THE ANTIGENIC STRUCTURE OF THE MUCOUS MEMBRANE OF THE NORMAL HUMAN STOMACH *

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The study of tissue and organ antigens of man has been going on for more than a half-century. Over this time the specificity of cytoplasmic granules, alcohol and aqueous extracts of organs has been studied and much valuable information obtained concerning the nature of various organ antigens [5-7, 12, 13]. But the antigenic structure of the stomach has been little studied. In addition, serologic methods which were used previously did not permit discrete analyses of complex antigenic mixtures.

In the present work we have studied the structure of the mucous membrane in the normal human stomach by immunoelectrophoresis according to Grabow in an original micromodification.

METHODS

The object of study was the gastric mucosa from cadavers of persons who died of trauma. The mucosa was removed from the washed stomach and kept at minus 15°.

Antisera were obtained after combination immunization of rabbits with water-salt extracts of gastric mucosa. The first course consisted of six injections of 8-10 ml of antigen which was injected subcutaneously, intraperitoneally or intramuscularly twice a week. The second course was given after one month and comprised three injections: one intraperitoneally and two intramuscularly with five ml at intervals of 3 days. Blood was taken from the ear vein seven days after the last immunization. The antiserum was concentrated seven to ten times in ammonium sulfate [1]. In several experiments rabbit antiserum to human lung tissue was used. This was prepared in a similar manner.

In the preparation of an aqueous salt extract for immunization the ratio of tissue: physiologic saline was 1 :10 also for antigens used in the gel precipitation reaction. Antigen for the immunoelectrophoresis experiments were prepared in a ratio of 1 :4 in veronal-medinal buffer, pH 8.6, $\mu = 0.05$. All antigens and antisera were preserved with merthiolate in a dilution of 1 :10,000. The protein concentration in the antigens, determined by the Kjeldahl method, was 3.5-5 mg/ml.

Immunoelectrophoresis was performed in the following manner. Melted 1.5% agar in veronal-medinal buffer, pH 8.6, $\mu = 0.025$ was poured onto a glass plate 9 x 12 cm in size. A bridge of filter paper connected the plate with the chamber electrodes. Transverse troughs were cut in the agar, 1 x 3 mm in size, along the midline about seven mm from each other for antigen, and were filled with the organ extract under study.

Electrophoresis was performed with a gradient potential of 3-4.5 volts per cm of agar and current strength of 10-15 milliamp for 45-60 min at room temperature. After termination of the electrophoresis a longitudinal trough was carved out with a razor for the immune serum, 40 mm in length and one mm in width to a distance of three mm from the antigen trough. Immune serum was poured into the longitudinal trough and the plate was kept in a moist chamber for one to two days. This micromodification was used in one experiment to carry out tests of 10-11 antigens and 9-10 immune sera at the same time. Gel precipitation reactions were performed in Petri dishes [4].

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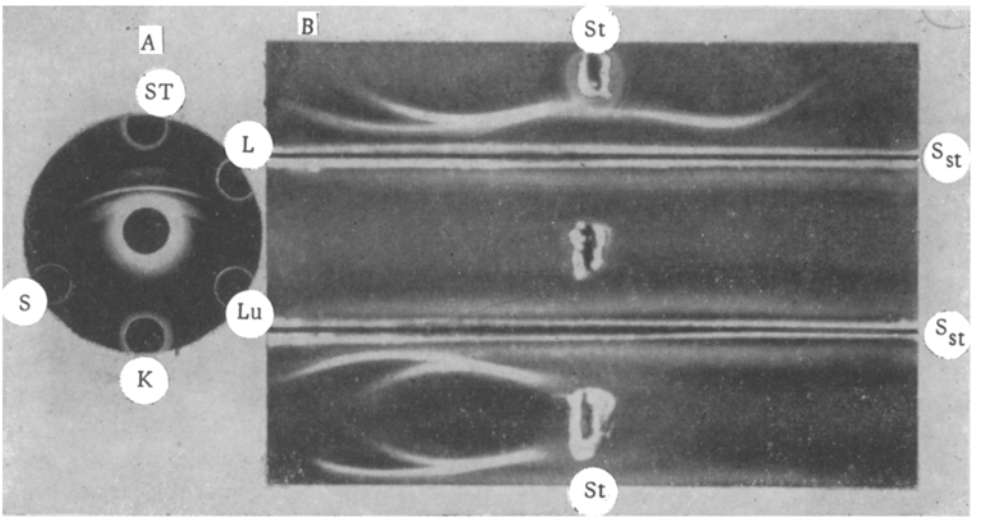

