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Hemoglobin-Reactive Substance in Human Serum

Under the title, "Demonstration of hemoglobin-reactive substance in human serum," a paper has been published by A. H. Tuttle (*1*) reporting on a phenomenon that we also had noticed when we were studying the influence of hemolysis on quantitative paper electrophoresis of human serum (*2*). We then identified this "hemoglobin-reactive substance" as haptoglobin, a well-characterized protein of the α_2-globulin group on which much work has been done, mostly by European investigators (*3*).

The observation by Tuttle that no complex formation occurs in the serum of the newborn is very interesting and substantiates our conclusion, for only traces of haptoglobin can be demonstrated in such serum. I may also add that the electrophoretic mobility of this complex is slightly inferior to that of α_2-globulin. If it is not taken into account, the phenomenon described may be a major source of error when even slightly hemolytic human serums are submitted to paper electrophoresis (*4*).

ROGER J. WIEME
Department of Internal Medicine, University of Ghent, Ghent, Belgium

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Reports published by M. F. Jayle *et al.* (*1*) and by R. J. Wieme (*2*) brought attention to the fact that the presence of small amounts of hemoglobin in serum being studied by electrophoresis may result in significant errors in quantitative determinations of the α_2- as well as the β-globulin fractions. Their experiments indicated that errors occurred as a result of complex formation between hemoglobin and an α_2 globulin, probably haptoglobin. Similar independent studies that were carried out in our laboratory during this time have been published (*3*); they confirm these observations.

Ordinarily, this important source of error in quantitative paper electrophoresis of serums has not been taken into account, perhaps because of the fact that the work of these European investigators is not generally known. Unfortunately, I was not aware of these reports at the time the results of my experiments were published, and accordingly, appropriate references were inadvertently omitted. It is the purpose of this communication to call attention to the experiments reported by Jayle and by Wieme.

A. H. TUTTLE
Division of Pediatrics, University of Tennessee, Memphis

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Serum Components in the Newborn

Previous investigations were concerned with the patterns of distribution of serum proteins (*1*), lipoproteins (*2*), and glycoproteins (*3*) in normal adult human beings by means of paper electrophoresis. The present study extends these observations to the newborn infant (*4*).

Umbilical cord blood samples were obtained from ten infants at birth. The serum was analyzed by paper electrophoresis, parallel paper strips being stained simultaneously for proteins (Amidoschwarz *2, 5*), lipoproteins (Oil Red O, *2, 6*), and glycoproteins (Periodic acid Schiff, *3, 7*). Chemical analyses performed included estimation of serum total protein, albumin and globulin (biuret method), total and esterified cholesterol (Sperry-Schoenheimer method *8*), protein-bound polysaccharide (*9*), and hexosamine (*10, 11*).

Quantitative differences between newborn infants and adults were observed in all categories studied (Tables 1 and 2). The serum protein pattern of newborn infants was characterized by lower values of albumin and elevation of gamma globulin. The lipoprotein profile of the newborn differed from that of the adult in relative decrease in beta-lipoprotein and increase of the O-fraction (lipid not migrating in the electric field), while the proportion of alpha-lipoprotein was unchanged. Glycoproteins in cord blood stained less intensely than they do in adult blood; there was relatively more beta-glycoprotein and less alpha-2 glycoprotein. Similarly low values for the newborn were obtained in chemical estimation of serum total and esterified cholesterol, polysaccharides, and hexosamine in cord blood. Rough correlation was observed between the total area of glycoprotein stained, measured by the planimeter, and the levels of total serum polysaccharide or hexosamine that were determined chemically. Total protein was somewhat low, but there was no alteration of the albumin-globulin ratio as determined by the usual salting-out technique.

A newborn infant of a mother with familial hypercholesteremia (xanthoma tendinosum, xanthelasma, coronary heart disease) was subjected to a similar investigation. No significant difference from the pattern of normal infants was observed in the distribution of proteins, lipoproteins, or glycoproteins. It was noted, however, that the lipid bands stained with greater intensity. The serum total cholesterol level was elevated, in

Table 1. Electrophoretic patterns of proteins, lipiproteins, and glycoproteins of newborn infants compared with those of adults. The figures give the percentage of total stainable material. Proteins stained with Amidoschwarz, lipoproteins with Oil Red O, and glycoproteins with Periodic acid Schiff.

Measurement	Proteins					Lipoproteins			Glycoproteins		
	Albumin	α-1-globulin	α-2-globulin	β-globulin	γ-globulin	α	β	O-fraction	α-1	α-2	β
Cord blood											
Mean	41.3	7.1	11.6	12.6	27.4	36.9	39.9	23.2	30.4	52.1	17.5
S.D.	5.6	2.1	2.6	4.0	4.4	6.6	5.2	9.4	4.1	3.4	4.7
Adult											
Mean	52.5	4.2	12.2	14.0	17.1	35.3	52.3	12.3	28.8	41.6	29.6
S.D.	3.7	1.5	3.6	2.9	3.0	6.7	7.2	3.8	3.8	3.6	3.1
Statistical significance of differences of mean values between newborns and adults											
p	0.0005				< 0.0001		0.0005	0.0035		< 0.0001	< 0.0001

Table 2. Chemical analysis of serum components of newborn infants compared with analysis of those of adults.

Measurement	Total protein (g%)	Albumin (g%)	Globulins (g%)	Total cholesterol (mg%)	Esterified cholesterol (mg%)	Total polysaccharides (mg%)	Hexosamine (mg%)
Cord blood							
Mean	6.2	4.1	2.1	79.4	58.6	69.3	74.6
S.D.	.7	.2	.3	15.2	10.3	9.8	11.1
Adult							
Mean				211	157	103 (*14*)	93 (*10*)
S.D.				31	25		