various digestive enzymes, including pancreatic enzymes. The results now obtained indicate that these disturbances under the conditions of chronic stress inhibit the formation and possibly also the activity of the principal pancreatic enzymes. However, this inhibition is observed only at certain times of adaptation and it gradually disappears during repetition of the action of the stressors, suggesting the increasing efficiency of the mechanisms responsible for maintenance of the neurohormonal, metabolic, and enzymic status of the body in situations of stress. The results described in this paper suggest that when scientifically based diets suitable for use during chronic exposure to stress factors are drawn up the state of the pancreatic enzyme spectrum in the different stages of adaptation must be taken into account.

LITERATURE CITED

1. A. A. Viru, Functions of the Adrenal Cortex during Muscular Activity [in Russian], Moscow (1977).
2. Sh. K. Kurbanov, Uzbek. Biol. Zh., No. 6, 57 (1975).
3. Sh. K. Kurbanov, Byull. Éksp. Biol. Med., No. 11, 1335 (1976).
4. A. A. Lazdynysh and M. R. Lazdynya, Izv. Akad. Nauk Latv. SSR, No. 1, 68 (1975).
5. É. Sh. Matlina and G. N. Kassil', Usp. Fiziol. Nauk, No. 2, 13 (1976).
6. Z. T. Tursunov et al., Uzbek Biol. Zh., No. 4, 27 (1975).
7. Z. T. Tursunov et al., Biol. Nauki, No. 2, 35 (1977).
8. A. M. Ugolev et al., Investigation of the Digestive Apparatus in Man [in Russian], Leningrad (1969).
9. I. A. Iskin, Principles of Physiology of the Endocrine Glands [in Russian], Moscow (1975).
10. N. A. Yudaev, in: Chemical Bases of Processes of Vital Activity [in Russian], Moscow (1962), p. 235.

LIPID PEROXIDES AND THROMBOSIS

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On incubation of rabbit platelets with ADP, adrenalin, serotonin, and thrombin, the level of lipid hydroperoxides in the platelets, determined as malonic dialdehyde, increases parallel with the increased aggregative power. An even higher concentration of malonic dialdehyde is observed in the platelets of animals with pulmonary thrombosis. Dynamic studies showed that the accumulation of malonic dialdehydes in the platelets reflects the initial stage of development of thrombosis; this can be used for the diagnosis of the early stages of intravascular thrombosis.

KEY WORDS: aggregation of platelets; malonic dialdehyde; lipid peroxides; pulmonary thrombosis.

The role of peroxidation products of lipids in the mechanisms of thrombosis has received insufficient study. It is stated [12, 13] that lipid peroxidation products formed by the oxidative destruction of unsaturated fatty acids may injure the platelet membrane and induce irreversible aggregation.

Since platelet aggregation is the trigger mechanism of development of thrombosis, it has been suggested that the accumulation of lipid hydroperoxide in the platelets must be a reliable sign of commencing thrombosis.

To test this hypothesis, the writers studied the dynamics of accumulation of lipid peroxidation products in the platelets of rabbits with experimental pulmonary thrombosis of immune etiology.

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TABLE 1. Content of Malonic Dialdehyde in Intact Platelets and after Treatment with Aggregating Agents

Statistical index	MDA level, nmoles/10^9 platelets					
	intact platelets		on aggregation of platelets with undermentioned agents			
	original	with $FeCl_3$ $1 \cdot 10^{-4}$ M	ADP $5 \cdot 10^{-6}$ M	Adrenalin $1 \cdot 10^{-5}$	Serotonin $1 \cdot 10^{-5}$	Thrombin 0.4 unit/ml
$M \pm m$ (n=25)	0,32±0,02	2,73±0,11	3,94±0,18	3,81±0,21	3,59±0,17	6,82±1,22
P		<0,01	<0,01	<0,01	<0,01	<0,01

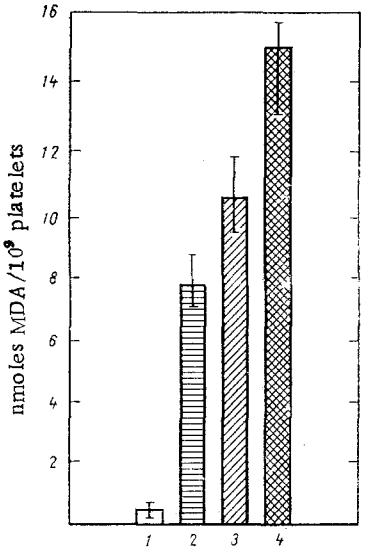

Fig. 1. Dynamics of MDA content in platelets of rabbits during development of pulmonary thrombosis. 1) Original content; 2, 3, 4) 3, 12 and 24 h, respectively, after development of thrombosis.

