen by a modified Cullen and Van Slyke (15) method. Hematocrit determinations of blood cell volume were carried out on samples containing equal and constant amounts of oxalate.

The Frei test on patients suspected of lymphogranuloma inguinale was performed with antigen prepared by the method described by Frei (16) from material obtained from suppurating inguinal buboes. The minimum reaction accepted as a positive response was the development of a papule with a surrounding area of erythema at least 6 millimeters in diameter and persisting at least 3 days. The cutaneous reaction was examined after 48 or 72 hours. We are indebted to Drs. W. and H. Curth, who carried out and interpreted the Frei tests.

Results of serum electrolyte partitions in normal persons. The limits of variation in normal serum sodium by the method used here have been found by Loeb and his collaborators to lie between 137.0 and 144.0 m.eq. per liter (unpublished studies). Potassium, calcium and magnesium total 12 ± 1 m.eq. per liter, giving values of 149.0 to 156.0 m.eq. per liter for total base. Total base usually exceeds total determined acids by 1 to 4 m.eq. per liter. We rarely obtain higher values for total determined acids than for total base in normal subjects by the methods described, i.e. B — A is rarely negative. This experience, however, is contrary to that of Peters and Man (17) who find negative B — A values, in normal subjects, as large as — 6.5 m.eq. per liter, associated with total base values (determined by the method of Hald) as low as 142.0 m.eq. per liter.

RESULTS

Hyperproteinemia in lymphogranuloma inguinale

Whereas the concentration of serum proteins in normal subjects exceeds 7.5 per cent only in occasional instances ((18, 19); personal observations), 31 of 35 patients with lymphogranuloma inguinale were found to have serum protein values of 7.6 per cent or more, in 10 instances exceeding 9.0 per cent (Table I).[2] Our data indicate that hyperproteinemia is likely to be more marked in cases with chronic complications, particularly in females with rectal stricture, though such patients do not invariably present high serum protein values.

TABLE II

Lymphogranuloma inguinale: Serum electrolyte partitions in 18 cases

Case	Cl	HCO_3	Protein	PO_4	Na	K	Ca	Total base	Total acid	B—A	Non-protein nitrogen
	m. eq. per liter	*m. eq. per liter*	*m. eq. per liter*	*m. eq. per liter*	*m. eq. per liter*	*m. eq. per liter*	*m. eq. per liter*	*m. eq. per liter*	*m. eq. per liter*	*m. eq. per liter*	*mgm. per 100 cc.*
E. A.	102.6	26.8	22.6	2.1	134.7	5.5	4.6	146.8	154.1	−7.3	26
C. K.	98.5	23.4	22.1	2.5	131 6	4.4	5.3	143.3	146.5	−3.2	30
P. S.	106.8	24.4	20.6	1.9	138.8	4.9	5.3	151.0	153.7	−2.7	32
E. J.	101.7	27.7	20.8	1.8	137.5	4.4	5.4	149.3	152.0	−2.7	27
E. F.	102.9	27.8	20.8	2.1	138.7	4.0	5.0	149.7	153.6	−3.9	26
A. Br.	106.7	24.3	20.6	2.3	139.0	5.0	5.4	151.4	153.9	−2.5	25
K. W.	104.0	24.0	20.3	1.7	135.6	4.5	5.1	147.2	150.0	−2.8	20
A. Bo.	105.8	26.3	20.0	1.8	139.3	4.7	5.3	151.3	153.9	−2.6	30
E. G.	101.3	28.0	19.9	1.6	139.7	3.8	5.4	150.9	150.8	+0.1	31
G. J.	103.4	28.0	19.4	2.1	140.2	4.0	5.1	151.3	152.9	−1.6	25
D. P.	103.2	28.7	19.2	1.7	142.6	5.4	5.1	155.1	152.8	+2.3	24
E. R.	106.3	26.3	19.1	2.0	143.3	4.1	5.4	154.8	153.7	+1.1	32
Es. D.	105.5	27.4	18.7	1.3	142.0	4.2	5.2	153.4	152.9	+0.5	27
C. D.	103.3	25.8	18.1	1.8	138.2	4.5	5.3	150.0	149.0	+1.0	36
M. G.	103.3	29.1	18.7	1.7	142.0	4.8	5.4	154.2	152.8	+1.4	28
C. H.	103.8	26.4	17.9	1.7	137.8	4.8	5.2	149.8	149.8	0.0	24
R. L.	105.0	28.5	17.7	1.2	141.2	4.1	5.2	151 5	152.4	−0.9	34
I. T.	103.4	27.8	17.4	1.9	141.5	4.5	5.3	153.3	150.5	+2.8	29

The hyperproteinemia was found to be due to an increase in the euglobulin and pseudoglobulin I fractions. The rise in euglobulin was striking, being 10 times our normal mean value in one instance in which euglobulin constituted more than 50 per cent of the total globulins. The albumin content of the serum was moderately or definitely decreased in several cases. The fibrinogen content of the plasma did not exceed 0.6 per cent in the 6 cases examined.

