BEHAVIOR OF "U" ANTIGEN TOWARDS THE ACTION OF CERTAIN PHYSICAL AND CHEMICAL FACTORS

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We have previously shown that the tissues of man, mice, and certain other animals contain a universal corpuscular antigen, reacting mainly with sera against leukemic human spleen [4]. This antigen was called "U" antigen. It is present in larger amounts in the spleen of patients with hemocytoblastosis than in the spleen of persons dying from trauma [3]. The method of differential ultracentrifugation was used for the detection of "U" antigen. The object of the present investigation was to study some of the physical and chemical properties of "U" antigen.

METHOD

Antigens for the gel-diffusion reaction were prepared by grinding the tissue in a mortar with quartz sand (1 g tissue and 3 ml physiological saline). The supernatant fluid after centrifugation at 4000 rpm for 10 min was used as antigen. Rabbits were immunized with saline extracts of the spleens of persons dying from acute or subacute hemocytoblastosis or killed accidentally [4]. The antigens were studied by a micromodification of the gel-diffusion technique as developed in the laboratory directed by L. A. Zil'ber [1]. Immunoelectrophoretic experiments were carried out by Wieme's micromodification [6] of Grabar's method [5]. Electrophoresis was carried out with a potential difference of 150 V between the electrodes (potential gradient about 20 W/cm) and with a current of a strength of 48-50 mA.

RESULTS

In the first place it was necessary to find out whether "U" antigen is thermostable or thermolabile. Saline extracts obtained from the organs of mice, dogs, pigs, lemmings, monkeys (Macacus rhesus) or human subjects were heated to 100° for 20-30 min. As a result of this treatment most of the proteins in the extracts coagulated to form a large residue, from which a small volume of a translucent, opalescent fluid could be aspirated. This fluid was used as antigen in the gel-diffusion reaction.

Serum against leukemic spleen (No. 46), when reacting with untreated antigens obtained from organs of man or the monkey, formed several precipitation lines, but when reacting with the same antigens treated by boiling, they formed, as a rule, only one line corresponding to the "U" antigen. This line gave the phenomenon of incomplete identity with the line situated opposite the wells into which were poured the untreated and heated antigens obtained from the organs of mice, dogs, and pigs (Fig. 1A).

The "U" antigens detected in the organs of the same animals before and after heating were serologically identical. This identity, however, was apparently incomplete, for when another antileukemic serum against leukemic spleen (No. 64) and a serum against normal human spleen (No. 50) reacted with "untreated" mouse antigens, a precipitation line appeared which formed a spur in relation to the line situated opposite the wells containing heated mouse antigens (Fig. 1B).

Consequently, "U" antigen is a complex antigen consisting of a thermostable and a thermolabile component. The latter loses its antigenic properties during boiling, but this procedure does not precipitate the "U" antigen.

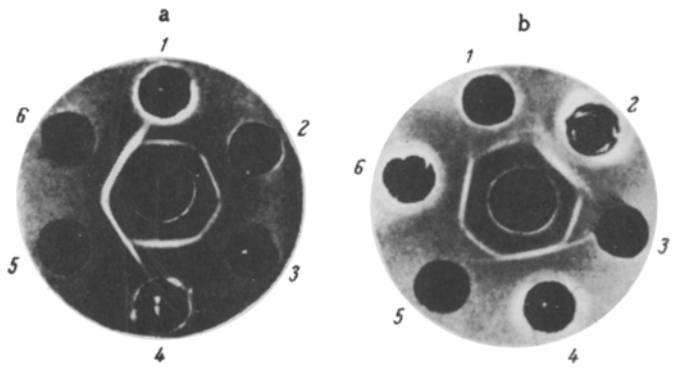

Fig. 1. Detection of "U" antigen in untreated and heated antigens prepared from human and animal organs. A) In the central well—serum against leukemic human spleen (No. 46): 1) extract of organs of mice (untreated); 2) extract of organs of mice (heated); 3) extract of pig's liver (untreated); 4) extract of pig's liver (heated); 5) extract of leukemic human spleen (heated); 6) extract of monkey's liver (heated); B) in the central well—serum against leukemic human spleen (No. 64); 2, 4, and 6) extracts of organs of mice (untreated); 1, 3, and 5) extracts of organs of mice (heated).

