DYNAMICS OF ANTIBODY FORMATION AND FRACTIONAL COMPOSITION OF BLOOD SERUM GLUCOPROTEINS IN IMMUNIZATION WITH TISSUE ANTIGENS

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According to data in the literature [1, 2] certain protein fractions of the blood serum connected with the body's protective function, as for example the γ-globulins, contain a large amount of carbohydrate components (fructose, neuramino acid and others) which play an important role in the body's metabolic processes.

In the literature available we did not find data concerning the study of carbohydrates which are linked with proteins in the blood serum during antibody production. There are only isolated indications of a possible change in the serum glucoproteins of rabbits immunized with Candida albicans [3].

Moreover, a study of this problem, in our opinion, is of definite interest since it may broaden the concept of the nature of the shifts which occur in the body during antibody production.

In the present work the fractional composition of the blood serum glucoproteins at different times after immunization with tissue antigens was studied. At the same time the titer of the complement fixing and precipitin antibodies of the antisera under investigation was determined.

EXPERIMENTAL METHOD

Antisera was obtained from 13 healthy rabbits (males) weighing 3-3.5 kg. Three preparations of human aorta tissue were used as the immunizing antigen. Immunization was carried out by intraperitoneal injection of 2 ml of a homogenized suspension of aorta tissue in physiological solution in a ratio of 1 :10 every day for 8 days. 10-12 ml of blood was removed from the ear vein beginning with the third day and ending on the 70th day after immunization with intervals of 7-10 days.

The investigation of the blood serum glucoproteins was done by the horizontal electrophoresis method on Wattman 1 paper in veronal buffer (pH 8.6) for 18-19 hours at a voltage of 175 V. The glucoprotein fractions were separated under the control of the distribution of blood serum protein fractions obtained on proteinograms. The glucoprotein fractions were identified using the specific RAS-color with Shiff's reagent [4]; the phoregrams were developed by the method of Laurell and Skoog [5]. The protein fractions were stained with bromphenol blue with subsequent elution with 0.02 M NaOH. The composition of the fractions was determined photometrically.

The titer of the complement fixing antibodies was determined in the complement fixation test from the 50% titer, the precipitin antibodies by the ring precipitation reaction. Water-salt extracts of human aorta tissue in a ratio of 1 :10 was used as the test antigen.

Blood Serum Glucoprotein Fractions in Rabbits Normally and after Immunization with Human Tissues (in %, M ± m)

Fraction		Amount of glucoproteins				
		normally	After immunization			
			8th day	14th day	21st day	28th day
Albumins		7,48±1,18	6,23±0,98 $P>0,1$	6,9±0,96 $P>0,1$	4,7±0,38 $P<0,05$	5,5±0,55 $P>0,1$
Globulins	α_1	23,78±0,74	28,03±2,87 $P>0,1$	24,7±2,11 $P>0,1$	29,1±2,01 $P<0,05$	27,0±2,53 $P>0,1$
	α_2	30,94±1,14	30,54±2,10 $P>0,1$	41,3±0,41 $P<0,001$	33,0±1,67 $P>0,1$	30,0±0,39 $P>0,1$
	β	19,78±1,13	15,34±1,78 $P>0,05$	15,9±1,12 $P<0,05$	17,7±2,07 $P>0,1$	15,1±1,9 $P>0,05$
	γ	17,16±0,94	16,58±2,84 $P>0,1$	17,3±1,77 $P>0,1$	16,4±1,9 $P>0,1$	11,8±1,8 $P<0,05$

EXPERIMENTAL RESULTS

An examination of the sera in the complement fixation test over the course of 68-70 days showed that the amount of complement fixing antibodies increased on the 8th-14th day (titer 1 : 160-1 : 640) and then gradually decreased by the 21st-28th day after the conclusion of immunization.

The maximum precipitin titer was noted also on the 8th-14th day (1 : 320-1 : 2560), a decrease in the titer was observed on the 28th-38th day of study.

An investigation of the glucoproteins and protein fractions in the animals' blood serum gave the following results.

Five glucoprotein fractions combined with the corresponding protein fractions—albumins and α_1, α_2, β, and γ-globulins were found in the blood serum of nonimmunized animals (10 rabbits). Only in certain cases did we not succeed in noting a sharp boundary line between α_1 and α_2-glucoproteins, whereas in of the corresponding protein fractions the absence of separation was observed more frequently.

In the table data is presented on the amount of glucoproteins in the blood serum over the course of 28 days after immunization.

Since in later periods of the study (up to the 70th day) we did not find significant changes in the indices being studied the results of these tests are not given in the table.

From an analysis of the data obtained during a study of the immune serum glucoprotein fractions it is seen that the degree of the change in the percent composition of the individual fractions proved to be different. Thus, we could not find significant changes in the glucoproteins entering into the make-up of the albumins. The data obtained on the 21st day was an exception—the amount of glucoproteins entering into the composition of the albumins was increased.

A significant increase in the glucoprotein α_1-fractions of the immune sera was noted on the 21st day of the study ($R < 0.05$).

A considerable increase in the glucoprotein α_2-fractions was found in antisera obtained on the 14th day ($R < 0.001$). In the remaining periods the amount of the glucoproteins α_2-fractions was within the normal limits.

For the glucoprotein β-fractions the greatest decrease was observed on the 14th ($R < 0.05$), and for the γ-fractions—on the 28th day of the study ($R < 0.05$).

In the parallel determination of the blood serum protein fractions it was also shown that the greatest changes in the indices being studied were observed in the first 28 days after immunization was stopped.

The average content of the albumin fractions on the 8th, 14th, 21st and 28th day was respectively 51, 51.1, 54.99 and 40.88% with a norm of 56.07%.

The γ-globulins content during the same periods of investigation was respectively 19.02, 18.20, 17.89 and 20.41% with a norm of 14.76%.

The percentage content of the α_1, α_2 and β-protein fractions of immune sera hardly varied from the norm.

Thus, from the data presented it follows that in anti-tissue immune sera the titer of the complement fixing and precipitin antibodies reaches a maximum on the 8th-14th day. A decrease in the complement fixing antibody titer occurs on the 21st-28th day, the precipitin—on the 28th-38th day of study.

The greatest changes in the content of the serum glucoproteins and protein fractions were observed in the first 28 days after immunization.

A comparative analysis of the content of the serum glucoprotein and protein fractions showed that in all cases the degree of their dislocation was the same, which, evidently, to a certain degree is caused by a change in the quantitative relationship and connection between the protein and carbohydrate part which enter into the composition of the glucoproteins of the immune sera.

On the basis of this data it is possible to accept the hypothesis on the implication of glucoproteins in the body's immunological reaction to intraperitoneal immunization.

LITERATURE CITED

1. E. L. Rozenfel'd, In the book: The Chemical Bases of Vital Processes [in Russian], Moscow, (1962), p. 50.
2. M. Bichter, H. Blumer, F. Cua-Lim, et al., Canad. J. Biochem., 40 (1962), p. 105.
3. J. Biguet, P. Tran Van-Ky, R. Havez, et al., Ann. Inst. Pasteur, 102, (1962), p. 328.
4. E. Köiw and A. Grönwall, Scand. J. clin. Lab. Invest., 4 (1952), p. 244.
5. C. B. Laurell and N. Skoog, Ibid., 8, (1956), p. 21.