The hyperproteinemia found in lymphogranuloma inguinale corresponds with that described in kala-azar (23, 24) and leprosy (25, 26) in degree, fractional distribution and incidence. A

[2] The occurrence of hyperproteinemia in lymphogranuloma inguinale appears not to have been recorded in the literature. It is interesting to note, however, that Rowe (20), in an extensive investigation of serum proteins in disease, obtained his highest value (10.4 per cent, after stasis) in an undiagnosed case of "enlarged inguinal glands." Since inguinal buboes are now known to be a characteristic manifestation of lymphogranuloma inguinale, it seems not improbable that Rowe's patient was affected with this disease.

Turner, in a study of serum proteins in pellagra (21), noted a moderate increase in serum globulin in 4 cases with co-existing rectal stricture. Lymphogranuloma inguinale is now known to be a common cause of inflammatory rectal strictures.

Nicolau (22) noted an increase in the refractive index and in the viscosity of the serum in several cases of lymphogranuloma inguinale and considered that these changes might indicate an increase in serum albumin. Our findings are not in accord with this inference.

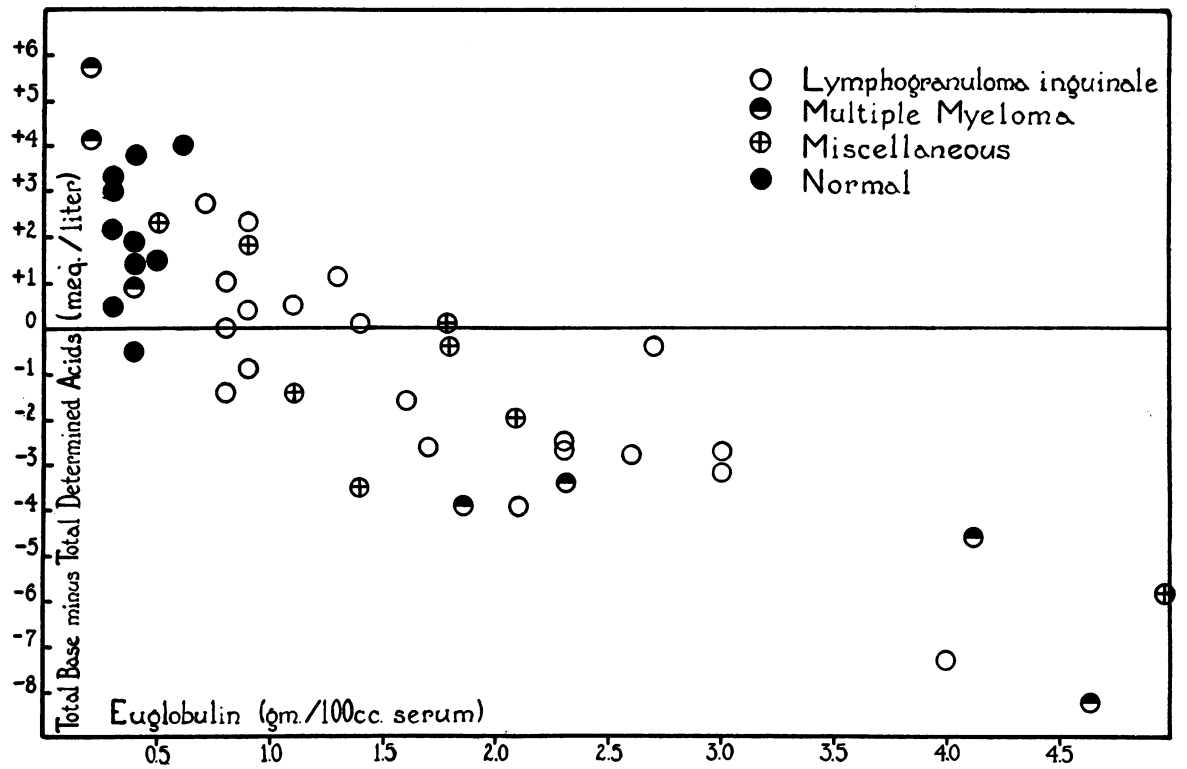

FIG. 1. SCATTER DIAGRAM IN WHICH B — A IN MILLI-EQUIVALENTS PER LITER IS PLOTTED AGAINST EUGLOBULIN CONTENT IN GRAMS PER 100 CC. SERUM IN 10 NORMAL SUBJECTS, 18 CASES OF LYMPHOGRANULOMA INGUINALE, 4 CASES OF MULTIPLE MYELOMA AND 8 MISCELLANEOUS CASES WITH HYPERGLOBULINEMIA.