In the next series of experiments the behavior of "U" antigen was studied when the pH of the extracts was changed. The initial antigens were prepared from the liver or spleen of mice. The reaction of the medium was changed by carefully adding 1-3% NaOH solution or 3-5% acetic acid solution to the extracts to pH values of 3.0, 4.1, 4.5, 5.0, 5.5, 6.0, 6.5, 7.0, 8.0, 8.7, and 9.7.

With a change in the pH of the extracts to the acid side, a precipitate was usually formed and this was separated by centrifugation at 6000 rpm for 10 min. The precipitate was resuspended in physiological saline equal in volume to $\frac{1}{3}$-$\frac{1}{4}$ of the original extract, after which the pH of the suspension of the precipitate and the supernatant fluid was adjusted to 7.0-7.5 with 1-3% NaOH solution.

With a change in the pH to the alkaline side, to between 7.5 and 9.7, the "U" antigen continued to appear just as clearly as in the neutral medium. "U" antigen was precipitated in an acid medium between the limits of pH 3.0 and 3.5 after boiling for a short time (for 1 min). Subsequent neutralization of the suspension of the precipitate with 3% NaOH solution transferred the "U" antigen into solution.

The behavior of "U" antigen was next studied when treated with organic solvents: alcohol, acetone, ether, and chloroform. The procedures connected with treatment of the antigens with alcohol were carried out at −5°, and those connected with the use of acetone, ether, and chloroform at room temperature. One volume of saline extract prepared from the organs of mice or rats was mixed with one volume of 96° (96% by volume of ethyl alcohol in water) ethyl alcohol or with three volumes of acetone, chloroform, or ether. The mixtures were kept in a refrigerator for 30 min. The mixture of antigen with ether or chloroform was then filtered through filter paper while the mixture of antigen with acetone or alcohol was centrifuged at 6000 rpm for 15-30 min and the supernatant fluid was decanted. The residue obtained on the filter paper or in the bottom of the centrifuged tubes was dried in vacuo and resuspended in phosphate buffer (pH 6.8), equal in volume to $\frac{3}{4}$ of the original antigen. The suspensions were studied in the gel-diffusion reaction. Sera against leukemic spleen formed one line opposite each of the wells containing these suspensions, giving the phenomenon of identity with the line corresponding to the "U" antigen of the original tissues. Consequently, although the "U" antigen was precipitated by treatment with alcohol, acetone, ether, or chloroform, irreversible denaturation did not take place in these conditions, and after removal of the organic solvents the antigen again went into solution.

It was not one of the objects of this investigation to determine the chemical nature of "U" antigen, but on the assumption that it is a constituent of a protein, we attempted to precipitate it with ammonium sulfate.

The initial antigens were prepared from a mixture of the liver and spleen of mice or from human liver. Many proteins, although they coagulate on boiling, remain in suspension in a weakly alkaline medium. The saline

