On the 6th-7th day the deficit of synapses was reduced to 34.1% ($P < 0.05$). The increase in the number of synapses took place on account of small synapses with 1-2 and 3-4 intersections with the control grid (Fig. 1d); the number of large synapses was a little reduced. The number of curved contacts increased. In this series of experiments, just as on the 1st-4th day, synapses with asymmetrical composition predominated but there was an appreciable increase in the number of symmetrical contacts. Medium-sized synapses predominated although there was a fair number of large contacts. The number of curved synapses increased to 4.9 ± 0.34 ($P < 0.01$) per field of vision (106.5% of the control), and the number of straight contacts in this period amounted to 57.1% of the control ($P < 0.05$). The appearance of small synapses and the increase in the number of contacts with symmetrical composition are evidence of the activation of synaptogenesis [1, 4].

Rapidly developing generalized changes in the ultrastructure of synapses and their death were thus observed in the cortex and these changes play an important role in the pathogenesis of postresuscitation encephalopathy. Meanwhile the plastic properties of the synaptic apparatus develop rapidly and become apparent in the neocortex after resuscitation. During the first 3 days mainly compensatory hypertrophy of interneuronal contacts is found, but on the 6th-7th day synaptogenesis is well marked. Structural changes in the synaptic apparatus reflect compensatory and reparative changes in neocortical neurons and constitute an important mechanism leading to restoration of brain functions in the postresuscitation period.

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EFFECT OF PROSTAGLANDINS E_1 AND E_2 ON PLATELET SHAPE AND AGGREGATION

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KEY WORDS: shape of platelets; prostaglandins E_1 and E_2; membrane-bound calcium.

In the process of platelet aggregation an essential role is played by prostaglandins (PG). It has been shown that PGE_1 inhibits [6, 8] whereas PGE_2 stimulates platelet aggregation induced by ADP [6]. The mechanism of this effect has not yet been fully explained. The work of Born has shown the importance of changes in the shape of these cells for the development of aggregation [1]. Changes in platelet shape have been shown to lead to marked aggregation [8].

With these facts in mind it was decided to study the effect of PGE_1 and PGE_2 on the shape of platelets, including platelets activated by exogenous ADP and changes in pH.

EXPERIMENTAL METHOD

Platelets were obtained from rat blood taken from the abdominal aorta. A 3.8% solution of sodium citrate was used as anticoagulant (ratio of blood to anticoagulant 9:1). Platelet-rich plasma was obtained from citrated blood by centrifugation at 250g for 6 min at room temperature. Platelet-rich plasma diluted 1:6 with Tyrode's solution (without magnesium), pH 7.4, was used in the experiments. Fluorescence and transmittance of the platelet suspension were

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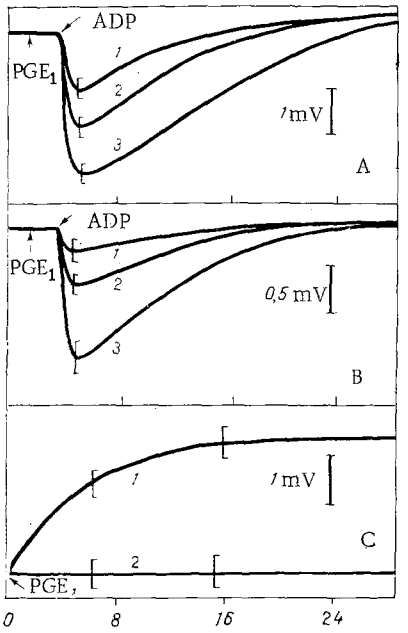

Fig. 1. Action of PGE_1 on change in shape of platelets. A) Effect of PGE_1 on ADP-induced change in shape of platelets: 1) $2.8 \cdot 10^{-6}$ M; 2) $2.8 \cdot 10^{-7}$ M; 3) 0. B) Effect of PGE_1 on changes in chlortetracycline fluorescence induced by ADP (10^{-7} M); 1) $2.8 \cdot 10^{-6}$ M; 2) $2.8 \cdot 10^{-7}$ M; 3) 0. C) Effect of PGE_1 on transmittance of a suspension of platelets incubated in Tyrode's solution, pH 9.0; 1) $2.8 \cdot 10^{-6}$ M. 2) 0. Abscissa, time (in min); ordinate, A, C) transmittance, B) intensity of fluorescence.