In the cases of multiple myeloma with nitrogen retention in which sulfates were determined, the values obtained for sulfates have been added to the sum of acid-equivalents.

The figure shows a trend in the direction of increasing excess of total determined acids over total base in bloods containing increasing amounts of euglobulin.

similar increase in euglobulin may occur in malaria (27), filariasis (28), syphilis (29, 30), tuberculosis (31, 32), rheumatoid arthritis (33, 34) and other chronic infections; but the incidence of patients presenting hyperproteinemia appears to be considerably lower, and values over 9.0 per cent are exceptional. In multiple myeloma (35), as is well known, the concentration of proteins in the serum may reach extraordinarily high levels.

It is apparent that the hyperproteinemia occurring in lymphogranuloma inguinale is not the result of a decrease in the volume of circulating fluids since this would not account for the alteration in A:G ratio. Moreover, hematocrit determinations of blood cell volume, carried out in 3 cases, gave normal values. None of the patients in this series presented the clinical picture of dehydration. It is probable that the hyperglobulinemia observed in lymphogranuloma inguinale, like that noted in other chronic suppurative infections, reflects the response of the organism to certain types of infection.

Hyperproteinemia is neither as constant nor as specific a manifestation of lymphogranuloma inguinale as is the Frei reaction. It is apparent, however, that the possibility of lymphogranuloma inguinale should be considered in cases presenting unexplained hyperproteinemia, particularly in negroes, and that this possibility should be ruled out by means of the Frei test.[3]

[3] In a disease as widespread as lymphogranuloma inguinale appears to be in the negro population of the United States (36, 37), it is of course possible that the association with hyperproteinemia may be wholly coincidental, the exciting cause being in reality some co-existing infection such as syphilis, chancroid or tuberculosis. The

Serum electrolyte partitions in lymphogranuloma inguinale

Complete serum electrolyte partitions were carried out in 18 cases of lymphogranuloma inguinale (Table II). In 10 instances the sum of the determined acid equivalents exceeded the total base, i.e. B — A appeared to be negative. Every case of lymphogranuloma inguinale in this series in which the euglobulin fraction was increased to 1.4 per cent or more exhibited this apparent discrepancy in acid-base equivalence.[4] There was, in fact, a fairly well defined tendency for the discrepancy in acid-base balance to be more marked as the euglobulin content increased, a relationship approximately linear. This trend is illustrated in a scatter diagram in which the euglobulin content of sera containing normal and increased amounts of euglobulin is plotted against total base minus total determined acids (Figure 1).

Consideration of the individual constituents of the acid-base balance in these cases of lymphogranuloma inguinale (Table II) reveals little of significance with the exception of protein where this was elevated. In several instances, the sodium content of the serum fell to 136 m.eq. per liter or below, for reasons wholly obscure. While the fall in sodium may contribute to the apparent excess of total determined acids over total base, in most cases the discrepancy in acid-base equivalence was associated with sodium values within normal limits. In several cases, the serum albumin was definitely decreased (Table I). For the most part, the decrease was associated with proteinuria (1), notably in Cases E. A. and C. K.

TABLE III

Multiple myeloma: Serum electrolyte partitions; serum protein fractions in 6 cases

