SEROLOGICAL ACTIVITY OF TISSUE LIPIDS IN MAN

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The immunological specificity of lipids has been studied little as yet. A number of works have been published [7, 8, 9] in which alcoholic extracts of different organs of various animals have been examined serologically. The authors have proved indirectly that lipids possess a species and organ specificity. Recently, some investigators from other countries have studied the active lipid components isolated from tumor tissue [10, 11, 12]. When rabbits were immunized with the mitochondrial fraction of rat lymphosarcoma, complement-fixing antibodies were formed which demonstrated a relatively high titer against the lipid components of this fraction. The antisera obtained did not react in the complement-fixation reaction (CFT) with lipid haptens from the tissues of rabbits, guinea pigs, mice, humans, or large cattle. The authors concluded from this [13] that lipids possess a species specificity. Ravich-Shcherbo and associates brought forth evidence in a series of papers of the antigenicity of lipids and proved the presence of a species immunological specificity in substances of a lipid nature [5]. The sera of rabbits, which had been immunized with an emulsion of dog cardiolipid in a physiological solution, gave a strong positive reaction in the CFT with dog lipids and a weaker reaction with lipids of other animals (bovine, rabbit) and humans.

The dearth of knowledge about the immunological specificity of lipids no doubt arises from the fact that a study of lipids is difficult because of their marked anticomplementary effects and their insolubility in a physiological solution – the usual medium for all serological reactions. Immunological results of a complete serological investigation of lipids cannot help being influenced by the application of organic solutions – alcohol, ether, acetone, benzene, etc. – in which the lipids are easily dissolved.

The purpose of the present work was a description of several new procedures, which facilitated a serological study of lipids, and a statement of the results of their immunological specificity.

METHODS AND RESULTS

The experimental lipids were extracted with chemically pure ether from normal and malignant tissue from the stomach and spleen of humans. The lipid fractions were dissolved in ether, warm alcohol, and acetone without a sediment, and in water they gave unstable emulsions. The presence of glycerin was demonstrated by a positive acrolein reaction. Serological analyses were done on the free lipids, which were extracted directly from the tissues in a Soxhlet's apparatus, and on the bound lipids, which were extracted with ether and then weakly hydrolyzed with 0.1 N acetic acid. The obtained ether extracts were dehydrated with heated sodium sulfate for removal of all water-soluble substances and were evaporated until dry in a water bath at 40°, after which the dry residues, brought to uniform weight, were dissolved in a specific volume of pure ether.

Portions of Whatman No. 1 chromatographic paper were weighed on a torsion balance (10-15-20 mg) and were saturated with equal quantities of the ether solution of lipids. The ether quickly evaporated, and the lipid-

treated paper was taken as the test antigen for the serological investigation. For serological properties of the paper-fixed lipids we used the quantitative CFT at 50% titer, developed in the Heidelberger laboratory and slightly modified by A.P. Konikov [3]. At the suggestion of V.S. Gostev we applied the "paper" azo-antigen of N.A. Shagynova [6] in the CFT at 50% titer. Squares of paper lipid-antigen were cut into small pieces, and the experimental serum, complement (always in excess), and physiological solution were added to them. The standard hemolytic system was used for the reverse titration of free complement not bound by a specific complex. The degree of hemolysis was determined by an electrophotocolorimeter. The number of free units of complement were calculated according to the formula of Crook:

$$\log X_2 = \log X_1 - n \log \frac{y}{100 - y},$$

where X_2 is the volume of mixture capable of producing 50% hemolysis; X_1 is the volume of experimental suspension; $n = 0.2$ (constant value); y is the percent of hemolysis. The unit of complement was that amount which produced 50% hemolysis under the conditions of the experiment. In order to avoid protracted calculations, a direct graphic relationship was established between the electrophotocolorimetric readings and the number of free units of complement. The difference in the units of free complement in the control and experimental test tubes served as an indicator of the number of units of bound complement taking part in the formation of a specific compound.

In the investigation of the serological activity of human tissue lipids, serum from horse No. 343, which had been immunized with human tissues, was used as an antiserum. A natural lipid substance was taken as a control – butter, which could not react with "antihuman" serum because of a different species specificity. The results demonstrated a specificity: human tissue lipids, fixed on paper, bound complement with "antihuman" serum, while butter, impregnated on paper, did not react with this antiserum.