Fig. 1. Organ-specific Antigens of Human Gastric Mucosa. A) organspecificity of antiserum against human mucosa after adsorption with normal organ and human serum; S_{zh}) antiserum to gastric mucosa; St) gastric mucosa; s) spleen; l) liver; lu) lung; k) kidney; B) two types of immunophoregram of gastric mucosal organ-specific antigens; S_{st}) antiserum specific for stomach; St) gastric mucosa.

The method of adsorption of aqueous salt extracts of tissue by immune sera has been previously described [3]. The results of the experiments were photographed with a "Zenit" apparatus at low angle illumination.

RESULTS

Antiserum to gastric mucosa after adsorption with aqueous salt extracts of kidney, spleen and thyroid gland reacted only with antigens from gastric mucosa and gave no reaction with antigens from other organs taken from the same cadaver (see Fig. 1, A). Consequently, the antiserum was organ specific for gastric mucosa.

In the first experiments the structure of gastric mucosal organ antigens was studied with specific immune sera with immunoelectrophoresis.

All antigens from normal stomach which were studied gave, after gel electrophoresis, four bands of precipitation with the organ-specific antistomach sera.

The most frequent variants are shown in Fig. 1, B. Two bands of precipitate are seen to the left of the site of antigen application, in the region corresponding to the electrophoretic mobility of albumin and α-globulin of human serum. These antigens are called, respectively, gastric antigens I and II (compare Fig. 1, B, above). Still another band of precipitate is seen to the right of the site of antigen application in the region of the γ-globulins. This antigen is designated as gastric antigen III.

In some of the samples studied only two organ-specific antigens—I and II, appeared, in the region of albumin and α-globulin (see Fig. 1, B, below).

Subsequently, the organ-specific gastric antigens I and II were studied in greater detail. By changing the electrophoretic conditions we could discover still another organ-specific antigen II A, which has an electrophoretic mobility close to that of serum α-globulin (Fig. 2, A).

With increase in the time of electrophoresis (to 145-210 min) the precipitation band of antigen I in a number of cases splits into two parallel bands (see Fig. 2, B). Evidently, the organ-specific antigen is composed of two antigens, serologically and electrophoretically identical but differing only in their diffusion coefficient. It is possible that they also differ in molecular weight.

It is known that in addition to organ specific antigens cells contain tissue antigens common to the tissue antigens of other organs.