Case	Age	Sex	Date	Cl	HCO_3	Protein	PO_4	Na	K	Ca	Total base	Total acid	B—A	Non-protein nitrogen	Total protein	Albumin	Globulin	Euglobulin	Remarks
	years			*m. eq. per liter*	*m. eq. per liter*	*m. eq. per liter*	*m. eq. per liter*	*m. eq. per liter*	*m. eq. per liter*	*m. eq. per liter*	*m. eq. per liter*	*m. eq. per liter*	*m. eq. per liter*	*mgm. per 100 cc.*	*grams per 100 cc.*	*grams per 100 cc.*	*grams per 100 cc.*	*grams per 100 cc.*	
J. B.	37	♂	February 1, 1935	98.6	28.9	21.5	2.4	135.6	4.5	4.7	146.8	151.4	−4.6	28	10.0	3.2	6.8	4.1	Diagnosis proven at autopsy
			February 13, 1935	99.0	27.9	23.6	2.4	133.8	4.2	4.7	144.7	152.9	−8.2	26	10.9	3.5	7.4	4.6	
			April 24, 1935											36	10.3	4.2	6.1	3.6	
			February 16, 1936	97.6	22.5	20.0	0.9	127.8	3.7	4.1	137.6	141.0	−3.4	45	9.2	3.1	6.1	2.3	
A. T.	58	♂	May 11, 1936	99.5	23.4	19.5	1.7	133.4	4.8	4.9	145.1	149.1	−4.0	37	8.6	3.8	4.8	1.8	Bone marrow biopsy: plasma cell myeloma
P. F.	59	♂	October 29, 1933	113.8*	26.8	18.7	2.8	152.4*	5.1	8.5	167.3	162.1	+5.2	125	9.9	2.9	7.0		Mg determined: 1.3 m. eq. per liter. Sulfate not determined. Clinical findings summarized elsewhere [(38), Table III, Case 4]
S. E.	61	♂	November 8, 1935	106.2	21.3	13.4	3.8	141.5	5.0	6.8	155.3	144.7	+10.6	83	5.5	4.1	1.6	0.2	Diagnosis proven at autopsy
			February 17, 1936	114.6	18.6	11.9	3.4	143.8	5.0	6.7	157.5	148.5	+9.0	80	5.0	3.6	1.9		Sulfate determined: 4.9 m. eq. per liter
			February 24, 1936																
A. C.	47	♂	March 1, 1934	94.1	24.3	16.3	2.0	131.1	3.2	6.5	142.8	136.7	+6.1	65	6.9				Clinical findings summarized elsewhere [(38), Table III, Case 6]
G. D.	40	♂	April 22, 1936	101.5	23.1	19.1	3.3	139.1	4.5	7.1	152.7	147.0	+5.7	73	7.7	5.4	2.3	0.4	Sulfate determined: 4.8 m. eq. per liter. Diagnosis based upon typical x-ray findings and clinical course; Bence Jones proteinuria

* Contamination with NaCl?

Wassermann reaction of the blood was positive in 12 of our 35 cases, the Dmelcos test was positive in 6 patients. Evidence of tuberculosis was noted in only 2 cases, but no systematic investigation in this direction was carried out. Parenteral administration of arsenic, bismuth and gold compounds may affect the level of serum proteins, it is said, but hyperglobulinemia was observed in 24 patients in this series before such treatment was begun.

[4] The term "euglobulin" as used in this paper applies to the protein complex precipitated from serum by a concentration of 1M sodium sulfate, according to Howe's procedure. It is appreciated that the chemical identity of the euglobulin fraction is not established, that the salting-out process does not allow of sharp fractionation and that separation of the several protein fractions probably causes reversible changes in the several protein component systems.

In Case C. K., the marked proteinuria was due to glomerulonephritis, was associated with edema and was probably unrelated to the co-existing lymphogranuloma inguinale. The decrease in serum albumin in these cases would, of course, result in a decrease in protein acid-equivalents and hence of itself tend to make B — A more positive.

Serum electrolyte partitions in multiple myeloma and in a group of miscellaneous diseases

The results of serum electrolyte partitions in 6 cases of multiple myeloma are summarized in Table III.[5] In Cases J. B. and A. T., there was definite hyperproteinemia, due chiefly or solely to an increase in euglobulin. The apparent excess of total determined acids over total base in these cases varied from 3.4 to as much as 8.2 m.eq. per liter. In the remaining cases in this group, the acid-base balance was complicated by co-existing renal insufficiency (indicated by definite nitrogen retention, as noted in Table III) with accumulation of sulfates in the blood. In Cases S. E. and G. D., in which hyperproteinemia was not noted, the amount of sulfate in the blood was determined (Table III). It will be seen that adding the values for sulfate to the sum of total determined acids in these cases still leaves a positive value for B — A. In Case P. F., in which B — A appeared to be positive although the serum protein was 9.9 per cent, the amount of sulfate in the blood associated with the marked nitrogen retention present was not determined. It is not known whether, in this instance, total determined acids plus sulfates exceeded the total base.

Table IV summarizes our results on 11 cases of hyperglobulinemia of diverse and, in part, unknown etiology. The results are, in general, consistent with those obtained in lymphogranuloma inguinale and multiple myeloma, the sum of total determined acids exceeding the total base. In Case E. P., in which the euglobulin fraction was within normal limits, and on one occasion in Case A. S., B — A was positive. In Case J. D., the equivalents of total determined acids equalled approximately the total base.

