6. T. K. Chatterjee, R. K. Bhatnager, J. Cannon, and J. Long, Eur. J. Pharmacol., **98**, No. 2, 293 (1984).
7. P. B. Timmermans, M. J. Mathy, and B. Wilffert, Eur. J. Pharmacol., **97**, 55 (1984).
8. R. S. Lefkowitz, D. Mullikin, and M. Caron, J. Biol. Chem., **251**, 4686 (1976).
9. C. J. Lynch, R. Charles, P. F. Blackmore, and J. H. Exton, J. Biol. Chem., **260**, 1593 (1985).
10. D. Murray and R. Meyn, Cancer Res., **46**, No. 5, 2324 (1986).
11. J. C. Reeve, K. Wright, and P. Workman, Brit. J. Cancer, **50**, No. 2, 155 (1984).
12. C. C. D. Roothan, Rev. Mod. Phys., **23**, No. 2, 69 (1951).
13. V. K. Sharma and S. P. Banerjee, J. Biol. Chem., **252**, 7444 (1977).
14. C. Taylor and S. Merrit, TIBS, **11**, No. 6, 228 (1986).

IMMUNOCHEMICAL IDENTIFICATION AND PHYSICOCHEMICAL CHARACTERISTICS OF THE SPECIFIC α_2-GLOBULIN OF HUMAN GRANULOCYTES

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Polymorphonuclear leukocytes, or granulocytes, are universal effector cells whose potential is largely determined by their ability to release biologically active substances stored in the granules of these cells [10, 12, 13]. Granulocytes play a key role in defense against infections, in tissue damage and the development of vascular changes during inflammation, and in antitumor defense. Specific proteins secreted by granulocytes activate proteolytic cascade systems in the blood plasma [2, 11], interact with receptors on the surface of immunocompetent cells [2, 10], participate in the regulation of hematopoiesis [10] and in disturbances of blood clotting and fibrinolysis [11], and they possess powerful collagenolytic potential [13].

The list of granular proteins of neutrophils includes antimicrobial cationic components — myeloperoxidase, lysozyme, and lactoferrin, and neutral proteinases — elastase, cathepsin G, collagenase, gelatinase, B_{12} binding protein, transcobalamin, etc. More recently several new granulocytic proteins have been described [3, 4, 14, 15].

The aim of this investigation was to identify the specific α_2-globulin of human granulocytes and to undertake their immunochemical and physicochemical study.

EXPERIMENTAL METHOD

To obtain antisera rabbits were immunized with an extract of leukocytes and the antisera were adsorbed with blood plasma from healthy donors and with extract of liver tissue, under the control of immunodiffusion analysis with polyspecific antisera against human plasma proteins and liver extract. Leukocytes were isolated from whole blood, and pus was obtained by opening an abscess. The method of preparation of the leukocyte lysate and extracts of pus and tissues was described previously [4, 9].

Antisera against leukocytic elastase, lactoferrin, lysozyme, fibronectin, amyloid P-component, C-reactive protein (from "Calbiochem," USA), and standard test systems for soluble leukocytic antigens [4], obtained at the Research Institute of Physico-

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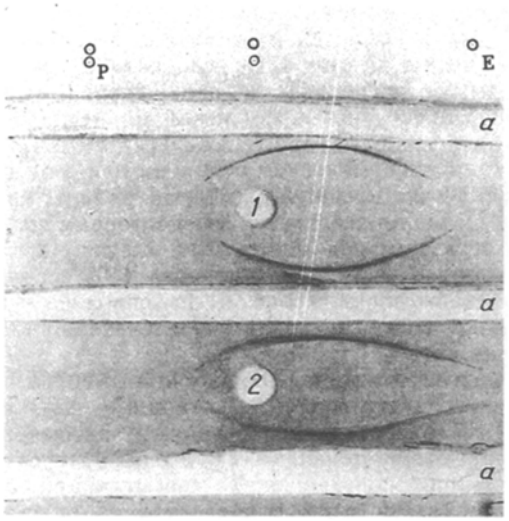

Fig. 1

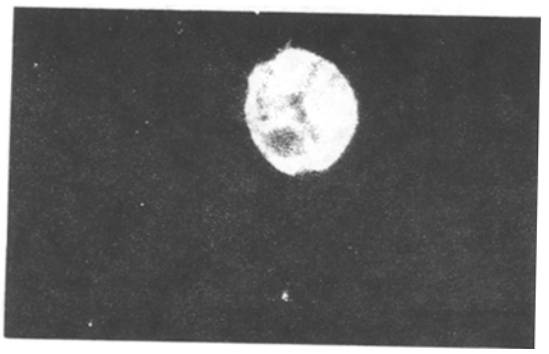

Fig. 2

Fig. 1. Immunoelectrophoretic analysis of α_2-GG. 1) Extract of leukocytes, 2) extract of leukocytes treated with neuraminidase, a) monospecific antiserum against α_2-GG. Reference substances for immunoelectrophoresis: p) pyronine, E) Evans' blue.

