A COMPARISON OF THE PROCESSES OF FORMATION OF ANTIBODIES AND NON SPECIFIC PROTEINS IN ANIMALS

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In the literature data are often encountered which attest that the assimilation of protein in the body can follow various pathways. Breakdown of introduced proteins is not essential for their utilization. Assimilation of proteins in the body can evidently take place at various stages of protein breakdown and during synthesis of protein of organs and tissues structural units of various sizes may be incorporated [11, 10, 4, 1].

The question of what specific protein antibodies are synthesized from in the body is an interesting one. At the present time there hardly exists any doubt that serum proteins do not serve as direct precursors of antibodies [2, 8].

However, whether antibodies utilize for their formation amino acids only, as several authors propose [8], or appear as derivatives of certain intermediate proteins formed in the body, has thus far not been ascertained.

The fact, discovered by Schoenheimer and coworkers [9], that like other serum and tissue proteins, antibodies take part in metabolic reactions and utilize dietary nitrogen, allows one to suppose that the pathway of antibody synthesis does not differ essentially from the pathway of synthesis of any non specific protein.

At the same time, the formation of antibodies in the body differs in the peculiar dynamics of their synthesis, and also in the fact that a protein is being formed with a sharply defined specificity.

In a previous paper we called attention to the fact that the sharp acceleration of production of specific antibody protein which follows revaccination is accompanied by an activation of the synthesis of non specific serum proteins [3].

By comparing the processes of formation of antibody and ordinary serum proteins, we expected to elucidate to a significant degree the question whether antibodies are synthesized only from amino acids as structural units, or whether for their formation, proteins and peptides may be utilized directly without preliminary breakdown into amino acids, as the synthesis of tissue proteins proceeds, which was shown by a series of the above mentioned investigators. Along with this we attempted to ascertain whether there is any connection between the capacity to form antibodies, i.e., the extent of synthesis of specific proteins and the extent of synthesis of non specific proteins, particularly serum proteins.

The investigations were carried out on various groups of immune rabbits, which along with methionine labelled with S^{35} were injected with labelled liver and serum proteins. The extent of synthesis was estimated from the radioactive amino acid content of the antibody and serum proteins.

METHODS

The proteins of a tissue homogenate and of serum were assayed for radioactive S^{35} methionine by the method described in a previous paper [1].

We immunized the rabbits with typhoid vaccine.

We carried out the experiment on 3 groups of immunized rabbits. Into animals of the first group we injected S^{35} methionine intraperitoneally according to the calculation of 1 g of weight from 4,000 to 30,000 impulses. Similarly we injected rabbits of the second group intraperitoneally with S^{35} methionine labelled liver homogenate (from 1500 to 10,000 impulses per 1 g in a volume of 25 cc), and injected the rabbits of the third group with labelled serum (from 500 to 4000 impulses per 1 g in a volume of 10 – 15 cc).

In order to equalize the period of absorption of the injected materials, as much as possible, we divided the dose of serum into two parts and injected it on 2 successive days; the liver homogenate was injected as a single dose.

We took blood from all rabbits on the 1st, 3rd, 5th, 8th and 12th day after simultaneous injection of the antigen and labelled material. We isolated the antibodies of the serum by precipitation with "diagnosticum" on the 3rd, 5th, 8th and 12th day.

After the introduction of the antibodies we precipitated the serum proteins with trichloracetic acid, and washed free of radioactivity in the usual way; we washed the antibody 5 times with cold physiological saline. Then we dried this and other samples with ether and ground them to a powder. We weighed out samples of 2 mg. We counted the radioactivity, in the antibody and the serum proteins, on aluminum disks with an end-window counter. The squared deviations of the individual determinations did not exceed 5%.

On the 3rd, 5th, 8th and 12th day, instead of a count of the radioactivity of the antibodies, we determined their titer.

In order to find out whether parenterally injected proteins could be utilized in the formation of antibodies without preliminary breakdown into amino acids, we attempted to apply the marker dilution method [7,1,5]. Therefore each of the three groups of rabbits was subdivided into two subgroups. Rabbits of the first subgroup received only the appropriate labelled substrate, but the second subgroup received in addition "ballast" unlabelled methionine.

We considered that an absence of dilution of the radioactivity of the proteins in the rabbits of the second subgroup would show that the incorporation of parenterally injected proteins into the composition of the organs and tissues takes place without preliminary breakdown into amino acids. Dilution of the radioactive marker in the proteins of the organs of the animals would indicate preliminary, profound breakdown of the injected proteins to amino acids and subsequent partial resynthesis with utilization of radioactive and ballast methionine.

However, since the determination of the radioactivity of antibodies and serum proteins was carried out, basically, many hours after the injection of the methionine, during which time it quickly left the body, the method of dilution of the marker proved poorly suited for these investigations (see Table). In addition, the non-uniformity of the results which were obtained in carrying out these experiments is evidently explained by the dependence of the extent of protein synthesis on the individual reaction of the rabbit to the injected antigen.