The diseases represented are so varied as to suggest that the association of marked hyperglobulinemia with a negative B — A is a phenomenon of general significance.

DISCUSSION

The consistency of the association of the discrepancy in acid-base equivalence with hyperglobulinemia is noteworthy since it has been the experience of this laboratory, using the analytical methods previously described, that B — A is negative otherwise only occasionally, either in normal subjects or in disease. As to the cause of

[5] Reports of studies on acid-base balance on 2 cases of multiple myeloma, neither with hyperproteinemia, were found in the literature (39, 40). In both instances very high figures were reported for B — A (37.0, 39.1 m.eq. per liter in one instance, 38 and 39 in the other). Differences in method preclude direct comparison with the results recorded here.

TABLE IV

Miscellaneous cases with hyperglobulinemia. Serum electrolyte partitions, serum protein fractions

Case	Age	Sex	Diagnosis	Cl	HCO_3	Protein	PO_4	Na	K	Ca	Total base	Total acid	B — A	Non-protein nitrogen	Total protein	Albumin	Globulin	Euglobulin
	years			*m.eq. per liter*	*m.eq. per liter*	*m.eq. per liter*	*m.eq. per liter*	*m.eq. per liter*	*m.eq. per liter*	*m.eq. per liter*	*m.eq. per liter*	*m.eq. per liter*	*m.eq. per liter*	*mgm. per 100 cc.*	*grams per 100 cc.*	*grams per 100 cc.*	*grams per 100 cc.*	*grams per 100 cc.*
J. P.	48	♂	Lymphosarcoma (autopsy)	99.8	21.1	25.4	2.3				137.1*	148.6	−11.5	25	12.1	2.9	9.2	
J. C.	50	♂	Undiagnosed	101.6	29.4	19.8	1.8	136.2	4.4	4.5	147.1	152.6	−5.5	28	9.5	2.2	7.3	5.0
														26	9.3	3 4	5.9	
N. L.	48	♂	Undiagnosed	97.5	26.3	19.9	2.3	133.8	4.2	5.6	145.6	146.0	−0.4	29	9.0	3.4	5.6	1.8
B. S.	37	♀	Undiagnosed	105.0	25.4	20.4	1.4	138.5	3.3	4.9	148.7	152.2	−3.5	27	8.9	4.2	4.7	1.4
H. F.	23	♀	Undiagnosed	100.8	25.9	19.6	2.5	137.7	4.5	4.8	149.0	148.8	−0.2	25	8.8	3.5	5.3	
J. H.	63	♀	Lymphosarcoma (biopsy)	97.3	32.7	20.3	2.6	139.5	5.1	5.4	152.0	152.9	−0.9	32	8.7	4.6	4.1	
A. S.	34	♀	Tb. lymphadenitis (biopsy)	100.8	25.8	19.4	2.1	134.9	4.0	5.0	145.9	148.1	−2.2	24	8.5	4.0	4.5	
				106.3	23.3	18.2	1.9	139.4	4.7	5.4	151.5	149.7	+1.8	29	7.9	3.9	4.0	0.9
A. B.	41	♀	Cirrhosis of liver (autopsy)	100.0	29.7	17.9	2.0	136.7	4.4	4.6	147.7	149.6	−1.9	27	8.4	2.4	6.0	2.1
E. P.	38	♀	Undiagnosed	105.3	24.0	18.7	2.2	140.4	4.6	5.5	152.5	150.2	+2.3	25	8.0	4.3	3.7	0.5
M. A.	33	♀	Leprosy (biopsy)	107.1	25.0	17.3	1.9	138.8	4.9	4.9	150.6	151.3	−0.7	29	7.5	3.7	3.8	1.1
J. D.	25	♂	Cirrhosis of liver; jaundice	106.8	24.5	15.5	1.9	137.8	4.3	4.7	148.8	148.7	+0.1	26	7.2	2.2	5.0	1.7

* Total base in this case determined by a modification of Fiske's method.

this discrepancy, we are unable for the present to do more than suggest certain possibilities and indicate which seems to us most probable. Changes in pH of the serum or alteration in the content of organic acid radicles of the blood as possible factors may be excluded at the outset, since our analyses give no indication of such alterations in the blood.

