FUNCTIONAL MORPHOLOGY OF THE MESENTERIC MICROCIRCULATION IN EXPERIMENTAL HYPOPARATHYROIDISM

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The study of the state of the microcirculation in hypoparathyroidism can shed light on many unexplained aspects of the pathogenesis of the visceral disturbances and the particular features of lesions in different organs associated with this condition, for the microcirculation is the final link in the chain of events responsible for the transport function of the cardiovascular system and transcapillary exchange, and it creates the vitally essential tissue homeostasis [7].

The current importance and usefulness of such investigations are determined by the fact that hypoparathyroidism, as a model of hypocalcemia — a feature observed in many pathological states — may shed light on the role of microcirculatory disorders in certain diseases accompanied by disturbance of calcium homeostasis in the body [4].

EXPERIMENTAL METHOD

Experiments were carried out on 68 noninbred male albino rats weighing 100-120 g. Hypoparathyroidism was induced by extirpation of the parathyroid glands. After removal the glands were examined morphologically. The tests were carried out 5, 9, 15, and 30 days after the operation. The degree of hypoparathyroidism was judged from the blood calcium level, determined photometrically. Animals undergoing mock operations served as the control. The state of vascular permeability was determined on membrane preparations of the mesentery by studying the outflow of colloidal ink particles and of isothiocyanate-labeled homologous γ-globulin [1, 3].

Mast cells were counted in 10 fields of vision in preparations of the mesentery stained with toluidine blue and eosinophilic leukocytes were counted in preparations stained by the Unna—Giemsa method in 10 fields of vision with a 20× objective. Biogenic amines in the mast cells were detected in membrane preparations of the mesentery by a fluorescent method using paraform [10] and orthophthaleic aldehyde [11], with the aid of the FMÉL-1A photometric attachment. Two-dimensional films of the mesentery were stained by the usual morphological methods. Ultrathin sections were examined in the BS-613 electron microscope under an accelerating voltage of 80 kV. The serum histamine concentration was determined by Udenfriend's method in Shore's modification on the Hitachi MPF-4 spectrofluorometer.

EXPERIMENTAL RESULTS

Investigation of vascular permeability after intravenous injection of ink revealed marked deposition of ink particles on the walls of the microvessels on the 5th, 9th, 15th, and 30th days (Fig. 1a). All four degrees of labeling were observed. Increased permeability also was observed on the 5th day in rats undergoing mock operations, but only of the I and II degrees, and the number of labeled vessels was 7.5 times smaller than in the hypoparathyroid animals. Later no disturbances of permeability were observed in animals after mock operations just as in intact rats (Table 1).

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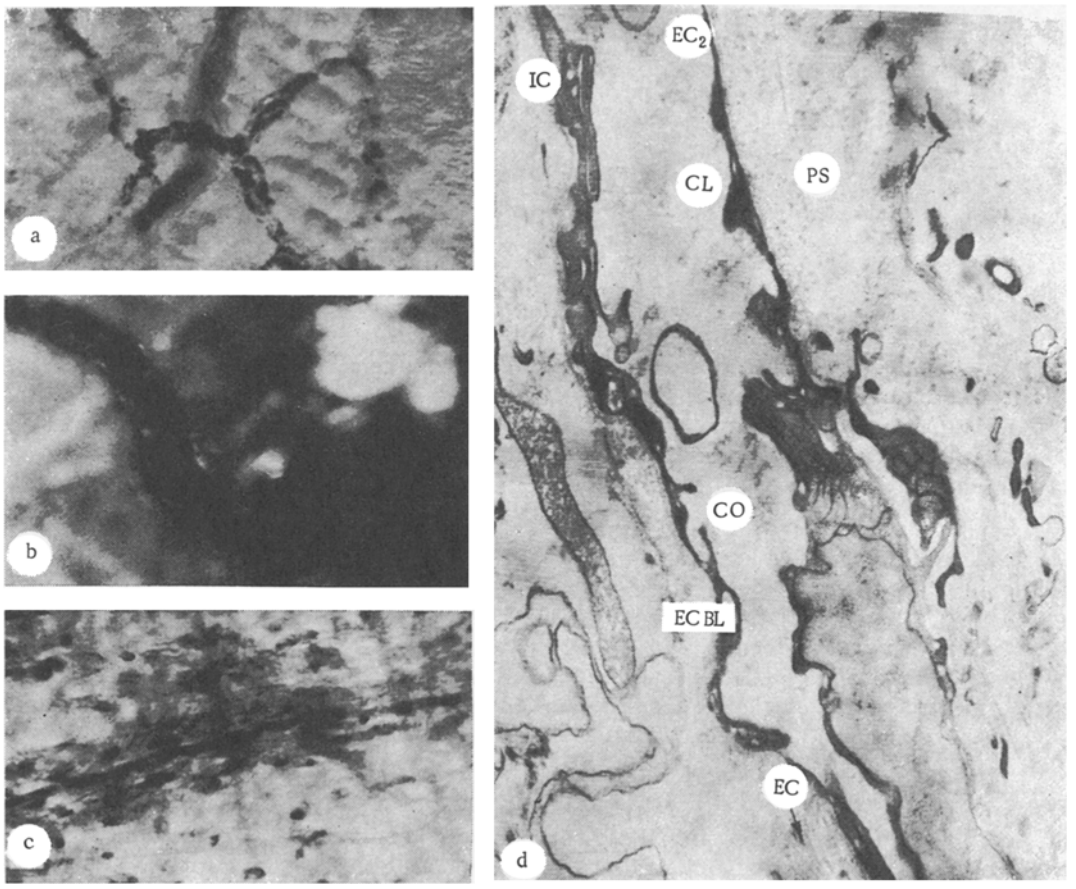

