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Immunochemical Quantitation of Serum β_{1A} -Globulin in Health and Disease

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The β_{1A} globulin concentration was quantitatively determined in the sera of healthy subjects of different age and sex, as well as in the sera of patients with various diseases, using single radial immunodiffusion on cellulose acetate strips.

Very low levels were found in the cord blood, but a significant increase was observed during the first days of life. Lower values were found in women if compared with men or children.

Six out of seven cases of acute nephritis had low levels of this protein. A reduction of β_{1A} globulin concentration was also present in sera of patients with liver cirrhosis (7 out of 14 cases).

Unter Anwendung der radialen Immundiffusion auf Celluloseacetat wurde die Konzentration des β_{1A} Globulins im Serum von Normalpersonen verschiedenen Alters und beiderlei Geschlechts und Patienten mit diversen Krankheitsformen quantitativ untersucht. Die Konzentration dieses Proteins war sehr niedrig im Nabelschnurblut, aber schon in den ersten Lebenstagen signifikant erhöht. Frauen zeigten tiefere Werte als Kinder und Männer. Die Konzentration dieses Proteins war im Laufe von akuter Nephritis und in 7 von 14 Leberzirrhosefällen erniedrigt.

Since a drop in serum concentration of the complement component C'_{3A} (β_{1C} - β_{1A} globulin) has been found to occur in children with glomerulonephritis, much interest has emerged in studies on this protein (1—5). However, the concentration of β_{1A} globulin has been determined semiquantitatively: quantitative data have been presented only recently by SANDOR and Coworkers (6).

The present investigation was undertaken to study the influence of age and sex on the serum content of β_{1A} globulin in normal subjects, and its modifications in pathological states.

Material and Method

The concentration of β_{1A} globulin was determined in 218 subjects. To avoid the presence of antigens with different molecular weights, the sera were studied only after the β_{1C} globulin had been completely converted in to β_{1A} globulin (i. e. by incubation at 37° C for 3 days) (Fig. 1).

Quantitative determinations were carried out by single radial immunodiffusion on cellulose acetate. The method has been described elsewhere (7). The specific anti- β_{1C}/β_{1A} antiserum

Fig. 1

Immunoelectrophoresis of healthy human serum with anti- β_{1A}/β_{1C} rabbit antiserum (above = native serum; below = after 3 days at 37° C). The fresh serum shows a double arc corresponding to the β_{1C} and β_{1A} globulins; the aged serum shows the single β_{1A} arc

(Behringwerke, Marburg/Lahn, Germany) was diluted 1:10. Strips of cellulose acetate 3.9 × 31.5 cm (Schleicher & Schüll, Dassel Germany) were used: 50 sample determinations can be carried out on each strip (Fig. 2). The linear relation existing between the β_{1A} globulin concentration and the area of the precipitate at the end of the diffusion is illustrated in figure 3. Calibration curves were obtained using a standard human serum with a known concentration of β_{1A} globulin (Standard Human Serum, Behringwerke Op. Nr. 166).

Fig. 2

Appearance of β_{1A} globulin precipitin rings after immunodiffusion on cellulose strip (staining with Ponceau S and Nigrosine W. S.)

Fig. 3

Relation between serum β_{1A} globulin concentration and area of precipitate

Results

The serum concentration of β_{1A} globulin in different physiological states are summarized in table 1.

Low levels were found at birth in the cord blood, but a significant increase was observed in the newborns as early as the fourth day of life. The highest levels were found in children from 2 to 9 years old. Similar values were observed in adult men, but a significant decrease was present in women, both in the menstruating and

Tab. 2
 β_{1A} -globulin concentrations in the sera of patients with various diseases

Disease	No. of cases	Mean (mg/100 ml)	Range
Acute nephritis	7	39.4	9-77
Chronic nephritis	4	84.0	66-100
Nephrosis	6	111.8	84-139
Acute hepatitis	7	92.2	60-110
Cirrhosis	14	70.4	29-123
Rheumatic fever	15	102.3	52-191
Collagen disease	11	99.6	50-134
Paraproteinemia	15	98.0	46-141
Allergic disease	4	97.0	83-123
Acute myeloid leukemia	5	118.8	72-173
Chronic lymphocytic leukemia	5	91.4	77-114
HODGKIN's disease	4	129.0	97-192
Lymphosarcoma	1		118

post-menopausal group. Significant differences were also found between menstruating women and men, but not between post-menopausal women and men.

The serum levels of β_{1A} globulin in various pathological states are reported in table 2. Very low values were present in 6 out of 7 cases of acute nephritis. Of 14 patients with hepatic cirrhosis, 7 had persistently low levels of serum β_{1A} globulin. High values were observed in 2 out of 4 patients with HODGKIN's disease and in 1 out of 5 cases of acute myeloid leukemia.

