That is why an ascorbic acid deficit in the fetus, first, inhibits collagen synthesis in the developing limbs and damages mesenchymal cells in them, and second, damages the limb ganglia. The combined action of these two factors probably leads to disturbance of morphogenesis of the limbs.

LITERATURE CITED

1. A. P. Dyban and I. M. Akimova, Arkh. Anat., No. 8, 3 (1966).
2. A. P. Dyban, I. I. Tikhodeeva, and N. V. Khromov-Borisov, Vest. Akad. Med. Nauk SSSR, No. 12, 78 (1975).
3. I. I. Karuzina and A. I. Archakov, in: Modern Methods in Biochemistry [in Russian], Moscow (1977), p. 49.
4. I. I. Matusis, Vitamins and Antivitamins [in Russian], Moscow (1975).
5. V. V. Popov, B. L. Vaisman, and V. B. Puchkov, Byull. Éksp. Biol. Med., No. 5, 613 (1981).
6. I. B. Chatterjee, G. C. Chatterjee, et al., Biochem. J., 76, 279 (1960).
7. A. H. Conney, G. A. Bray, et al., Ann. N. Y. Acad. Sci., 92, 115 (1961).
8. S. Fabro, R. L. Smith, and R. T. Williams, Biochem. J., 104, 565 (1967).
9. S. Fabro, R. L. Smith, and R. T. Williams, Biochem. J., 104, 570 (1967).
10. G. B. Gordon, S. P. Spielberg, et al., Proc. Natl. Acad. Sci. USA, 78, 2545 (1981).
11. W. G. McBride, Teratology, 14, 71 (1976).
12. D. Neubert et al., in: Role of Pharmacokinetics in Prenatal and Perinatal Toxicology, Stuttgart (1978), p. 359.
13. P. Sato and S. Udenfriend, Vitam. Horm., Vol. 36, p. 33.
14. H. J. Schumaher, in: Methods for Detection of Environmental Agents That Produce Congenital Defects, Amsterdam (1975), p. 65.
15. H. J. Schumaher et al., Br. J. Pharmacol., 25, 338 (1965).
16. T. H. Shepard, Catalog of Teratogenic Agents, Baltimore (1976).
17. H. P. Spencer and C. T. Theisen, Teratology, 21, 71A (1980).
18. T. H. Vickers, Br. J. Exp. Pathol., 48, 579 (1967).

QUANTITATIVE DETERMINATION OF THE INTENSITY OF FLUORESCENCE OF 5-HT ORGANELLES IN THE STUDY OF PLATELET FUNCTION

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The role of platelets in the pathogenesis of thrombohemorrhagic complications of meningococcal infection has been insufficiently studied. The role of the granular apparatus of the platelets in disturbances of their function has been particular little studied, especially in endotoxinemia accompanying the generalized form of meningococcal infection.

The work of da Prada et al. (1965-1978), who used luminescence microscopy [5], showed that it is possible to study the state of the granular apparatus of platelets with the aid of a fluorescent marker (mepacrine, acridine orange – AO, etc.), which is selectively taken up by the serotonin-containing granules (5-HT organelles, dense bodies, β-granules). The quantity of marker taken up reflects the functional state of these granules, which play an essential role in hemostasis, for they release serotonin, ADP, Ca^{++}, and platelet factor 3 in response to activation.

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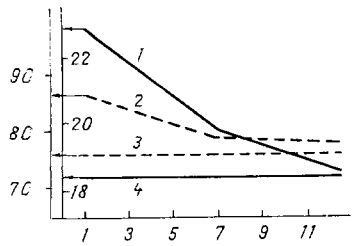

Fig. 1

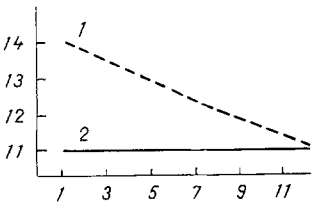

Fig. 2

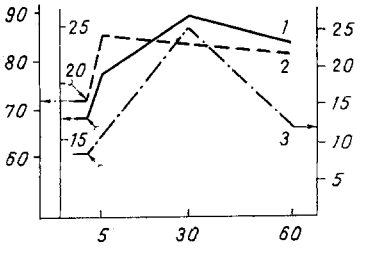

Fig. 3

Fig. 1. Intensity of fluorescence of platelets from normal subjects (3, 4) and patients with meningococcal infection (1, 2). 1, 4) Group A platelets; 2, 3) Group B platelets. Abscissa, time of treatment (in days); ordinate, intensity of fluorescence (in relative units).