EXPERIMENTAL METHOD

Experiments were carried out on 60 chinchilla rabbits of different weights and sexes. Experimental thrombosis was produced by intravenous injection of antilung immunoglobulins into the animals after suppression of the fibrinolytic activity of the blood [1]. The degree of development of thrombosis was judged from changes in the hemodynamic indices of the pulmonary circulation obtained by rheopolycardiography and catheterization of the chambers of the right heart [4], and also by the thrombotic index calculated as a percentage after sacrifice of the animals [5].

Tests were carried out on suspensions of platelets and on platelet-enriched plasma. The latter was obtained from blood taken from the carotid arteries of animals through a polyethylene catheter, stabilized with 3.8% sodium citrate solution (9 : 1) or 1.5% EDTA solution in 0.7% NaCl, and centrifuged for 20 min at 150g. Platelets were isolated from the platelet-enriched plasma by repeated centrifugation of the plasma for 20 min at 2000g. The residue of platelets was washed twice with 0.154 M NaCl solution at 4°C and then resuspended in Tris-maleate buffer, pH 5.9. The platelet count was determined with the MBI-1 microscope (objective 20, ocular 15), using a pale green filter.

Washed platelets were used for investigation not later than 30 min after they were obtained. The formation of lipid peroxide was judged from the accumulation of malonic dialdehyde (MDA) in the platelets after their interaction with thiobarbituric acid [15]. The total quantity of MDA was calculated by the equation:

$$\text{MDA (nmoles)} = \frac{2 \times E_{548}}{0.145} = 14 \times E_{548},$$

where 2 is the final volume of the solution in the cuvette, 0.145 is the molar absorption coefficient; E_{548} the optical density.

Lipid peroxidation was activated by incubation of the platelets with ferric ions ($FeCl_3$) in a final concentration of $1 \cdot 10^{-4}$ M at 37°C.

Platelet aggregation was studied by a photometric method [6] with graphic recording on the KSP-4 potentiometer. Solutions of the disodium salt of ADP (in a final concentration of $5 \cdot 10^{-6}$ M), adrenalin ($1 \cdot 10^{-5}$ M), serotonin ($1 \cdot 10^{-5}$ M), and thrombin (0.4 unit/ml) were used as inducers of aggregation.

The results were subjected to statistical analysis with the use of B. G. Kaplan's Tables [3].

EXPERIMENTAL RESULTS

Normally platelets contain only negligible quantities of MDA (Table 1). On the addition of ferric ions to the residue of platelets the yield of MDA increased. Incubation of platelets with aggregating agents, especially thrombin, led to a considerable increase in their MDA content.

Other workers [12, 13] also obtained similar data for washed human platelets. After comparing the action of different inducers of aggregation on the formation of lipid peroxidation products in donors, these workers concluded that thrombin is the most effective prooxidant. Later, however, it was shown that MDA formation under the influence of ADP may be just as intensive if Ca^{++} and Mg^{++} ions are present in the medium [15].

A clear disturbance of lipid peroxidation was found in the platelets of animals with pulmonary thrombosis (Fig. 1). As early as 3 h after injection of antilung immunoglobulins into the rabbits, besides morphological evidence of damage to the pulmonary microcirculation, an increase was observed in the MDA content in the platelets, which was seven times higher than initially, and in six rabbits it was increased by almost 20 times.

During the development of thrombosis the MDA content in the platelets of the animals rose progressively: by 3 h after the development of thrombosis the MDA level in the platelets was 7.9 ± 1.1 nmoles/10^9 platelets, rising after 12 h to 10.8 ± 1.0 nmoles/10^9 platelets. The MDA content in the platelets 24 h after occlusion of the main branches of the pulmonary vessels had increased to 14.9 ± 1.2 nmoles/10^9 platelets, and after 3 and 5 days it was 17.3 ± 1.6 and 19.8 ± 1.8 nmoles/10^9 platelets respectively, or 65 times higher than the initial MDA level in these animals. Clearly definite correlation is observed between the MDA level and the platelets and the degree of thrombosis in the pulmonary vessels: The higher the MDA content the more active the course of thrombosis.