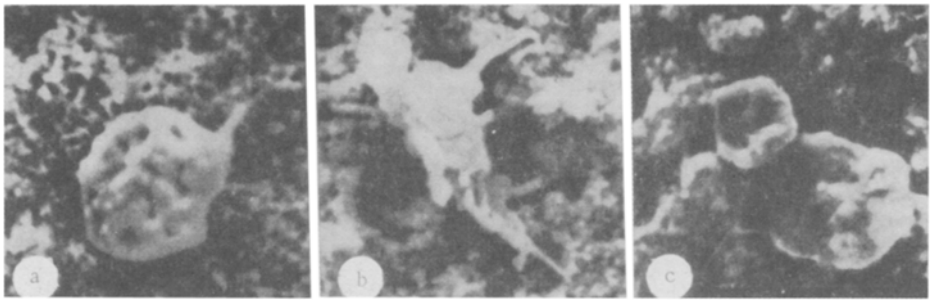

Fig. 2. Restoration of native shape of platelets incubated in Tyrode's solution, pH 9.0, on addition of PGE_1. a) Tyrode's solution, pH 7.4; b) the same, pH 9.0; c) the same, pH 9.0, + PGE_1.

measured on a Hitachi MPF-4 fluorescence spectrophotometer. Chlortetracycline (final concentration 10^{-5} M) was used as the calcium-sensitive fluorescent probe. Fluorescence was excited by light with a wavelength of 400 nm (width of slit 4 nm) and light was recorded with a wavelength of 530 nm (width of slit 10 nm). Fluorescence was measured at an angle of 90° with the anterior edge of the square quartz cuvette (length of edge 1 cm). The temperature in the cuvette was kept constant (35°C) by means of a special cuvette holder and the UT-15 water ultrathermostat. The contents of the cuvette were constantly mixed by means of a vibratory mixer. Transmittance was recorded at a wavelength of 620 nm.

Preparations of platelets for scanning electron microscopy were obtained by the method in [7]. The Hitachi S-500 scanning electron microscope was used.

The ADP used in the investigation was from Reanal and the chlortetracycline from Calbiochem (USA). PGE_1 and PGE_2 were generously provided by Professor Bergström (Sweden). The remaining reagents used were of Soviet manufacture and were of the optically pure and chemically pure grades.

EXPERIMENTAL RESULTS

Addition of PGE_1 to the platelet suspension caused no change in their shape. However, preliminary incubation of the platelets with PGE_1 for 2 min inhibited changes in their shape induced by ADP (Fig. 1A). PGE_1 also blocked changes in the membrane-bound calcium level in the platelets, which are known to take place [4] during ADP-induced changes of shape (Fig. 1B). Considering the initiating role of calcium in changes of shape [5], it can be tentatively suggested that the inhibitory action of PGE_1 is exerted through blocking of calcium mobilization.

However, the results can also be explained by competition between PGE_1 and ADP for the binding sites on the platelets, for it has been shown that PG reduces the quantity of ADP bound with platelets [2]. To verify the suggested mechanism of action of PGE_1 on platelets, the method of activating the platelets had to be changed so as to rule out the effect of ADP.

The pH of the incubation medium is known to have a strong influence on the state of platelet function. Alkalification of the incubation medium leads to a change in the shape of these cells and an increase in their ability to aggregate, whereas acidification blocks aggregation induced by various agents [3]. The effect of pH on platelets is explained by changes in Ca^{++} binding accompanying changes in pH.

A stable change in platelet shape was obtained by alkalification of the incubation medium to pH 9.0 (Fig. 2b). Addition of PGE_1 under these conditions stimulated normalization of the shape of the cells (Figs. 1C and 2c). The increase in transmittance observed after addition of PGE_2 (Fig. 1C), it will be noted, was equal to the change in transmittance of the platelet suspension during changes in shape induced by ADP (Fig. 1A, 3).

The results thus indicate that the effect of PGE on platelets is in fact to cause activation of the mechanism restoring the shape of the cell.