Fig. 2. Effect of treatment on number of Group B platelets in patients with meningococcal infection. 1) Patients with meningococcal infection; 2) healthy subjects. Abscissa, time of treatment (in days); ordinate, number of platelets (in %).

Fig. 3. Effect of lipopolysaccharide of meningococcus A on intensity of fluorescence of platelets of Groups A and B and on number of Group B cells. 1) Group A platelets; 2) Group B platelets; 3) number of Group B cells. Arrow indicates addition of lipopolysaccharide. Abscissa, incubation time (in min); ordinate: on left – intensity of fluorescence (in relative units), right – number of Group B cells (in %).

It has also been shown that the intensity of fluorescence of the platelet and its density depend on the number of 5-HT granules [4], which may vary under different physiological conditions and in certain diseases [1, 3, 6].

This paper gives the preliminary results of a study of the ingestive capacity of the platelets of patients with a generalized form of meningococcal infection at the height of the disease and during treatment and recovery, and also the results of a study of the action of meningococcal endotoxin (lipopolysaccharide of meningococcus A) on platelets of a healthy blood donor (experiments in vitro).

EXPERIMENTAL METHOD

Quantitative estimation of fluorescent marker AO ingested by platelets was carried out on the LYUMAN IUF-1 luminescence microscope with photometric attachment. The source of exciting radiation was a mercury-quartz DRK-120 lamp. An RFT digital voltmeter was used as the recorder.

Preparations of living platelets were made from platelet-rich plasma (PRP), stabilized with sodium citrate in the ratio of 9:1; 0.1 ml of PRP was diluted with an equal volume of physiological saline and incubated for 30 min with 0.1 ml of AO in a concentration of 5 mg in 10 ml.

A drop of the fluorochromed sample was placed on a defatted slide and covered with a coverslip. Those areas of the preparation where the platelets were arranged in a monolayer were examined under the microscope. The intensity of fluorescence of the background and platelet was measured. The difference between these values corresponded to the quantity of AO taken up by the platelet. Fluorescence of 25-50 platelets was measured in each preparation. For greater accuracy the measurements were made on two parallel films.

Platelets from 35 patients aged from 18 to 60 years with a severe mixed form of meningococcal infection (meningitis with meningococcemia) at the height of the disease (1st-3rd day), on the 5th-8th day, and during the period of convalescence (17th-22nd day) were studied. Investigation of the patients' blood clotting system showed the presence of disseminated intravascular blood clotting (DIC syndrome).

The control group consisted of 24 healthy blood donors aged from 20 to 25 years.

In the experiments in vitro, 0.1 ml of lipopolysaccharide of meningococcus A was added to specimens of fluorochromed PRP, up to a concentration of 1 μg/ml in the sample, and incubated for 5-80 min. The method of measurement was that described above.

EXPERIMENTAL RESULTS

Depending on the intensity of fluorescence the platelet pool of the healthy donors was heterogeneous: The great majority of cells (80-90%) were platelets whose intensity of fluorescence ranged from 15 to 30 relative

units (Group A); a smaller number of platelets (from 10 to 20%) had brighter fluorescence – from 50 to 125 relative units (Group B). In the group of blood donors the intensity of fluorescence of the Group A platelets was on average 18.3 ± 0.64 relative units, and in Group B 77.3 ± 3.2 relative units. The brighter platelets (Group B) accounted for 11.0 ± 1.03% of the total number of platelets.

