BIOCHEMISTRY AND BIOPHYSICS

THE HALF-LIFE OF THE DIFFERENT SERUM PROTEIN FRACTIONS IN THE CIRCULATING BLOOD

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The possibility of artificially labeling protein preparations, the natural serum proteins in particular, permits a study of the duration of life of the different protein fractions and thus, to judge the intensity of the processes of their synthesis and destruction.

Certain researchers, using protein preparations labeled with I^{131}, determined the half-life of albumin and γ-globulin in humans and in a number of animals [6, 7, 8]. On the basis of these data it was computed that in the rabbit, for example, 0.6 g of serum albumin and globulins is synthesized and destroyed daily [9].

The utilization of different protein preparations, however, does not permit one to answer the question of the duration of life of all the protein fractions of blood serum; furthermore, preparative separation of the protein fractions may tell on the properties of the proteins. Natural homologous serum proteins were therefore employed.

The aim of our work was to determine the half-lives of the different serum protein fractions, labeled with I^{131} and S^{35}, in the circulating blood of rabbits.

Intravenously injected serum proteins are cleared from the blood stream according to a double exponential curve [10, 13, 15, 16]. The first part of the curve characterizes the process of establishing a dynamic equilibrium between the proteins of the blood and the proteins of the extracellular fluid, while the second phase is dependent upon the processes of metabolism and the destruction of the serum proteins. According to our data, the time of half-clearance of the protein fractions labeled with both I^{131} and S^{35} from the vascular system of the rabbit in the first distributive phase equals, on the average, $3\frac{1}{2}$-5 hours (the first exponential portion of the clearance curve). After 24 hours the reduction in the protein content of the blood is retarded and depends upon processes of their destruction. We therefore carried out the determination of the duration of the half-life of the different serum protein fractions of the blood in the second stage of their clearance from the blood stream (the second exponential portion of the curve), which begins a day after intravenous injection of the labeled serum proteins.

EXPERIMENTAL METHODS

Two series of experiments were conducted: the first on 5, the second on 4 rabbits of different color and sex weighing $1.77\sigma \pm 0.22$ kg. In the first series of experiments, serum from the blood of donor rabbits (8-15 ml) labeled with I^{131} outside of the organisms was injected into the experimental rabbits; in the second series the serum of donor rabbits (15-20 ml) labeled within the organisms with S^{35} was used. Following a single injection of the labeled serum, blood was taken from the experimental rabbits every 24 hours for a period of 5 days.

The serum specimens were studied by the method of paper electrophoresis. Applied to a strip of paper was 23.1 ± 0.1 mg of serum. The protein separation took place over a period of $2\frac{1}{2}$ hours using 19 v/cm and 1 mA/4 cm. A barbital buffer was used (pH = 8.6, μ = 0.023). The apparatus is described in the work of E. P. Smolichev [2]. The paper was stained with a solution of bromophenol blue. The stained electrophoregram was cut into smaller strips 0.5 cm wide. Each of these strips was again cut into 3 parts and placed in a standard target (15 mm) under a Type B end counter apparatus, and the radioactivity was computed for a period of 5-10 minutes. According to the results of the computation the average background values were calculated. Then the concentration of the dye eluted from each strip with 0.01 NaOH was determined in a multistage photometer. Electrophoretic curves were constructed on the basis of the results expressed in units of absorbance. The total radioactivity of the separate protein fractions was obtained by adding the activities of the individual strips which went to make up a fraction. In computing the radioactivity of the protein fractions a correction was made for the value of the radioactive trace [2]. The activity of the whole serum (23.1 mg), which was applied to a standard target, was computed simultaneously.

The experimental results were expressed in the period of half-life of the different labeled protein fractions in the blood stream. Half-life was determined by means of a graph constructed with the semilogarithmic system of coordinates [1].

Iodination of the serum proteins was performed according to the method of Francis, Mulligan and Wormall [11]. In some of the experiments the serum was freed from the uncombined I^{131} by dialysis into an 0.9% NaCl solution, in others by passing the serum through the anionite EDE-10. The radioactivity of the serum after dialysis amounted to 200,000-250,000 counts/min./ml.

In order to obtain S^{35}-labeled serum the donor rabbits were injected with fractional doses (4-5 times) of 1.5-2 mc of methionine containing S^{35}. Twenty-four hours after the first injection of the isotope the rabbits were exsanguinated via the carotid artery and the serum was obtained. About 5% free methionine was contained in the serum. The radioactivity of the S^{35}-labeled serum amounted to 55,000-85,000 counts/min./ml.

The labeled serum specimens were kept in a refrigerator not more than a day and were heated to 36° prior to intravenous injection.

RESULT OF THE EXPERIMENTS

The results of the first series of experiments are listed in Table 1.