Peters and Man (17) presented evidence for the existence of lipoid-chlorine in serum and were able in this manner to account, in part, for negative values for B — A which they obtain in normal sera. It is possible that the presence of lipoid-bound chlorine might contribute to the apparent excess of total determined acids over total base observed in our cases of hyperglobulinemia. Of interest in this connection is the fact that euglobulin is thought to contain a large, loosely-bound lipoid component. However, none of our cases presented definite hyperchloremia, nor were we able to discern any correlation between the degree of excess of total determined acids over total base and the level of chloride in the blood.

Obviously, negative analytical errors in the estimation of total base (or of sodium where, as in this study, total base is calculated as the sum of determined cations) result in falsely low values for equivalents of base and consequently may cause an apparent excess of total determined acids. In only 3 of our 10 cases of lymphogranuloma inguinale in which B — A was negative (Table II) was the blood sodium below the minimum normal value obtained by the analytical method used (137.0 m.eq. per liter). The low values for blood sodium in these 3 instances were confirmed by repeated analyses.

Consideration of the data in Table II reveals that in addition to the above-mentioned cases in which the blood sodium was below normal, values for total base tend to be lower in our cases in which B — A was negative than in those cases in which B — A was positive. In fact, a fair negative correlation can be made out between total base levels in the blood and the degree of excess of total determined acids over total base. This raises the question as to whether or not the apparent discrepancy in acid-base equivalence noted by us in association with hyperglobulinemia should be attributed to loss of base resulting from disease. This explanation is contrary to the experience of this laboratory in a large number of cases with moderate, or even marked, loss of base occurring in a variety of diseases. With the apparent exception of the cases with hyperglobulinemia presented in this paper, loss of base has been found to be associated almost invariably with a compensatory loss of chloride and bicarbonate so that B — A remains positive irrespective of the level of total base in the blood. It should be pointed out, moreover, that whereas B — A has been found to be negative consistently in cases presenting definite hyperglobulinemia, we rarely find B — A negative in diseases exhibiting a loss in total base, except where there is a concomitant marked increase in serum globulin.

The discrepancy in acid-base equivalence is so constantly associated with hyperglobulinemia, in our experience, as to suggest some relationship between them, particularly since both phenomena are of themselves distinctly uncommon. As already pointed out, the individual electrolyte components of the blood, other than protein, exhibited no obvious deviation from the normal in such cases, except lowering of the sodium and of albumin in some instances. B — A was invariably positive in cases of lymphogranuloma inguinale or multiple myeloma in which the serum globulin was not increased.

The precise nature of the relation between the apparent excess of total determined acids over total base and hyperglobulinemia is uncertain but the authors attach significance to the fact that in no case of lymphogranuloma inguinale did the serum calcium exceed the limits of normal variation, despite increases in serum protein up to 11.2 per cent. In the absence of hyperphosphatemia, or of any evidence for a decrease in ionized calcium, this fact is interpreted to mean that the added euglobulin increment bound no calcium.

It is, of course, hazardous to draw from the capacity of a globulin to bind calcium any deductions concerning its capacity to bind total base. It is suggested, however, that the apparent discrepancy in acid-base equivalence noted by us in association with hyperglobulinemia would be explained if the serum globulin in such cases bound

significantly less base at the pH of the blood than does normal serum globulin. This would lead to erroneously high values for base bound to protein when the factor ordinarily used to calculate base bound to globulin is applied to such sera.

It will be appreciated that the application to sera with definitely increased globulin content of a factor derived from titration curves of normal serum globulin (7) involves the assumption that the base binding capacity of the serum globulin in hyperglobulinemia is identical at the pH of the blood with that of normal serum globulin. The validity of this assumption is open to doubt. As is well known, any appreciable increase in the globulin content of the serum involves a qualitative as well as quantitative change in serum globulin, since the euglobulin fraction almost invariably constitutes most or all of the added increment. Thus whereas in normal serum, euglobulin may comprise as little as 15 per cent or less of the globulin fraction, in multiple myeloma and in certain chronic infections 60 per cent or more of the serum globulin may consist of euglobulin. There is, moreover, evidence that in such pathological sera, the euglobulin may differ in properties (and, presumably, in structure) from normal euglobulin.

The authors are of the opinion that the excess of determined acids over total base encountered in sera with definitely increased globulin content is apparent only and is the result of erroneously high values for protein acid-equivalents. The error is introduced by the application to pathological serum globulin (which usually contains a large proportion of abnormal euglobulin), of a factor for calculating base bound to globulin which was derived from normal serum globulin.

