# IDENTIFICATION OF A THERMOSTABLE ANTIGEN FROM NORMAL AND LEUKEMIC HUMAN TISSUES

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A cross-reacting thermostable corpuscular human and animal antigen, the content of which was increased in the spleens of persons with hemocytoblastoses, was isolated from leukemic human spleens and purified by ultracentrifugation in a sucrose gradient and in a preformed CsCl gradient. In the gel-diffusion reaction it was found to be completely identical with human ferritin.

A cross-reacting corpuscular antigen, sedimented by centrifugation at 90,000 g, was discovered in 1962 in the organs of man and several animals with the aid of antisera against human leukemic spleens in the gel-diffusion reaction [3]. It was described conventionally as antigen Y. This antigen consists of thermolabile and thermostable components [4]. The latter retains its antigenic activity if tissue extracts are heated on a boiling water bath. The content of Y antigen in the spleens of persons with hemocytoblastoses is 4-16 times higher than in normal human spleens [2].

The present investigation was undertaken in order to identify the Y antigen.

## EXPERIMENTAL METHOD

The spleen of a person dying from leukemia was homogenized at 14,000 rpm for 3 min in 0.05 M tris-buffer, pH 7.0, in the proportion of 2 ml buffer per gram tissue. The resulting suspension, to which hyaluronidase was added (10-15 mg enzyme to 100 ml suspension), was left for 1 h on a magnetic mixer at 20°C and then centrifuged at 6,000 rpm for 20 min. The residue was discarded and the supernatant was again centrifuged at 30,000 g for 30 min, and the antigen was precipitated from the supernatant by further centrifugation at 90,000 g for 1 h. The residue was resuspended with a Potter's homogenizer in a small volume of 0.05 M tris-buffer, the suspension was centrifuged for 15 min at 6,000 rpm, and the resulting supernatant, in a volume of 1.5-2 ml, was applied to the surface of a sucrose gradient (20-60%) in 0.05 M tris-buffer and centrifuged at 29,000 rpm for 3 h at 4°C (3·23 ml rotor, MSE Superspeed-65 centrifuge). After centrifugation, fractions containing most of the Y antigen were diluted 3-4 times and centrifuged at 105,000 g for 1 h. The residue was resuspended in 0.05 M tris-buffer and centrifuged for 15 min at 6,000 rpm. The supernatant was centrifuged in a preformed CsCl gradient with density 1.5-1.7 g/ml at 36,000 rpm for 16 h at 4°C (3·5 ml rotor, Superspeed-65).

The factions giving opalescence were diluted with 0.05 M tris-buffer, sedimented by centrifugation at 105,000 g for 1 h, and the residues were resuspended and investigated by the gel-diffusion test and in the electron microscope.*

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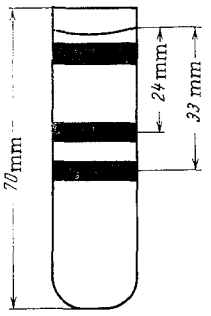


Fig. 1

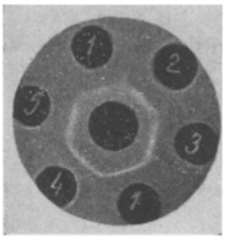


Fig. 2

Fig. 1. Diagram showing distribution of zones in 20-60% sucrose gradient after centrifugation of a suspension of the residue obtained by centrifugation of leukemic spleen homogenate at 90,000 g in it.

Fig. 2. Identification of Y antigen with human ferritin. In center, antiserum against Y antigen. 1) Original homogenate of human leukemic spleen; 2) fraction from bottom of tube after centrifugation of yellow zone of sucrose gradient of human leukemic spleen homogenate in CsCl gradient; 3) human ferritin; 4) yellow zone of sucrose gradient of monkey spleen homogenate; 5) yellow zone of sucrose gradient of normal human spleen homogenate.

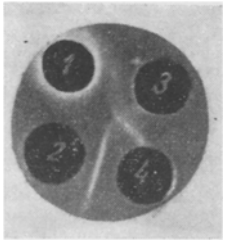


Fig. 3. Comparison of Y antigen with horse ferritin by G. I. Abelev's test. 1) Antiserum against Y antigen; 2) antiserum against horse ferritin; 3) horse ferritin; 4) homogenate of bovine spleen.