RESULTS

In the table are presented the average values of the radioactivity content of serum proteins and antibody of rabbits of all three series with and without injection of ballast labelled methionine.

When the data presented in the table and in Fig. 1 are examined one's attention is called to the fact that following the injection of tagged methionine there is observed a low radioactivity content in the antibody and serum proteins. The proteins of rabbits injected with tagged liver homogenate and serum were characterized by a significantly higher radioactivity and it is noted that the antibody scores highest following the injection of the rabbits with tagged liver homogenate, while the serum proteins score highest following injection of serum. Furthermore, as in seen from the table, in all the experiments the same effect was observed – the radioactivity content of the antibody and serum proteins was significantly higher if the radioactive isotope was introduced in the form of tagged protein, rather than in the form of amino acids. This indicates that the process of synthesis of both the non-specific and specific proteins utilizes not only amino acids but also larger structural units of the protein, for if it utilized only amino acids injection of them would caused antibody and serum proteins to show a higher degree of labelling than would the injection of labelled protein.

In spite of the fact that the method of marker dilution, under the conditions of our experiments, did not

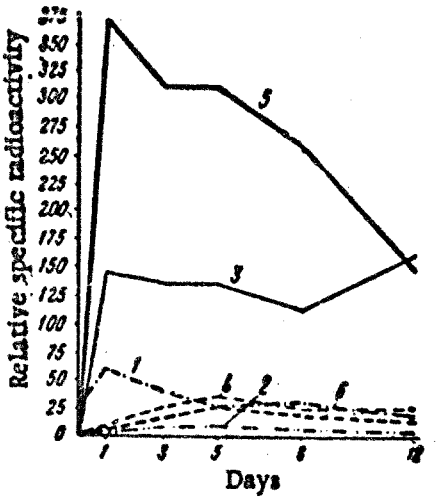

Fig. 1. Average values of radioactivity of antibodies and serum proteins after injection of labelled methionine, liver homogenate, and serum.
1) Serum Proteins + methionine (16 rabbits); 2) Antibodies + methionine; 3) Serum proteins + liver homogenate (14 rabbits); 4) Antibodies + liver homogenate; 5) Serum proteins + labelled serum (6 rabbits); 6) Antibodies + labelled serum.

give the expected results, the data obtained allow the conclusion to be drawn that antibody is not synthesized from amino acids alone; for their formation larger fragments may be utilized, possibly even whole protein molecules.

As was mentioned above, in all rabbits injected with antigen and radioactive material, the course of inclusion of the marker in the antibody is reflected in the curve of increasing titers. In addition, in the rabbits with moderate antibody titers low radioactivity characterized not only the antibody but also the serum proteins. In Fig. 2 there are presented the values for radioactivity content of antibody and serum proteins in rabbits with titers to 1:800 and in rabbits with titers from 1:1600.

On comparing the increase in antibody titer and the content of radioisotope in the non-specific serum proteins after injection of labelled proteins, the relation between the degree of immunogenesis and the degree of labelling of the non-specific proteins is clearly evident. An analogous relation is evident also following the injection of labelled amino acids into rabbits of various immunological reactivity (Fig. 2 B), however in this case it is less clearly expressed, which is perhaps explained by the low radioactivity of the antibodies and proteins, which makes the differences less noticeable.