The problems arising in connection with the estimation of *B* protein in *hyper*proteinemia appear, in fact, to be analogous in some ways to those which came to the fore when acid-base balances were first attempted in blood with *decreased* protein content. Since the decrease in serum protein affected chiefly or solely the albumin fraction, and since *B* albumin greatly exceeds *B* globulin at the pH of the blood, errors were introduced in estimating *B* protein by the use of a common factor which was derived from sera with normal albumin: globulin ratios. This source of error was eliminated by determining the albumin and globulin fractions and applying to them separate factors for the estimation of *B* albumin and *B* globulin (7).

The data presented in this paper suggest that a further correction of the factor ordinarily used to calculate *B* globulin is necessary when that factor is applied to sera with markedly increased globulin content; otherwise the values obtained for protein acid-equivalents in such sera are too high and B — A appears to be negative.

CONCLUSIONS

1. The concentration of serum proteins was found to exceed 8.0 per cent in 26 of 35 patients with lymphogranuloma inguinale. The euglobulin and pseudoglobulin I fractions, as determined by Howe's method, were increased.

2. Electrolyte partitions of the sera of 18 patients with lymphogranuloma inguinale revealed an apparent excess of determined acid equivalents over total base in those cases presenting definite hyperglobulinemia. A similar apparent discrepancy in acid-base equivalence was observed in association with hyperglobulinemia due to multiple myeloma and, occasionally, to other causes. The apparent discrepancy in acid-base equivalence was not noted in cases of lymphogranuloma inguinale or of multiple myeloma not presenting definite hyperglobulinemia.

3. The discrepancy in acid-base equivalence is probably the result of erroneously high values for protein acid-equivalents; the error being introduced when the factor ordinarily employed to estimate base bound to globulin is applied to sera with markedly increased globulin content.

4. It is suggested that when the factor now in general use for estimating base bound to globulin is applied to sera with definite hyperglobulinemia, a correction is necessary.