TABLE 1
Half-life in the Blood Stream of the Different Serum Protein Fractions Labeled with I^{131} (in days)

No. of the Experiment	Protein fractions					Proteins of whole serum
	Albumin	Globulins				
		α_1	α_2	β	γ	
1	6.2	2.2	2.0	2.5	2.2	4.9
2	3.0	2.8	3.3	2.9	3.5	4.5
3	4.5	3.8	4.4	6.0	4.1	4.5
4	5.3	3.0	3.5	2.2	3.6	3.7
5	5.2	4.1	4.3	4.4	5.0	4.0
M	4.8	3.2	3.5	3.6	3.7	4.3
$\sigma\pm$	1.19	0.77	0.96	1.59	1.02	0.47
$M\pm$	0.53	0.34	0.043	0.71	0.46	0.21

A statistical analysis of Table 1 shows that the half-life in the blood stream of all of the globulin fractions (α_2-, β and γ-globulins) is the same ($P > 0.05$) and equals, on the average, $3\frac{1}{2}$ days. The half-life of albumin is somewhat longer ($P < 0.05$), averaging $4\frac{1}{2}$ days. The mean half-life of the proteins of whole serum amounts to $4\text{-}4\frac{1}{2}$ days.

The results of the determinations in the second series of experiments are shown in Table 2.

TABLE 2

Half-life in the Blood Stream of the Different Serum Protein Fractions Labeled with S^{35} (in days)

No. of the Experiment	Protein fractions					Proteins of whole serum
	Albumin	Globulins				
		α_1	α_2	β	γ	
1	3.9	5.0	2.7	2.8	3.7	4.0
2	4.5	3.4	4.0	4.2	3.8	4.3
3	3.6	4.7	4.4	3.0	3.6	6.0
4	6.0	3.3	3.0	3.8	4.0	5.2
M	4.5	4.1	4.0	3.4	3.8	5.1
$\sigma\pm$	1.07	0.88	0.98	0.66	0.18	0.87
$M\pm$	0.53	0.44	0.48	0.33	0.09	0.44

We admitted the statistical handling of such a small number of experiments in view of the fact that each of the values presented in Table 2 is the average of 5 measurements.

A comparison of the data included in Tables 1 and 2 showed that the half-life of the protein fractions labeled within an organism with S^{35} and outside of an organism with I^{131} is the same and amounts to $4^1/_2$ days for albumin and $3^1/_2$-4 days for the globulins. If it is assumed that the volume of a rabbit's blood adds up to $^1/_{13}$th of its weight, that the hematocrit reading amounts to 41.5% [5], the concentration of protein in the serum to 6.3% g (60.5% albumin and 39.5% globulins [4]), the half-life of albumin to $4^1/_2$ days, that of the globulins to 4 days and that of the proteins of whole serum to $4^1/_2$ days, then about 0.3 g of serum proteins (0.18 g of albumin and 0.12 g of the globulins) per kg of body weight must be synthesized and destroyed daily in the rabbit organism. This agrees with the data of Dixon and Maurer [9].

SUMMARY

Determination of the half-life of the single serum protein fractions in the circulating blood of the rabbit has been carried out by means of electrophoresis on paper combined with radiometry. The half-life of natural serum proteins labeled outside the organism with I^{131} and within the organism with S^{35} has been studied. Both experimental series revealed that the average half-life of all the globulin fractions attained 3.5 to 4.0 days, those of albumin —4.5 days. 0.3 g of serum proteins (0.18 g of albumin and 0.12 g of globulin) per kg of weight are, therefore, daily synthesized and destroyed in the rabbit organism.

LITERATURE CITED

[1] I. A. Olvin, in the book: Transactions of the Stalinabad State Medical Institute, Stalinabad, Vol. 21, pp 195-230 (1956).

[2] E. P. Smolichev, ibid., pp. 237-253.

[3] M. S. Surovikina, ibid., pp. 33-37.

[4] M. S. Surovikina, in the book: Transactions of the Stalinabad Medical Institute, Stalinabad, Vol. 13, pp 85-88 (1954).

[5] E. C. Albritton, Standard Values in Blood, Philadelphia and London, p. 42 (1953).

[6] A. Bauman, M. A. Rothschild, R. S. Yalow and S. A. Berson, J. Clin. Invest., V. 34, pp 1359-1368 (1955).

[7] F. J. Dixon, P. H. Maurer and M. P. Deichmiller, Proc. Soc. Exp. Bio. and Med., V. 83, N. 21, pp 287-288, (1953).

[8] F. J. Dixon, D. W. Talmagl, P. H. Maurer and M. J. Deichmiller, J. Exptl. Med., V. 96, No. 4, pp. 313-318 (1952).

[9] F. J. Dixon and P. H. Maurer, Exptl. Med., V. 101, No. 3, pp. 233-244 (1955).

[10] L. L. Forker, L. L. Chaikoff and W. O. Reinhardt, J. Biol. Chem., V. 197, pp 625-636 (1952).

[11] G. Francis, N. Mulligan and A. Wormall, Nature, V. 167, pp 748-751 (1951).

[12] P. H. Maurer and A. Niklas, in the book: Radioaktive Isotope in Klinik und Forschung, Munchen — Berlin, S. 202-211 (1955).

[13] L. R. Melcher and S. P. Masouredis, J. Immunol. V. 67, pp 393-402 (1951).

[14] A. Niklas, W. Maurer and H. Krause, Biochem. Ztschr. Bd. 325, S. 464-476 (1954).

[15] K. Wasserman and H. S. Mayerson, Amer. J. Physiol. V. 165, pp 15-26 (1951).

* In Russian.