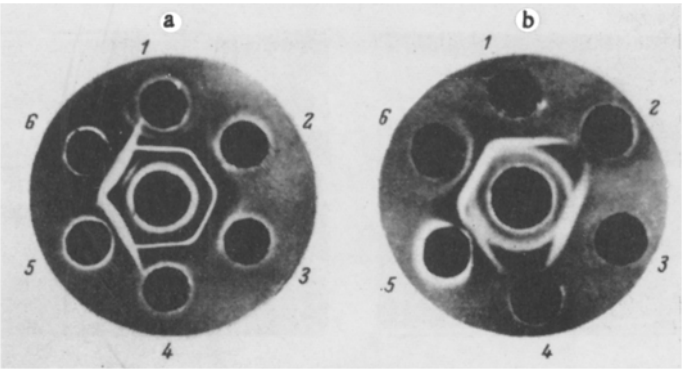

Fig. 2. Detection of "U" antigen with serum against heated preparations obtained from leukemic human liver (No. 81). A) In the central well—serum No. 81: 1) extract of mixture of liver and spleen of a mouse (untreated); 2) extract of rat's liver (untreated); 3) extract of cow's spleen (untreated); 4) "U" antigen isolated from pig's liver (heated and saturated with ammonium sulfate); 5) extract of monkey's liver (untreated); 6) extract of human spleen (untreated); B) in the central well—serum No. 81; 1) extract of mouse's liver; 2 and 4) supernatant fluid obtained after centrifugation of extract of mouse's liver at 90,000 g for 1.5 h; 3 and 6) suspension of residue obtained after centrifugation of extract of mouse's liver at 90,000 g for 1.5 h; 5) heated extract of mouse's liver.

extracts were therefore preliminarily alkalinized with 3% NaOH solution to pH 8.1-9.0, and then heated to 100° for 30 min on a water bath. The heated extracts were centrifuged at 6000 rpm for 15 min. The fairly large precipitate thus formed was discarded, and the pH of the supernatant fluid was adjusted to 7.0-7.2 by acidification with 3% acetic acid solution. An equal volume of a saturated solution of ammonium sulfate was then added to the resulting preparation drop by drop with constant mixing (to semisaturation). The precipitate was separated by centrifugation at 6000 rpm for 15 min and resuspended in physiological saline, equal in volume to $\frac{1}{4}$ of the original preparation. The suspension of the precipitate and the supernatant fluid were dialyzed in cellophane bags against physiological saline, and then evaporated to a volume equal to $\frac{1}{2}$-$\frac{1}{3}$ of the volume of the original preparation. The preparations of the precipitate and supernatant fluid were tested by the gel-diffusion reaction. "U" antigen was detected only in the fraction of the precipitate which formed one or two precipitation lines when reacting with sera against leukemic spleen. One of the these lines gave the phenomenon of complete identity with the line corresponding to the "U" antigen of the original preparation. With 50% saturation of the sera and tissue extracts, the globulins and certain other proteins usually appeared in the precipitate. It is evident that "U" antigen was also associated with a protein.

The preparations obtained by heating the extracts of leukemic human liver and precipitation of "U" antigen with ammonium sulfate were used to immunize rabbits. The first cycle of immunization consisted of four injections. The preparations were injected simultaneously subcutaneously and intramuscularly every 3rd day in doses of 2-3 ml. For the second and third cycles heated extracts of leukemic human liver, freed from coagulated proteins by centrifugation (without subsequent salting out), were used. The preparations were injected every 3rd day three times subcutaneously and intramuscularly in doses of 5-6 ml.

After the second or third cycle of immunization, the sera of 2 rabbits could be used to detect "U" antigen (Fig. 2). For example, the serum obtained from rabbit No. 81 after the second cycle of immunization, when reacting with saline extracts of human and monkey's organs, formed 2-3 precipitation lines, one of which corresponded to "U" antigen. Opposite the wells containing antigens prepared from the organs of mice, cows, dogs, and pigs, this antiserum formed only one precipitation line, giving the phenomenon of incomplete identity with the line situated opposite the wells with saline extracts of human and monkey's organs (Fig. 2A). The precipitation line due to the "U" antigen present in the human organs in turn formed a "spur" in relation to the line corresponding to the "U" antigen of the monkeys' organs. The "spurs" found in these experiments were "true," for they appeared when the antigens were used after preliminary titration and contained equal concentrations of "U" antigen.