Fig. 2. Film from donor's blood. Reaction in zone of cytoplasm of a polymorph. Treated with antiserum against α_2-GG. Indirect immunofluorescence test. Objective 90× (oil immersion); ocular, homal 3×.

chemical Medicine, also were used. Immunodiffusion analysis was carried out in the modification [5] and immunoelectrophoresis was performed in 1% agarose gel ("Sigma," USA). The molecular weight of the antigen was determined by gel-filtration on Sephadex G-200 followed by immunodiffusion analysis of the resulting fractions with a standard test system; a kit from "Serva" (West Germany) was used as the standard proteins. The remaining parameters of the primary physicochemical characteristics of the antigen were determined as described previously [6, 7, 9].

Films of donors' blood were treated and studied by the indirect immunofluorescence method, as described previously [1, 8], and monospecific antiserum against granulocytic α_2-globulin was used as the intermediate antiserum. The intermediate antiserum and labeled antibodies were additionally adsorbed by an acetone powder of mouse liver [1]. Films treated with labeled antibodies without the use of the intermediate antiserum, and also films treated with polyspecific antiserum against blood plasma proteins and against kidney extract as intermediate antiserum, were tested as the control. The films were examined under phase-contrast. The preparations were photographed on RF-3 film (objective 90×, oil immersion; ocular homal 3×).

EXPERIMENTAL RESULTS

The adsorbed antiserum revealed one antigen with mobility of α_2-globulins in the leukocytic extract on immunoelectrophoresis (Fig. 1). A standard monospecific test system was obtained to this antigen, and by means of it its concentration was studied in the tissues and biological fluids. As Table 1 shows, the test α_2-globulin was present in the highest concentration in extracts of pus (titers to 1:64), it could also be found in hemolysate and synovial fluid, but could virtually not be detected in saliva, blood serum from healthy donors, or extracts of parenchymatous organs and muscle tissues.

On treatment of blood films obtained from a healthy human donor with adsorbed monospecific antiserum against the test protein, a specific indirect immunofluorescence reaction was found in the polymorphs. The reaction was localized in the cytoplasm around the nucleus and in the zone of the cytoplasmic membrane (Fig. 2). The character, intensity, and localization of the immunofluorescence reaction were similar to those for cytoplasmic granulocytic proteins — lactoferrin, leukocytic α-glycoprotein [8]. A positive reaction was not present in erythrocytes or in the control experiments. These results show that the α_2-globulin thus found is a specific component of granulocytes.

TABLE 1. Immunodiffusion Analysis of α_2-GG in Biological Fluids and Tissue Extracts

Material	Number of samples tested	Number of positive tests	Titers
Blood serum	20	1	(—)—(±)
Lymph	5	0	—
Saliva	3	0	—
Milk	5	0	—
Synovial fluid	3	2	(—)—1:1
Hemolysate	10	7	(—)—1:2
Liver	7	1	(—)—(±)
Kidney	11	0	—
Heart	8	0	—
Skeletal muscle	4	0	—
Extract of purulent, necrotic masses	5	5	1:8—1:64

TABLE 2. Physicochemical Characteristics of α_2-GG

Parameter	Result of determination
Molecular weight, kD	50±8
Relative electrophoretic mobility	0,71±0,1
Staining for lipoproteins	Negative
Binding with concanavalin A	Partially bound
Change in electrophoretic mobility under the influence of neuraminidase	Unchanged
Precipitation by ammonium sulfate (50% saturation)	Partially precipitated
Precipitation by rivanol	0,25 % — partially, 0,4 % — completely
Resistance to the action of enzymes:	
trypsin	Destroyed
papain	Resistant
hyaluronidase	"
Precipitation by TCA (2%)	»
Precipitation by sulfosalicylic acid (0.3M)	Not precipitated
	Precipitated

Comparative immunodiffusion analysis using standard antisera showed that the granulocytic α_2-globulin (α_2-GG) differs immunochemically from lactoferrin, leukocytic elastase, lysozyme, fibronectin, fetal hemoglobin, C-reactive protein, and the amyloid P-component of blood serum, and also from soluble leukocytic antigens described previously [3, 4, 8].

Table 2 gives the results of primary physicochemical characterization of α_2-GG. The molecular weight of this antigen in extract of pus was 50 ± 8 kD. Incubation with neuraminidase did not change the electrophoretic mobility of this protein (Fig. 1), evidence that sialic acids are not present in its composition; however, binding with concanavalin A-sepharose indicates the glycoprotein nature of α_2-GG.