Ferritin was obtained from human and horse spleens by ultracentrifugation [5]. Splenic homogenates were heated for 10 min to 80°C in a water bath, and filtered successively through gauze and paper filters; the filtrates were centrifuged for 2 h at 78,000 g (a more complete yield of ferritin can be obtained by centrifugation at 120,000 g), and the residues were resuspended in distilled water; the suspensions were centrifuged at 6,000 rpm for 30 min, the supernatants were centrifuged at 105,000 g for 1 h, and the residue was again resuspended and centrifuged at 105,000 g for 1 h. The ferritin suspensions were centrifuged in a 20-60% sucrose gradient and in a preformed Cs-Cl gradient with density 1.5-1.7 g/ml under the same conditions as were used to purify the Y antigen.

A specimen of standard horse ferritin and rabbit antiserum against it were generously provided by N. A. Dorfman (Department of Virology, Moscow University). Antisera against Y antigen were obtained by immunizing rabbits with extract of human leukemic spleen heated in a boiling water bath [3]. The gel-diffusion test was carried out in a micromodification of Ouchterlony's method [1].

## EXPERIMENTAL RESULTS

To purify the Y antigens, centrifugation in a sucrose gradient and preformed CsCl gradient was used. If the suspension of unpurified antigen (residue resuspended after centrifugation at 90,000 g) was applied to the surface of a 20-60% sucrose gradient and centrifuged for 3 h at 29,000 rpm, the characteristic picture shown schematically in Fig. 1 was observed in the gradient. At the top of the tube was a zone stained red with hemoglobin. Further down the tube 2 distinct zones were clearly visible: the first was 24 mm from the meniscus and was colored yellow or yellowish-brown, the second was grayish-white and was 33 mm from the meniscus. Investigation of the sucrose gradient fractions showed that no Y antigen was present in the red zone. About 90% of this antigen was found in the yellow zone and a very small quantity in the bottom zone. When a suspension of normal spleen was analyzed, the activity was also highest in the yellow zone, but it was much weaker.

The electron-microscopic study of this fraction was impossible because of the large number of unidentified components. For further purification of the Y antigen the suspension of the residue obtained by centrifugation of the yellow zone at 105,000 g was centrifuged in a preformed CsCl gradient. In this case, 2 maxima of activity were found in the gradient, when tested both by immunodiffusion and by optical density methods, in the yellowish-brown zone with a buoyant density of 1.58 g/ml and also at the bottom of the tube, where the yellow fraction was located. In individual cases the whole of the colored fraction and the antigenic activity were concentrated at the bottom of the tube. Differences in the distribution of maxima of activity indicated heterogeneity of the protein with which the Y antigen is bound.

Electron-microscopic investigation of fractions taken both from the zone with a density of 1.58 g/ml and from the bottom of the tube showed characteristic particles measuring 100-115 Å and similar to ferritin. Neither ribosomes nor their fragments could be found in these fractions. It was postulated that the Y antigen is identical with ferritin. To test this hypothesis, ferritins were isolated from human and horse spleens by ultracentrifugation and then recentrifuged successively in a 20-60% sucrose gradient and a preformed CsCl gradient with density 1.5-1.7 g/ml. A standard preparation of horse ferritin was centrifuged in parallel tests in sucrose and CsCl density gradients. In a sucrose gradient, the human and horse ferritins each formed one wide, diffuse zone located 21-25 mm from the meniscus. The width of the zone was due to the heterogeneity of the ferritin [6]. After centrifugation in a CsCl gradient, all the ferritin was found at the bottom of the tube. Direct comparison of the Y antigen with human ferritin in Ouchterlony's reaction demonstrated their complete identity (Fig. 2). Human ferritin neutralized antibodies against Y antigen contained in human, monkey, and bovine tissues.

Antiserum against Y antigen did not reveal this antigen in horse ferritin or in native extracts of horse spleen. However, antiserum against Y antigen reacted with equine ferritin to form a precipitation line identical with that formed by the reaction between equine ferritin and antiserum against it, and intersecting the line corresponding to Y antigen (Fig. 3). Conversely, antiserum against equine ferritin reacted with extracts of bovine and human tissues to form a precipitation line identical with Y antigen but not identical with equine ferritin. These results indicate that equine and human ferritins possessed identical determinants, but their degree of identity is less than that between the antigenic determinants of ferritins from man, monkey, cow, mouse, and several other animals. This feature is responsible, on the one hand, for the ability of equine and human ferritins to induce cross-reacting antibodies and, on the other hand, the impossibility of detecting Y antigen in equine ferritin by the direct test.

The investigation thus showed that the Y antigen discovered in human tissues is in fact ferritin possessing identical determinants with the ferritins of several other animals. The increase in content of Y antigen in the tissues of patients with hemocytoblastoses was evidently due to the more rapid breakdown of defective erythrocytes with the increased liberation of iron into the tissues; the iron induces increased synthesis of apoferritin, with which it combines to form ferritin.

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