TABLE 1

CFT at 50% Titer With 15 mg "Paper" Lipid-Antigen (in units of complement)

Date of experiment	Lipid test antigen	Control antigen	Normal horse serum		Horse serum No. 343	
			units of complement			
			free	bound	free	bound
June 19-20, 1956	Saline extract of human spleen	24.2	24.2	0	13.55	10.65
	Butter	24.2	24.2	0	24.2	0
	Control: complement	28.3				
	serum		28.5		24.2	
August 1-2, 1956	Saline extract of human stomach	5.53	5.15	0.38	0	5.53
	Butter	5.43	6.00	0	4.65	0.78
	Control: complement	10.6				
	serum		9.85		9.85	

For further proof of the specificity of the reaction of human tissue lipids and antiserum, another control was set up, namely; the reaction between lipids and normal horse serum, which did not possess an antibody against human tissues. In this case the complement was not bound. The same results were obtained in other CFT with human lipid tissues and horse sera as well as rabbit sera. Hence, human tissue lipids reacted specifically with "antihuman" serum.

In view of the fact that milk lipids, such as the butter, could be completely different according to their serological properties, in further experiments we replaced the butter with lipids, extracted by exactly the same technique as in the first experiments, from stomach and spleen tissues of large cattle. "Antihuman" horse serum

No. 343 and normal horse serum were used as the antisera in these experiments. The findings, presented in Table 2, indicated that human lipid tissues reacted decisively with horse serum No. 343 against human tissue, specifically binding complement to it. But bovine tissue lipids did not react with this serum; neither human lipids nor bovine lipids reacted with normal horse serum not possessing a corresponding antibody.

TABLE 2

CFT at 50% Titer With "Paper" Lipid-Antigens of Humans and Large Cattle and Antihuman Horse Serum No. 343

Species source of lipids	Lipids from saline tissue extracts	Control antigen, units of complement	Normal horse serum		Horse serum No. 343	
			units of complement			
			free	bound	free	bound
Human	Malignant stomach	15.2	15.33	0	11.86	3.34
	Normal stomach	19.6	18.87	0.83	16.17	3.43
	Spleen	19.5	18.9	0.6	17.4	2.1
Large cattle	Normal stomach	10.4	10.28	0.12	10.0	0.4
	Spleen	11.24	12.0	0	11.10	0.14
Control	Complement	19.3				
	Serum		18.13		18.43	

The obtained data give rise to the assumption that there was a species specificity present in lipid fractions. For proof of such a possibility a number of cross-reactions were established with tissue lipids of different animals.

Precipitating sera used in the investigation were obtained from the Scientific Research Institute of Forensic Medicine of the Ministry of Public Health USSR. From Table 3 it is seen that lipids extracted from the stomach tissue of the cow reacted with precipitating anti-bovine serum and did not react with anti-dog serum. On the other hand, lipids from the stomach of the dog bound complement with anti-dog serum and did not react with serum against large cattle. From the results of December 28th and 29th, 1956, it is seen that in the system of tissue lipids of dogs + serum against large cattle there occurred a rather noticeable nonspecific binding of complement (7.62 units), but the specific binding of complement was considerably less (19.3 units).

TABLE 3

Cross-Reactions of CFT at 50% Titer With Tissue Lipids of Various Species Origins

Date of experiment	Species source of lipids	Control, unit of complement	Precipitating serum against tissue			
			dogs		large cattle	
			units of complement			
			free	bound	free	bound
December 26-27, 1956	Dogs	24.5	18.7	5.8	23.5	1.0
	Cow	25.2	23.7	1.5	19.3	5.4
	Control:					
	complement	25.2				
	serum		25.4		24.9	
December 28-29, 1956	Dogs	30.0	10.7	19.3	22.38	7.62
	Cow	30.0	30.0	0	13.68	16.32
	Control:					
	complement	30.0				
	serum		30.0		30.0	

Because of the strong anticomplementary action existing in our system of precipitating sera, we tested the serological properties of tissue lipids by the other quantitative serological reaction, the specific increase

of protein in the "paper" antigen, which was developed in our laboratory by V.S. Gostev. D.G. Grigorian studied the serological properties of desoxyribonucleoproteins in this reaction [2].