PGE_2 by itself caused changes in shape of the platelets (Fig. 3A) and a fall in the membrane-bound calcium level (Fig. 3C, 2). These changes, it must be pointed out, were stable in character unlike the reversible changes induced by ADP (Fig. 3B, 4). Simultaneous addition of PGE_2 and ADP prevented restoration of the native shape of platelets modified by ADP (Fig.

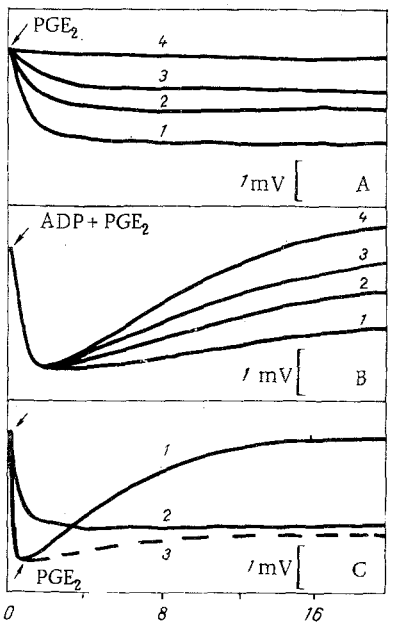

Fig. 3. Action of PGE_2 on platelets. A) Effect of different concentrations of PGE_2 on transmittance of platelet suspension: 1) $1.4 \cdot 10^{-5}$ M; 2) $0.7 \cdot 10^{-5}$ M; 3) $0.35 \cdot 10^{-5}$ M; 4) $0.1 \cdot 10^{-5}$ M. B) Changes in transmittance of platelet suspension on combined addition of ADP (10^{-7} M) and PGE_2 in doses of: 1) $1.4 \cdot 10^{-5}$ M; 2) $0.7 \cdot 10^{-5}$ M; 3) $0.35 \cdot 10^{-5}$ M; 4) 0. C) Changes in chlortetracycline fluorescence under the influence of PGE_2 in a dose of $1.4 \cdot 10^{-5}$ M (2), ADP in a dose of 10^{-7} M (1), and ADP + PGE_2 (3). Abscissa, time (in min).

3B). Under these conditions normalization of the membrane-bound calcium level also was blocked (Fig. 3C, 3).

PGE_2 thus blocks recovery of the native shape of platelets, possibly by inactivating the mechanism maintaining membrane-bound calcium at a definite level in the cell.

PGE_1 thus leads to recovery of the native shape of platelets, whereas PGE_2 alters that shape. Taking this into account and also the importance of changes in the shape of platelets for aggregation, it can be postulated that prostaglandins of the E group exert their action on aggregation through their effect on mechanisms, probably calcium-dependent, controlling the shape of platelets.

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ROLE OF THE PARASYMPATHETIC NERVOUS SYSTEM IN PROLIFERATION OF CORNEAL EPITHELIAL CELLS IN RATS AFTER INJURY TO THE SUBMAXILLARY SALIVARY GLAND

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KEY WORDS: corneal epithelium of rats; cell proliferation; botulinus toxin; muscarinic acetylcholine receptor; trauma to submaxillary salivary gland.

Proliferation of the corneal epithelial cells is enhanced after injury to internal organs as a result of the production of nonspecific growth stimulators [2]. However, trauma to organs leads to significant changes in the function of the autonomic nervous system. This evidently plays an important role in the changes taking place in cell proliferation after trauma, for proliferation is inhibited when autonomic dystonia develops, with predominance of the sympathetic division of the nervous system [3]. However, there is little information on the influence of the parasympathetic division of the nervous system on cell proliferation [1, 5]. To study this problem, however, is interesting because after injury to the preganglionic (decentralization) and postganglionic (denervation) neurons dystrophic changes do not develop equally [4].

On the basis of existing data showing the high level of proliferation of the corneal cells and the presence of many adrenergic and cholinergic receptors among them, it was decided to study how corneal proliferation is modified after blocking, initially of preganglionic, and later of postganglionic neurons and how total pharmacologic stimulation and blockade of muscarinic acetylcholine receptors is reflected in proliferation, in the absence and during the formation of nonspecific growth stimulators as a result of trauma to the submaxillary salivary gland (SMSG).

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