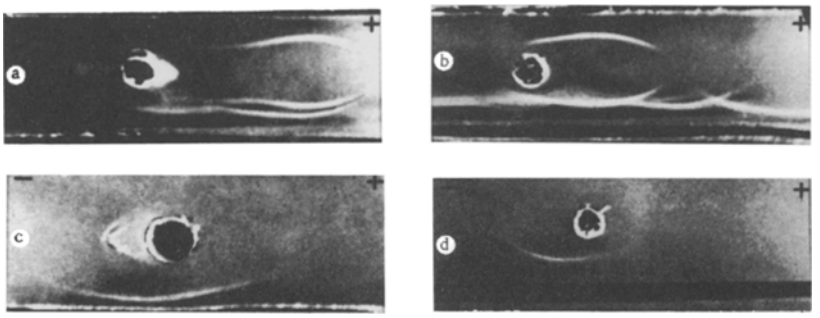

Fig. 3. Experiments in immunoelectrophoresis (identical experimental conditions). A) In agar–extract of liver of monkey (Macacus rhesus). Developed with: above–serum against heated preparations of "U" antigen exhausted with human embryonic kidney; below–serum against leukemic human spleen; B) in agar–extract of human liver. Developed with: above–serum against heated preparations of "U" antigen, exhausted with human embryonic kidney; below–serum against leukemic human spleen; C) in agar–extract of leukemic cow's liver. Developed with serum against heated preparations of "U" antigen; D) in agar–extract of mouse's liver. Developed with serum against heated preparations of "U" antigen.

Consequently, in man the "U" antigen is a constituent of a protein which contains an antigenic component specific for man, a component common to man and the monkey, and finally, a component common to man, monkeys, and several other animals. All these components are evidently the determinant groups of the same protein. They could not be separated experimentally by immunoelectrophoresis.

The antigens prepared from organs of human subjects, monkeys, mice, and cows were subjected to electrophoresis. The results of electrophoresis were developed by means of sera against leukemic human spleen and against heated preparations of "U" antigen. During the study of the human and monkeys' antigens, serum against heated preparations of "U" antigen was exhausted by a saline extract of human embryonic kidney. In the gel-diffusion reaction this serum formed one precipitation line opposite each of the wells containing antigens prepared from human and animal organs; corresponding to "U" antigen, while it continued to reveal in the human organs all the three components characteristic of the protein with which "U" antigen was bound.

In electrophoretic experiments sera against heated preparations of "U" antigen also formed one precipitation line when reacting with each of the extracts of the organs from man, monkeys, mice, and cows. However, the electrophoretic mobility of the "U" antigen present in the organs of man and the different animals varied. For example, the "U" antigen of the organs of the mice moved very slightly towards the cathode during electrophoresis, the "U" antigen of the organs of the cows remained in the region of application of the antigen, the "U" antigen of the human organs moved slowly towards the anode, and finally, the "U" antigen of the monkeys' organs moved towards the anode also, but rather faster than the "U" antigen of the human organs, following the serum albumin (Fig. 3).

Consequently, in man and different animals the proteins with which the "U" antigen is bound differ not only in their antigenic structure, but also in their electrophoretic mobility.

The behavior of the "U" antigen during differential centrifugation and chemical fractionation indicates that it is bound with proteins of the same kind in human and animal tissues. The results of the study of the electrophoretic mobility of the "U" antigen do not conflict with this hypothesis, for the electrophoretic mobility of the same proteins isolated from the tissues of animals of different species is known to differ [2]. Possibly the antigenic structure of the protein with which the "U" antigen is bound reflects both species differences in the composition and configuration of the particular protein molecule, and also features common to animals at different levels of the zoological scale. The degree of similarity evidently depends to some extent on the degree of phylogenetic closeness of animals of different species.

It may be postulated that this antigenic structure is characteristic, not only of the protein with which "U" antigen is bound, but also of many proteins of animal origin, reflecting certain general principles of biosynthesis of the same proteins in animals of different species.

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