In patients with the generalized form of meningococcal infection the intensity of fluorescence of both groups of platelets was increased at the height of the disease: in Group A to 22.8 ± 0.58 relative units ($P < 0.05$) and in Group B to 86.7 ± 3.24 relative units ($P > 0.05$). On the 5th-8th day of treatment the intensity of fluorescence of the platelets decreased: in Group A to 19.6 ± 0.7 relative units and in Group B to 79.0 ± 4.0 relative units ($P > 0.05$). By the time of discharge from the hospital the intensity of fluorescence of both groups of platelets was completely back to normal (Fig. 1).

The number of brighter cells (Group B) as a proportion of the total number of cells counted in the preparation increased at the height of the disease to 14 ± 2.6%, on the 5th-8th day of the disease it was 12.5 ± 2.7%, but during the period of convalescence it returned completely to normal (Fig. 2).

According to these observations, the ingestive power of the platelets increased in patients with a severe form of meningococcal infection at the height of the disease, and the number of platelets with the maximal intensity of fluorescence also increased.

To study the role of endotoxinemia in the change in functional activity of the granular apparatus of the platelets in meningococcal infection a series of experiments was carried out on platelets of normal blood donors after incubation for different time intervals with lipopolysaccharide (LPS) of meningococcus A.

After incubation with LPS the donor's platelets acquired the ability to take up a larger quantity of AO, with the result that the intensity of their fluorescence increased (Fig. 3). For instance, before incubation with LPS the mean intensity of fluorescence of the Group A platelets was 16.7 ± 0.48 relative units, and of the Group B platelets 71.0 ± 3.12 relative units. After incubation for 5-15 min the intensity of fluorescence of the platelets was 20.4 ± 0.5 relative units ($P < 0.01$) and 86.5 ± 1.5 relative units ($P < 0.05$) respectively. Incubation for 30-45 min led to an even greater increase in the intensity of fluorescence of the Group A cells, namely to 25.7 ± 0.45 relative units ($P < 0.001$) whereas the intensity of fluorescence of the Group B platelets remained high (83.8 ± 10.4 relative units). The phenomenon can be explained by an increase in the number of granules in the platelets. Similar changes in the Bernard-Soulier syndrome were observed by Rendu et al. [6]. However, longer incubation of the platelets with meningococcal LPS did not lead to any further increase in the effect; on the contrary, it actually decreased a little. This result was probably due to disturbance of the structure of the granular apparatus of the platelets or to active release of AO (exocytosis).

The number of cells with bright fluorescence (Group B) increased with an increase in the time of their incubation with LPS ($P < 0.05$). However, after incubation for 60 min their number fell distinctly compared with the effect after incubation for 30 min (Fig. 3).

Heterogeneity of the platelet pool in the donors as regards density, size, and volume was observed by Karpatkin et al. [2], who linked this feature with the subjects' age: Lighter cells were regarded as more mature, whereas heavier or more dense cells were considered to be young. Later it was shown by fluorescence microscopy [5] that young cells contain more 5-HT granules than mature cells, and this determines the difference in the intensity of their fluorescence [4].

Using luminescence microscopy, we obtained new data on changes in the granular apparatus of platelets in the course of the disease in patients with meningococcal infection with a well-marked DIC syndrome. At the height of the disease the increase in fluorescence of the platelets was evidently due, besides to other changes in homeostasis (pH, hypoxia, disturbance of the water-electrolyte balance, etc.), to endotoxinemia.

Experiments in vitro to study the direct action of meningococcal endotoxin on healthy human platelets showed that endotoxinemia is one of the factors that can cause morphological and functional changes in platelets.

LITERATURE CITED

1. B. Boneu, C. Caranobe, J. Capdeville, et al., Thromb. Res., 12, 831 (1978).
2. S. Karpatkin and A. Charmats, J. Clin. Invest., 48, 1073 (1969).
3. H. P. Lorez and M. da Prada, Experientia, 34, 663 (1978).
4. H. P. Lorez, M. da Prada, and A. Pletscher, J. Lab. Clin. Med., 89, 200 (1977).
5. M. da Prada and A. Pletscher, Eur. J. Pharmacol., 32, 179 (1975).
6. F. Rendu, A. T. Nurden, M. Lebet, et al., Thrombos. Haemostas. (Stuttgart), 42, 694 (1979).