Thus as a result of this investigation the specific α_2-globulin of human polymorphonuclear leukocytes was identified. The biological role and clinical importance of granulocytic α_2-globulin require special study.

LITERATURE CITED

1. L. V. Beletskaya and T. A. Danilova, Immunoluminescence in Medicine [in Russian], Moscow (1977), pp. 145-183.
2. O. G. Ogloblina, Vopr. Med. Khim., No. 5, 2 (1988).
3. D. D. Petrunin, Yu. M. Lopukhin, M. N. Molodenkov, and G. A. Oliferenko, Byull. Éksp. Biol. Med., No. 4, 66 (1982).
4. D. D. Petrunin and Yu. M. Lopukhin, Gematol. Transfuziol., No. 11, 3 (1985).
5. N. I. Khramkova and G. I. Abelev, Byull. Éksp. Biol. Med., No. 12, 107 (1961).
6. O. P. Shevchenko, D. D. Petrunin, and Yu. S. Tatarinov, Byull. Éksp. Biol. Med. , No. 8, 213 (1978).
7. O. P. Shevchenko, D. D. Petrunin, Yu. S. Tatarinov, and T. M. Tsagareeva, Biokhimiya, No. 1, 142 (1980).

8. O. P. Shevchenko, L. V. Beletskaya, V. F. Kirsanov, et al., Byull. Éksp. Biol. Med., No. 7, 53 (1986).
9. O. P. Shevchenko, A. G. Merkulov, G. A. Kratasyuk, and A. P. Domogatskii, Byull. Éksp. Biol. Med., No. 11, 604 (1987).
10. J. Fallon and J. Gallen, Allergy, **77**, 653 (1986).
11. E. J. Gustafson and R. W. Colman, Sem. Thrombos. Hemostas., **13**, 95 (1987).
12. K. Havemann and M. Gramse, Proteinases: Potential Role in Health and Disease, New York (1984), pp. 1-20.
13. M. Hibbs, K. Hasty, A. Kang, and C. Mainardi, Collagen Rel. Res., **4**, 467 (1984).
14. M. Moreno, C. Goulu, M. Fondanechi, et al., Immunol. Lett., **9**, 215 (1985).
15. T. Vartio, Biochem. J., **228**, 605 (1985).

ORIGIN OF URINARY PORPHYRINS IN EXPERIMENTAL HEXACHLOROBENZENE-INDUCED PORPHYRIA

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Key words: hexachlorobenzene-induced porphyria; uroporphyrinogen decarboxylase; porphyrins in urine, liver, and kidneys

The basic pathobiochemical mechanism of porphyria, caused by the fungicide hexachlorobenzene (gamma-BHC) is inhibition of uroporphyrinogen decarboxylase (EC 4.1.1.37; UPD) in the liver [3, 7]. Very large amounts of uroporphyrin and smaller amounts of heptacarboxyporphyrin accumulate in the liver. Their excretion in the urine is greatly increased. Inhibition of UPD also has been found in the kidneys of rats with gamma-BHC porphyria [2, 7]. The hypothesis thus arose that the kidneys are the main source of porphyrins in the urine in this condition [2].

It has not yet been established whether inhibition of renal UPD develops in animals other than rats, treated with gamma-BHC. Nor has the quantity of porphyrins deposited in the kidneys been compared with the quantity of porphyrins in the liver. The discovery of large accumulations of uroporphyrin in the kidneys would support the hypothesis that porphyrins in the urine in gamma-BHC poisoning are synthesized primarily in the kidneys. The final elucidation of this problem would allow suggestions to be put forward regarding the origin of the porphyrins in the urine in the disease porphyria cutanea tarda in man, for gamma-BHC porphyria is a model of that disease.

The aim of this investigation was to discover whether chronic gamma-BHC poisoning leads to inhibition of renal UPD activity in mice, and to examine the relationship between the degree of uropoyphyrin accumulation in the liver and kidneys.

EXPERIMENTAL METHOD

Twelve male C57BL/6 mice weighing about 15 g and eight female Wistar rats weighing about 150 g were used. Six mice received a single dose of 0.25 ml inferon (12.5 mg of iron) intraperitoneally and were maintained on a standard diet ad libitum, containing 0.02% of gamma-BHC. The animals were killed 8 weeks later. Four rats were killed after being kept for 7 weeks on a standard diet containing 0.3% of gamma-BHC. The remaining six mice and four rats (control) received the standard diet without gamma-BHC. Immediately after decapitation of the animals the liver and kidneys were removed and homogenized in a glass homogenizer of the Potter–Elvehjem type with Teflon pestle. The homogenizing solution contained 0.1 M K_2HPO_4/KH_2PO_4 and 0.1 mM EDTA-Na_2, pH 6.8. The dilution of the homogenates was 1:5 (w/v).

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