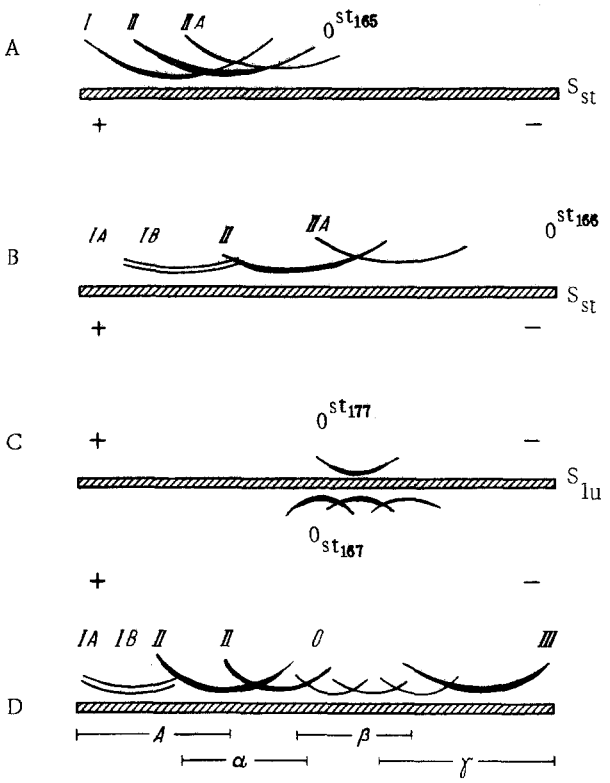

Fig. 2. Immunoelectrophoretic spectrum of organ-specific antigens. A) Appearance of organ antigen IIA in α-globulin region; B) discovery of complex structure of organ antigen I with increased period of electrophoresis, t = 145 min. Potential gradient 3.6 V/cm; C) appearance of tissue antigens of gastric mucosa which are common to lung tissue. St) Gastric mucosa; S_t antiserum to human lung, adsorbed with human serum. D) Antigenic spectrum of normal human gastric mucosa (diagram). IA, IB, II, IIA, III) gastric organ-specific antigens. In the region of the β-globulins are other gastric tissue-specific antigens.

In the following experiments the nature of these antigens was studied by use of anti-human lung antisera absorbed with normal human serum.

Antiserum to lung gave rather weak bands of precipitation with gastric antigens in the region of the β-globulins (see Fig. 2, C). This indicates the presence in gastric mucosa of certain tissue antigens, serologically related to the tissue antigens of lung and having an electrophoretic mobility of human serum β-globulins.

Thus, on the basis of the above stated structure of tissue antigens (see Fig. 2, D) the human gastric mucosa contains at least five organ antigens. Two of these (IA and IB) have an electrophoretic mobility similar to albumin, two (IIA and II) a mobility similar to α-globulin and one (antigen III) that of human serum β-globulin.

The nonspecific tissue antigens of the gastric mucosa, in common with tissue antigens from other organs, have an electrophoretic mobility similar to that of human serum β-globulins.

Our results are in accord with the data of various authors [10, 13] concerning the presence of a number of tissue and organ antigens in the cellular globulin fraction.

Recently, with immunoelectrophoresis the organ antigen was found in the α-globulin fraction of the thyroid gland [11] and in the globulin fraction in the liver [2].

The variation, mentioned above, in the antigenic spectrum of the immunophorogram of organ antigens in different individuals may have two bases. It is more probable that this variation reflects only the quantitative individual variation in content of organ antigens. But it cannot be excluded that this variation indicates an immunogenetic variability of tissue proteins in different individuals [8, 9]. The serological community of lung and stomach tissue at the expense of nonspecific tissue antigens is conditioned, evidently, by the soluble antigens of the connective tissue and the blood vessels.

LITERATURE CITED

1. G. I. Abelev, Z. A. Avenirova, and N. V. Éngel'gardt, Dokl. AN SSSR, 124, No. 6, (1959), p. 1328.
2. G. I. Abelev, N. I. Khramkova, and Z. A. Postnikova, Neoplasma (Bratisl). 9, (1962), p. 123.
3. G. I. Avdeev and I. S. Bashkaev, Byull. eksper. biol., No. 12, (1961), p. 76.
4. I. S. Bashkaev, Ibid., No. 5, p. 86.
5. P. N. Kosyakov, Antigenic substances in the organism and their significance in biology and medicine [in Russian], Moscow, (1954).
6. N. I. Kuznetsova, In book: Problems of immunology in normal and malignant tissue. [in Russian], Moscow., (1956), p. 156.
7. Z. I. Rovnova, Ibid., p. 142.
8. Zh. Dosse, Immunohematology [in Russian], Moscow, (1959).
9. J. Hirschfeld, Nature, 185, (1960), p. 931.
10. L. Korngold, Ann. N. Y. Acad. Sci., 69, (1957), p. 681.
11. N. R. Rose, R. S. Metzgar, and E. Isaacs, J. Immunol., 84, (1960), p. 849.

12. E. Witebsky, Zh. mikrobiol., 5, No. 2, (1928), p. 186.
13. E. Witebsky, N. R. Rose, and S. Shulman, Cancer Res., 16, (1956), p. 831.

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