It has now been shown that oxidative destruction of lipids is connected both with primary destruction of unsaturated fatty acids and with the accumulation of lipid peroxidation products [2, 10]. Since they attack primarily structures which are rich in lipids, namely cell membranes and subcellular organelles, lipid peroxidation products may play an important role in the mechanisms of cellular injury [10].

One of the first investigators to draw attention to the role of lipid hydroperoxides in platelet aggregation was the Japanese researcher Okuma [12]. He studied the functional state of platelets in the course of their physiological aging and showed that an increase in the aggregative power of the platelet with age is accompanied by a regular increase in their MDA content. Later he found that the accumulation of lipid hydroperoxides in platelets leads to injury predominantly of the lysosomal membranes, with liberation of their specific enzymes, and also with inactivation of certain enzymes dependent on sulfhydryl groups in the mitochondria and microsomes [13].

It is interesting that similar changes in the subcellular structure of the platelets are observed following the action of aggregating agents on them [9]. In the earlier investigations of Holmsen et al. [7] the classical picture of the "secretion reaction" of the platelets was described, and later other workers [8, 11] also observed that the initial period of platelet aggregation is characterized by the liberation of a large group of biologically active substances from them — ADP, serotonin, Ca^{++}, fibrinogen, etc. The mediators of this reaction have not yet been isolated, but it has been suggested that substances disturbing the permeability of the platelet membrane ought to play this type of role [8]. There is now every rea-

son to suppose that the most likely inducers of platelet aggregation are lipid endoperoxides. Their participation in the mediation of aggregation is due not only to their high membranotoxic properties, but also to their ability to induce changes in platelets analogous to the secretion reaction [13].

The present investigations show that accumulation of lipid peroxidation products does in fact take place during aggregation of platelets, but under natural physiological conditions this accumulation is very small and can be detected only in the presence of ferrous ions. Meanwhile, in thrombin-induced aggregation of platelets there is a marked increase in the yield of MDA, evidence of activation of lipid peroxidation processes.

Accumulation of lipid peroxidation products in the platelets of animals in the pre- and early thrombotic period deserves special attention. The absence of any clinical features of thrombosis at this time suggests that elevation of the MDA level in the platelets must be regarded as an early and pathognomonic diagnostic criterion of developing thrombosis.

LITERATURE CITED

1. S. V. Andreev and A. A. Kubatiev, Izobreteniya, No. 12, 140 (1977).
2. Yu. A. Vladimirov and A. I. Archakov, Peroxidation of Lipids in Biological Membranes [in Russian], Moscow (1972).
3. N. R. Paleev and I. M. Kasvitser, Atlas of Hemodynamic Investigations in the Clinical Management of Internal Diseases [in Russian], Moscow (1975).
4. K. D. Boruach, Ind. J. Med. Res., 62, 923 (1974).
5. I. R. O'Brien, J. Clin. Path., 15, 452 (1962).
6. H. Holmsen and H. Day, Biochem. J., 129, 67 (1962).
7. H. Holmsen, in: Biochemistry and Pharmacology of Platelets, Amsterdam (1975), p. 175.
8. T. Hovig, Thromb. Diath. Haemorrh. (Stuttgart), 8, 455 (1962).
9. C. Lea, in: Lipids and their Oxidation, Westport, Conn. (1962), p. 3.
10. D. Mills, in: Biochemistry and Pharmacology of Platelets, Amsterdam (1975), p. 153.
11. M. Okuma, M. Steiner, and M. Baldini, J. Lab. Clin. Med., 75, 283 (1970).
12. M. Okuma, M. Steiner, and M. Baldini, J. Lab. Clin. Med., 77, 728 (1971).
13. Z. Placer, L. Cushman, and B. Johnson, Anal. Biochem., 16, 359 (1966).
14. J. Smith, C. Ingerman, et al., J. Lab. Clin. Med., 88, 167 (1976).