# SPECIFICITY OF TISSUE POLYSACCHARIDES FROM CARCINOMA OF THE HUMAN STOMACH

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It was shown by the use of Ouchterlony's agar diffusion reaction and by specific absorption that tissue polysaccharides of carcinoma of the human stomach obtained by Westphal's method possess specific differences from the tissue polysaccharides of normal organs.

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Immunologic investigations of malignant tissues from experimental animals and man have demonstrated specific features distinguishing cancer tissue [1, 5, 6 et al.]. The study of the nature of substances responsible for "cancer" specificity is extremely interesting. The writer has previously shown that tissue polysaccharides are serologically active compounds, and that tissue polysaccharides from gastric carcinoma have specific differences from the polysaccharides of the normal human stomach and of human embryonic tissue.

The object of this investigation was to compare the serologic properties of tissue polysaccharides of human gastric carcinoma and normal human organs (stomach, spleen, liver, intestine, embryonic tissue). Only isolated studies of this problem have so far been published [8, 9].

## EXPERIMENTAL METHOD

Polysaccharides were isolated from homogenates of human tissues by Westphal's method of hot phenol-water extraction [20], followed by precipitation with 5 volumes of 96% ethanol. After removal of protein impurities by treatment with a mixture of chloroform and N-butanol (3 : 1) or chloroform and octyl alcohol (3 : 1) and dialysis, the polysaccharides were freeze-dried.

The total content of carbohydrates was determined by the anthrone method [13], protein by Lowry's method [15], hexosamine by the Elson-Morgan method [7], sialic acids by Warren's thiobarbiturate method, and nucleic acids present as impurities by Spirin's method [10]. The results of chemical analysis showed that polysaccharide preparations from cancer tissue are essentially indistinguishable from the polysaccharides of normal tissue in their composition and belong to the class of carbohydrate-protein compounds known as tissue mucopolysaccharides [12].

Serologic analysis of the polysaccharide preparation was carried out by the complement fixation reaction to 50% titer [2, 17], and by the micromodification of the double diffusion in gel method [4] with absorption by Bjorklund's method [14]. Anticancer $\gamma$-globulins of batches Nos. 3, 9, and 12-15, isolated from immune sera of horses and asses were used as antiserum. Batches Nos. 3 and 9 were obtained by immunization of a horse and an ass with cancer tissue homogenate, and batches Nos. 12-15 by immunization with a mixture of a fraction (polysaccharide, DNP, lipid) of gastric carcinoma tissue with Freund's adjuvant [3].

This paper described the results of serologic analysis of 64 polysaccharide preparations isolated from human tissues (gastric carcinoma 18, normal stomach 16, spleen 7, 6-10-week embryos 6, liver 8, and intestine 9). Each of these preparations was obtained from 3 or 4 individuals belonging to different blood groups.

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TABLE 1. CFR to 50% Titer with γ-Globulins of Anticancer Sera

| Test antigen | Antigen for immunization | | | | | |
|---|---|---|---|---|---|---|
| | homogenate of tumor | | mixture of antigen fractions with Freund's adjuvant | | | |
| | batch no. of γ-globulins | | | | | |
| | 9 from ass | 3 from horse | 12 from horse | 13 from ass | 14 from horse | 15 from ass |
| Tissue polysaccharide of gastric carcinoma | 1:80 | 1:40 | 1:10 | 1:640 | 1:80 | 1:640 |
| Tissue polysaccharide of normal stomach | 1:80 | 1:40 | 1:10 | 1:640 | 1:80 | 1:320 |

Fig. 1. Comparison of antigenic composition of polysaccharide fraction from human gastric carcinoma tissue and tissue of normal human organs. Anticancer γ-globulins (batch No. 15) in center; 1, 4) tissue polysaccharides of gastric carcinoma; 2, 5) tissue polysaccharides of normal stomach; 3, 6) embryonic tissue polysaccharides.

## EXPERIMENTAL RESULTS

The highest content of antibodies against tissue polysaccharides in the complement fixation reaction (CFR) to 50% titer was found in anticancer γ-globulins of batches Nos. 13 and 15 (Table 1).

Since tissue polysaccharides of gastric carcinoma reacted constantly in the CFR to 50% titer with anticancer γ-globulins of batch No. 15 in a somewhat higher titer (1:640) than polysaccharides from normal tissues (1:160-1:320), this batch of γ-globulins was used to test the antigenic composition of the polysaccharides.

A study of the antigenic composition of polysaccharides of tumor tissue by double diffusion in gel showed that these polysaccharides differ in this respect from polysaccharides both of homologous normal tissue and of other organs, and also from normal human serum proteins. The difference was due to the appearance of an additional precipitation line (indicated by an arrow in Fig. 1), not identical with the precipitation lines formed by antiserum either with polysaccharides of normal organs or with normal human serum (Fig. 2a).