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BIBLIOGRAPHY

1. Williams, R. D., and Gutman, A. B., Hyperproteinemia with reversal of the albumin: globulin ratio in lymphogranuloma inguinale. Proc. Soc. Exper. Biol. and Med., 1936, **34**, 91.
2. Gutman, A. B., and Williams, R. D., Anticomplementary Wassermann reactions associated with hyperproteinemia, particularly in lymphogranuloma inguinale and multiple myeloma. J. Clin. Invest. (Proc.), 1936, **15**, 458.
3. Van Slyke, D. D., and Neill, J. M., The determination of gases in blood and other solutions by vacuum extraction and manometric measurement. I. J. Biol. Chem., 1924, **61**, 523.
4. Wilson, D. W., and Ball, E. G., A study of the estimation of chloride in blood and serum. J. Biol. Chem., 1928, **79**, 221.
5. Kuttner, T., and Lichtenstein, L., Microcolorimetric studies. II. Estimation of phosphorus: Molybdic acid-stannous chloride reagent. J. Biol. Chem., 1930, **86**, 671.
6. Howe, P. E., The use of sodium sulfate as the globulin precipitant in the determination of proteins in the blood—a micro method. J. Biol. Chem., 1921, **49**, 93.
7. Van Slyke, D. D., Hastings, A. B., Hiller, A., and Sendroy, J., Jr., Studies of gas and electrolyte equilibria in blood. XIV. The amounts of alkali bound by serum albumin and globulin. J. Biol. Chem., 1928, **79**, 769.
8. Butler, A. M., and Tuthill, E., An application of the uranyl zinc acetate method for determination of sodium in biological material. J. Biol. Chem., 1931, **93**, 171.
9. Baxter, B., Leland, J., and Kurepkat, M., Unpublished data.
10. Tisdall, F. F., and Kramer, B., Methods for the direct quantitative determination of sodium, calcium and magnesium in urine and stools. J. Biol. Chem., 1921, **48**, 1.
11. Loeb, R. F., Atchley, D. W., Richards, D. W., Jr., Benedict, E. M., and Driscoll, M. E., On the mechanism of nephrotic edema. J. Clin. Invest., 1932, **11**, 621.
12. Clark, E. P., and Collip, J. B., A study of the Tisdall method for the determination of blood serum calcium with a suggested modification. J. Biol. Chem., 1925, **63**, 461.
13. Schmidt, C. L. A., and Greenberg, D. M., Occurrence, transport and regulation of calcium, magnesium and phosphorus in the animal organism. Physiol. Rev., 1935, **15**, 297.
14. Loeb, R. F., and Benedict, E. M., Inorganic sulphates in human blood. J. Clin. Invest., 1927, **4**, 33.
15. Cullen, G. E., and Van Slyke, D. D., Determination of the fibrin, globulin and albumin nitrogen of blood plasma. J. Biol. Chem., 1920, **41**, 587.
16. Frei, W., Eine neue Hautreaktion bei Lymphogranuloma inguinale. Klin. Wchnschr., 1925, **4**, 2148.
17. Peters, J. P., and Man, E. B., Lipoid-chlorine in serum. J. Biol. Chem., 1934, **107**, 23.
18. Peters, J. P., and Eisenman, A. J., The serum proteins in diseases not primarily affecting the cardiovascular system or kidneys. Am. J. M. Sc., 1933, **186**, 808.
19. Berglund, H., Medes, G., Huber, G. C., Longcope, W. T., and Richards, A. N., The Kidney in Health and Disease. Lea and Febiger, Philadelphia, 1935.
20. Rowe, A. H., The albumin and globulin content of human blood serum in health, syphilis, pneumonia and certain other infections, with the bearing of globulin on the Wassermann reaction. Arch. Int. Med., 1916, **18**, 455.
21. Turner, R. H., The pathologic physiology of pellagra. II. The serum albumin and globulin. J. Clin. Invest., 1931, **10**, 71.
22. Nicolau, C. T., Modification hématologique dans le lymphogranulome inguinal bénigne (Maladie de Nicolas et Favre). Bull. et mém. Soc. méd. d. hôp. de Bucarest, 1931, **13**, 45.
23. Sia, R. H. P., and Wu, H., Serum globulin in kala-azar. Chinese M. J., 1921, **35**, 527.
24. Lloyd, R. B., and Paul, S. N., Protein graphs in kala-azar. Indian J. M. Research, 1928, **16**, 529.
25. Frazier, C. N., and Wu, H., Blood serum globulin in leprosy. Am. J. Trop. Med., 1925, **5**, 297.
26. Schlossmann, K., Ueber Gleichgewichtsstörungen der Eiweisskörper im Blutserum Lepröser. Ztschr. f. Immunitätsforsch. u. exper. Therap., 1930, **68**, 154.
27. Chopra, R. N., Mukherjee, S. N., and Sen, B., Studies on protein fractions of blood sera; malarial sera during and after the rigor stage. Indian J. M. Research, 1935, **22**, 571.
28. Chopra, R. N., Mukherjee, S. N., and Rao, S. S., Studies on protein fractions of blood sera; normal and filarial blood sera. Indian J. M. Research, 1934, **22**, 171.
29. Wiener, H. J., and Wiener, R. E., Plasma proteins. Arch. Int. Med., 1930, **46**, 236.
30. Wu, H., A new colorimetric method for the determination of plasma proteins. J. Biol. Chem., 1922, **51**, 33.
31. Lüthy, F., Klinische Untersuchungen über Serumeiweisskörper bei Lungentuberkulose. Schweiz. med. Wchnschr., 1927, **57**, 993.
32. de Michelis, U., and Olivetti, R., Rilievi sulla reazione di Takata-Ara nella tuberculosi polmonare in rapporto al comportamento dei proteidi serici. Minerva med., 1935, **1**, 214.
33. Aldred-Brown, G. R. P., and Munro, J. M. H., The

plasma proteins and non-protein nitrogen, and the sedimentation rate, in chronic rheumatic disorders. Quart. J. Med., 1935, **4**, 269.

34. Davis, J. S., Jr., Protein studies in atrophic (rheumatoid) and hypertrophic arthritis. J. Lab. and Clin. Med., 1936, **21**, 478.
35. Perlzweig, W. A., Delrue, G., and Geschickter, C., Hyperproteinemia associated with multiple myelomas. J. A. M. A., 1928, **90**, 755.
36. Von Haam, E., and Lichtenstein, L., Incidence and clinical manifestations of lymphogranuloma inguinale in New Orleans. New Orleans M. and S. J., 1935, **88**, 92.
37. Gray, S. H., Hunt, G. A., Wheeler, P., and Blache, J. O., Lymphogranuloma inguinale: Its incidence in St. Louis. J. A. M. A., 1936, **106**, 919.
38. Gutman, A. B., Tyson, T. L., and Gutman, E. B., Serum calcium, inorganic phosphorus and phosphatase activity in hyperparathyroidism, Paget's disease, multiple myeloma and neoplastic disease of the bones. Arch. Int. Med., 1936, **57**, 379.
39. Bulger, H. A., Dixon, H. H., and Barr, D. P., The functional pathology of hyperparathyroidism. J. Clin. Invest., 1930, **9**, 143.
40. Mainzer, F., Bence-Jonessche Proteinurie und Nierenfunktion. Ztschr. f. klin. Med., 1932, **119**, 363.