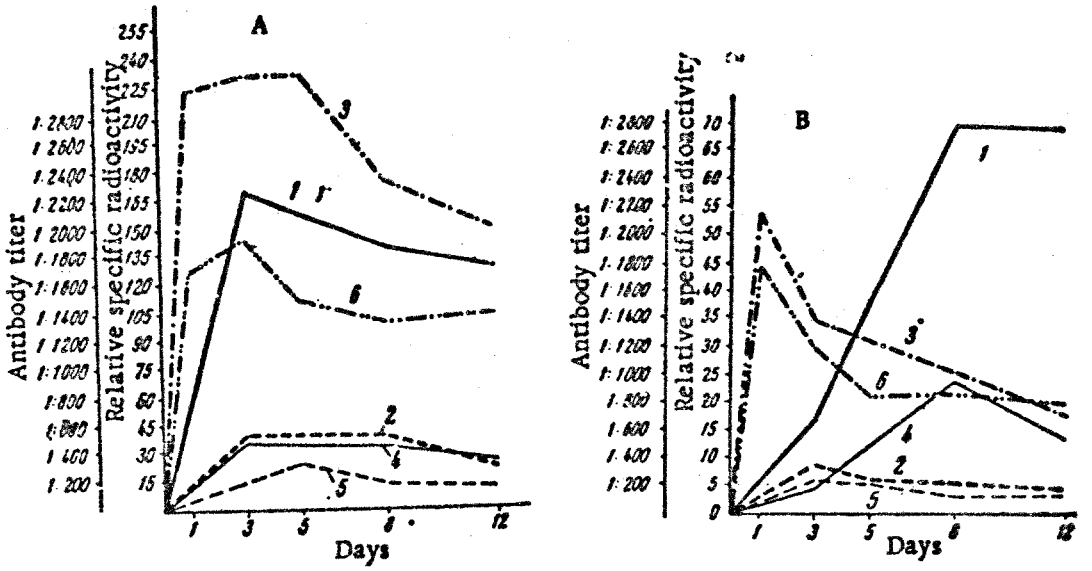

Fig. 2. Relation between antibody titers and radioactivity values of serum proteins and antibodies.
A. Injection of labelled proteins (Group I) 6 rabbits, titers from 1:1600; group II) 4 rabbits, titers to 1:800); B – Injection of labelled amino acid (group I – 7 rabbits, titers from 1:1600, group II) 3 rabbits, titers to 1:800).
1) Antibody titers of group I; 2) radioactivity of antibody of group I; 3) radioactivity of serum proteins of group 1; 4) antibody titer of group II; 5) radioactivity of antibodies of group II; 6) radioactivity of serum proteins of group II.

Radioactivity of Antibody and Serum Proteins Following Injection of S^{35} Methionine, Labelled Liver Homogenates and Labelled Serum, Alone and with "Ballast" Methionine

Material	No. of experiment	Radioactivity of injected material (imp/g)	Rel. Specific activity without ballast methionine										Rel. specific activity with ballast methionine									
				antibody				serum proteins						Antibody				serum proteins				
			no. of animals	3rd day	5th day	8th day	12th day	24 hours	3rd day	5th day	8th day	12th day	no. of animals	3rd day	5th day	8th day	12th day	24 hours	3rd day	5th day	8th day	12th day
S^{35} Methionine	I	10 000	2	24.5	20	8	6	120	85	50	55	44	2	15	13	5	7	110	62	36	31	22
	II	17 000	2	4.5	4	3.5	1	45	27	30	20	16	2	5	2	3.5	2.5	57	34	32	28	20
	III	30 000	2	2.5	2	2	1 5	30	18	13	20	12	2	2 5	2	2	2	31	20	10	12	9
	IV	4 000	2	11	12	14	11	39	35	45	30	14	2	8	18	14	8	52	36	42	33	20
		Average	8	10.5	9.5	7	5	58	41	34	31	22	8	7.5	9	6	5	62	38	30	26	13
Labelled liver homogenate	I	1 500	1	6.5	—	10	—	55	65	35	30	—	1	8	—	5	—	53	55	45	40	--
	II	2 900	2	23	—	—	—	104	110	65	80	—	2	20	—	—	—	150	115	160	160	--
	III	3 175	2	32	40	45	42	217	207	220	132	180	2	27	21	24	20	195	145	142	150	145
	IV	7 634	2	35	39	34	13	177	212	202	180	162	2	42	40	36	24	202	207	207	162	137
		Average	7	24	39	29	27	138	148	131	105	171	7	24	30	22	22	150	131	138	128	141
Labelled serum	I	500	1	25	—	25	25	515	385	—	—	125	1	0	—	10	10	470	280	—	—	170
	II	1 855	1	—	33	30	32	—	—	395	330	180	1	—	30	35	20	—	—	355	350	170
	III	3 110	1	16	28	12	12	195	275	200	150	100	1	25	25	26	13	310	310	305	210	150
		Average	3	20	30	22	23	355	330	297	240	135	3	13	27	23	14	390	295	330	280	160

The immunological reactivity of an animal is evidently closely connected with its individual metabolic rate. F. Dixon and coauthors, studying the survival of antibodies in various species of animals, came to the conclusion that the rapidity of γ-globulin metabolism depends on the rate of metabolism in the species in question [6]. Our data also allow one to conclude that the metabolic rate of an animal may determine its capacity for immunogenesis.

The ability of an organism to make antibody is evidently closely connected with the general level of synthesis of non-specific proteins.

In previous studies we remarked [3] that at the time of revaccination the synthesis of non-specific serum proteins is activated. At the present time there are no tests which can be used to determine the immunological reactivity of an organism before the introduction of the antigen.

The connection between the extent of synthesis of non specific protein in an organism and its capacity to produce antibody, which appears from our experiments, allows one to suppose that the determination by isotopic markers, of the extent of inclusion of structural units of protein in serum proteins may, in more refined studies of the question, serve as an indicator of the immunological reactivity of the subject.

SUMMARY

The utilization of liver and serum proteins and amino acid (S^{35} methionine) in the process of formation of specific (antibodies) and non specific proteins in rabbits was studied.

Thirty-six immunized rabbits were divided into 3 groups. Into the first group S^{35} tagged liver homogenate was introduced simultaneously with the antigen, into the second group S^{35} tagged serumand into the third group S^{35} tagged amino acid.

By comparing the amount and degree of incorporation of S^{35} in the antibodies and serum proteins it was established that in all groups of animals both proteins and protein fragments are utilized in the synthesis of antibodies and non specific serum proteins. Proteins are evidently utilized with greater ease and rapidity. The degree of antibody production is closely connected with extent of synthesis of non specific proteins in the individual animal.

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