By titration of antigens, and also by testing different dilutions of serum, no new precipitation lines were detected. Specific features distinguishing polysaccharides of cancer tissue were found in 16 of 18 investigated preparations of gastric carcinoma tissue.

Specific "cancer" antigen was identified by exhaustion of cancer antiserum by polysaccharides of normal tissues and by normal human serum.

The result of an experiment to study exhaustion of anticancer γ-globulins is shown in Fig. 2. Absorption by Bjorklund's method from the anticancer serum completely removed antibodies against normal tissue polysaccharides and normal human serum proteins. After exhaustion of noncancer antibodies the anticancer γ-globulins continued to react only with polysaccharides of human gastric carcinoma tissue (Fig. 2b).

Tissue polysaccharides obtained by Westphal's hot phenol-water extraction method are thus serologically active substances. In its antigenic composition the polysaccharide fraction of human gastric carcinoma tissue differs specifically in its behavior in the agar diffusion reaction from polysaccharides of normal tissues (stomach, spleen, liver, intestine, embryonic tissue).

The results of preliminary experiments performed with polysaccharide preparations from genetically homogeneous, autologous human tissues confirm the validity of these conclusions.

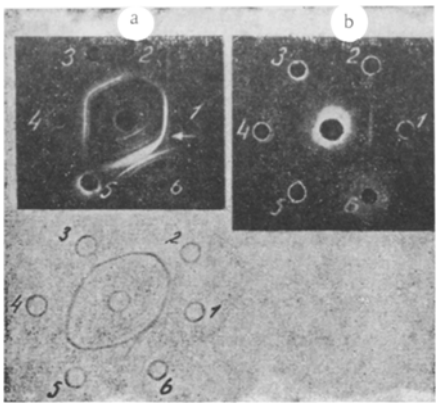


Fig. 2. Demonstration of specific antigen of gastric carcinoma tissue by anticancer serum before and after exhaustion. Anticancer $\gamma$-globulins of batch No. 15 before (a) and after (b) exhaustion with noncancer antibodies in center; 1) tissue polysaccharides of gastric carcinoma; 2) tissue polysaccharides of normal stomach; 3) polysaccharides of spleen; 4) polysaccharides of intestine; 5) polysaccharides of embryonic tissues; 6) normal human serum.

## LITERATURE CITED


1. G. I. Avdeev, I. S. Bashkaev, and B. É. Chechik, Proceedings of the International Cancer Congress [in Russian], Vol. 3, Moscow (1963), p. 298.
2. O. E. Vyazov (Editor), Laboratory Methods of Investigation in Noninfectious Immunology [in Russian], Moscow (1967), p. 176.
3. V. S. Gostev et al., Byull. Éksperim. Biol. i Med., No. 4, 94 (1964).
4. A. I. Gusev and V. S. Tsvetkov, Lab. Delo, No. 2, 43 (1961).
5. L. A. Zil'ber and G. I. Abelev, The Virology and Immunology of Cancer [in Russian], Moscow (1962), p. 289, 369.
6. P. N. Kosyakov, V. S. Korosteleva, and N. I. Kuznetsova, Byull. Éksperim. Biol. i Med., No. 9, 63 (1955).
7. N. K. Kochetkov (Editor), Methods in Carbohydrate Chemistry [in Russian], Moscow (1967).
8. A. M. Kuzin and N. I. Kuzina, Biokhimiya, 14, 432 (1949).
9. N. M. Mazina, Byull. Éksperim. Biol. i Med., No. 8, 82 (1958).
10. A. S. Spirin, Biokhimiya, 5, 656 (1958).
11. I. V. Tsvetkova and A. B. Kozina, Lab. Delo, No. 7, 409 (1966).
12. V. B. Khvatov, Abstracts of Proceedings of a Conference of Junior Research Workers at the Institute of Experimental Biology, Academy of Medical Sciences of the USSR [in Russian], Moscow (1968), p. 186.
13. R. Dreywood, Industr. Eng., Anal. Ed., 18, 499 (1946).
14. B. Bjorklund and V. Bjorklund, Proc. Soc. Exp. Biol. (New York), 79, 319 (1952).
15. O. Lowry et al., J. Biol. Chem., 193, 265 (1951).
16. O. Ouchterlony, Progr. Allergy, 5, 1 (1958).
17. E. Kabat and M. Mayer, Experimental Immunochemistry, Springfield (1958), p. 97.
18. I. Warren, J. Biol. Chem., 234, 1971 (1959).
19. E. Weiler, Brit. J. Cancer, 10, 553 (1956).
20. O. Westphal et al., Z. Naturforsch., 7B, 536 (1952).