A large variability of data was observed in rheumatic fever and in collagen diseases. Normal values were present in chronic nephritis, nephrosis, acute hepatitis,

Tab. 1
Serum β_{1A} globulin levels in different physiological conditions

	Men	Premenopausal women	Postmenopausal women	Children	Newborns (4th day)	Cord blood
Mean (mg/100 ml)	95.2	85.6	93.3	100.6	81.7	61.2
S. D.	15.50	18.41	23.22	17.61	6.79	13.12
S. E.	3.46	4.12	5.19	3.94	1.52	2.93

Analysis of variance

Source of variation	Degrees of freedom	Sum of squares	Mean square	F	P
Between groups	5	19655.2	3931	14.3	< 0.01
Within groups	114	31284.8	274.4		
Total	119	50940			

Tukey's Test

Groups	No. of cases	Mean	VS Cord blood	VS New borns	VS Premeno-pausal women	VS Postmeno-pausal women	VS Men
Children	20	100.6	S	S	S	S	NS
Men	20	95.2	S	S	S	NS	
Postmenopausal women	20	93.3	S	S	S		
Premenopausal women	20	85.6	S	NS			
Newborns (4th day)	20	81.7	S				
Cord blood	20	61.2					

S = P < 0.05 NS = P > 0.05

allergic diseases, paraproteinaemia, lymphatic leukemia and lymphosarcoma.

Discussion

β_{1A} globulin is detectable in all sera from foetus more than 15 weeks old (8). The in vitro synthesis of β_{1A} globulin was observed using liver obtained from foetus more than 15 weeks of age (9). However the present quantitative results indicate that at birth the serum levels of this protein are still low and increase very rapidly during the first days of life.

Estrogens seem to influence the β_{1A} levels as its values in menstruating women are clearly lower than that of postmenopausal women, adult men and children.

According to the semiquantitative data of WEST and Coworkers (1) and GOTOFF and Coworkers (2), low

levels were observed in acute nephritis and normal values in chronic nephritis and lipoid nephrosis.

Since this protein is synthesized outside the liver by mesenchymal tissues, the low values observed in 50% of cases of liver cirrhosis, especially in severe cases, are unlikely to be ascribed to the reduction of liver function. At present it is difficult to assess whether the low levels of serum β_{1A} globulin in liver cirrhosis, as in acute nephritis (10), are a consequence of in vivo breakdown and/or deposition on immune complexes and/or failure of formation.

The possibility of an increase of serum β_{1A} globulin could also be mentioned: as previously reported by SANDOR and Coworkers (6), this was found in cases of severe infection diseases or in tumor cases.

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Gaschromatographische Untersuchungen von Phenolsäuren im Urin

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Es wird über eine gaschromatographische Methode zur Trennung und quantitativen Bestimmung von Phenolsäuren berichtet. Hierzu werden die Phenolsäuren zuvor in die Methyl ester-Methyläther bzw. Trimethylsilyl ester-Trimethylsilyl äther umgewandelt. Die Methode ist zur Messung der Phenolsäuren im Urin geeignet.

Es werden zunächst die Ausscheidungen von *m*- und *p*-Hydroxyphenyllessigsäure bei Normalpersonen und bei Patienten mit Leberinsuffizienz bestimmt.

Ursachen der erhöhten Ausscheidung von *p*-Hydroxyphenyllessigsäure bei Leberinsuffizienz werden diskutiert.

The study of urinary phenolic acids by gas chromatography.

A gas chromatographic method is reported for the separation and quantitative determination of phenolic acids as their methyl ester-ethers, or their trimethylsilyl ester-ethers. The method is suitable for the determination of phenolic acids in urine.

The method was used to measure the excretion of *m*- and *p*-hydroxyphenylacetic acid in normal persons and in patients with liver insufficiency.

Reasons are discussed for the increased excretion of *p*-hydroxyphenylacetic acid in liver insufficiency.

Im Urin wird eine Vielzahl von Phenolsäuren ausgeschieden. Nach den papierchromatographischen Untersuchungen von ARMSTRONG (1), ROBINSON (2), SMITH (3) sowie HARTMANN und Mitarbeitern (4–6) sind etwa 40 Phenolsäuren, zum Teil in sehr geringer Konzentration zu finden.

Eine gleichzeitige quantitative Bestimmung ist mit papierchromatographischen sowie dünnschichtchroma-

tographischen Methoden nicht möglich, da sich auch bei 2-dimensionalem Lauf mehrere Substanzflecke überlagern.

Wir haben deshalb eine gaschromatographische Methode entwickelt, die es erlaubt, die wichtigsten Phenolsäuren voneinander getrennt zu erfassen und ihre Ausscheidung quantitativ zu messen. Methodische Hinweise erhielten wir aus den Arbeiten von WILLIAMS und SWEETLEY (7, 8),