The preparation of the "paper" lipid-antigen was the same as in the setting up of the CFT at 50% titer. An accurately weighed square of chromatographic paper with lipids fixed on it was placed on the depressed surface of a concave slide (Maksimov chamber) and 0.3 ml of whole serum was added to it. The "paper" antigen and the treated serum were then incubated at room temperature in a humid chamber for one hour, after which the unstably-fixed proteins were washed off with three changes of a physiological solution during the course of a minute. After washing, the nitrogen content was determined three times by the Conway method. The experiment was always accompanied by standard controls of the reagents, the nitrogen content in the adsorbent – the pure chromatographic paper – and the nitrogen content in the adsorbent impregnated with the experimental lipids and the untreated sera. A little protein was detected in the pure adsorbent, which was probably due to a small amount of protein impurities in the paper. We did not succeed in demonstrating 20 mg lipid fractions by the Conway method. A specific increment of protein was tested in tissue lipids from the stomach and spleen of large cattle, dogs, swine, and man. For antisera we tried precipitating rabbit sera against the tissue of man, dogs, swine, and large cattle, horse antiserum No. 343 against human tissue, and normal horse serum.

We were able to compare quantitatively the protein increment in the lipid system – the homologous serum in the species relationship from the same lipid system with other sera, both normal and that obtained against other animal species.

TABLE 4

Cross-Reactions of Protein Increase in mg, Using Lipid "Paper" Antigens of Unlike Species and Sera

Species origin of tissue lipid	Nitrogen in lipid antigen (in mg)	Adsorbent + lipid + precipitating sera against tissue				Specific protein increase (in %)
		dogs	large cattle	swine	man (serum No. 343)	
Dog	0	0.2100	0.1400	0.0875		50.0
Large cattle	0.0	0.0262	0.1925	0.1137		69.3
Swine	0.00875	0.1400	0.0787	0.2625		87.5
Man	0		0.2887	0.3062	0.5250	71.4

In Table 4 some cross-reactions are presented of the protein increase from dog, swine, bovine and human lipids. From Table 4 it is seen that dog lipids in the complex with serum against dog tissue gave a specific protein increase of 50% in comparison with the nonspecific increase in the system of these same lipids and sera against tissue of man, large cattle, and swine. We observed an analogous picture with bovine, swine, and human lipids – a definite specific protein increase of 71.4%, 87.5%, and 69.3% in the presence of homologous serum in the species relationship.

The given reactions of a specific increase of protein completely corroborated the results obtained in the CFT at 50% titer: lipids fixed on paper gave a definite specific reaction in the presence of homologous serum in the species relationship. Evidently, the ether-soluble fraction of animal tissues is characteristic of the species specificity. Extraction of the tissue "lipids" with ether after acid hydrolysis of the tissue extracts must have led to decomposition of the lipoproteins. We were not able to detect nitrogen in the consumption of the 20 mg lipid fraction, and this indicated that there were no protein impurities in the fraction. However, we could not exclude the possibility that there might be nitrogen present in the experimental fractions in such negligible amounts that we could not determine it by the Conway method. Therefore, in speaking of lipids at the present time, we are referring to the fraction in which there are possibly infinitesimal protein impurities.

A more elaborate clarification and study of the immunological specificity of lipids is the objective of our further investigations, but this does not prevent at the present time a study of the serological properties of the paper-absorbed lipids and other substances insoluble in physiological solution, which are used in the CFT at 50% titer and in the protein-increase reaction.

The "paper" lipid antigen technique is highly sensitive and can serve as a quantitative determination. It permits a comparison of the serological activity of different antigens independent of their anticomplementary

action; in reactions of a specific increase of protein it can utilize sera having a high anticomplementary action; the prerequisites of the designated reactions are more standardized than in the usual classical CFT; an appraisal of results is more objective and possesses a numerical interpretation in units of bound complement or in milligrams of protein.

SUMMARY

A method of serological quantitative reaction was developed with lipid test-antigens fixed on paper, i.e., complement fixation test with 50% titer and reaction of specific addition of protein in "paper" antigen. The serological activity of various tissue lipids (free lipids) isolated directly from the tissues and combined (obtained from the saline extracts after preliminary hydrolysis) was demonstrated by these methods. Tissue lipids of man, dog, swine and large cattle react only with homologous antiserum.

This allows the suggestion that the tissue lipid fraction is species-specific.

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