Fig. 1. Rat mesentery on 9th day after parathyroidectomy: a) deposition of colloidal carbon on capillary surface; b) outflow of labeled globulin into pericapillary space; c) perivascular edema with areas of loosening of fibrous structures. Heidenhain's azan stain; d) fragment of capillary wall. Capillary wall is thinner and its basal layer is loose in texture. Cytoplasmic outgrowths on surface of endothelial cells. Magnification: a) 280, b) 630, c) 200, d) 10,000. CL) Capillary lumen; EC_1, EC_2) endothelial cells; CO) cytoplasmic outgrowths; IC) interendothelial contacts; ECBL) extracellular component of basal layer; PS) pericapillary space.

TABLE 1. Calcium Level and Vascular Permeability of Mesentery in Parathyroidectomized Rats

Time after operation, days	Ca^{2+}, mg%	Number of labeled vessels	Distribution of vessels by degree of permeability			
			I	II	III	IV
Intact rats	8,2±0,15	0	—	—	—	—
5	4,4±0,11 $P<0,001$	12,5±1,14	1,3±0,63	5,0±0,73	3,0±0,66	3,2±1,14
9	4,16±0,47 $P<0,001$	12,3±0,98	2,0±0,86	2,3±0,61	2,8±0,65	5,2±1,4
15	5,1±0,11 $P<0,001$	14,5±1,45	4,7±0,61	5,0±0,8	2,3±0,61	2,5±0,56
30	7,1±0,12 $P<0,001$	6,0±0,81	1,8±0,75	3,0±0,97	0,7±0,39	0,5±0,34

Legend. Here and in Table 2, in all series of experiments n = 6.

TABLE 2. State of Mast Cells and Eosinophils in Mesentery of Parathyroidectomized Rats

Time after operation, days	Number of mast cells	Number of mast cells with different degrees of degranulation		Number of eosinophils	Histamine	Serotonin
		II	III			
Intact rats	26,3±1,76	7,7±0,95	3,2±1,14	251,8±6,1	22,17±0,23	22,1±0,19
5	29,2±1,85	8,3±1,89	7,8±0,79	100,6±7,4	13,39±0,58	3,92±0,19
	$P>0,05$	$P>0,05$	$P<0,02$	$P<0,001$	$P<0,001$	$P<0,001$
9	21,2±1,3	11,3±0,9	7,2±0,79	127,2±8,9	5,59±0,68	7,33±0,14
	$P<0,01$	$P<0,05$	$P<0,05$	$P<0,001$	$P<0,001$	$P<0,001$
15	20,5±0,76	8,16±0,3	5,5±0,4	189,0±12,8	4,29±0,1	7,72±0,15
	$P<0,01$	$P>0,05$	$P>0,05$	$P<0,001$	$P<0,001$	$P<0,001$
30	23,7±1,09	9,3±1,17	4,7±0,72	239,0±7,1	16,1±0,57	4,89±0,37
	$P<0,05$	$P>0,05$	$P>0,05$	$P<0,05$	$P<0,001$	$P<0,001$

Similar results were obtained when vascular permeability was investigated with isothiocyanate-labeled γ-globulin. The unequal character of spread of the label in the perivascular space at different stages of observation will be noted. For instance, on the 5th, 15th, and 30th days intensive fluorescence of γ-globulin was observed close to the vessels (Fig. 1b). In the period of maximal hypocalcemia, on the 9th day (Table 1) fluorescence was weaker but it was distinguished by diffuse blue penetration into the connective-tissue basis of the mesentery.

Histological investigation of the vessel walls of the mesenteric microcirculatory system showed that the pericapillary connective-tissue basis was saturated with plasma proteins, the collagen bundles were swollen and showed signs of focal granular degeneration, and the elastic fibers were thinner and fragmented (Fig. 1c). Individual regions of the capillary wall were thinner because of flattening of the endothelium, and the interendothelial contacts were sometimes moderately widened. Many cytoplasmic outgrowths could be seen on the luminal surface of the endothelial cells. The extracellular component of the basal layer was loose in texture (Fig. 1d).

An important place in the regulation of the local blood flow and, in particular, of vascular and tissue permeability, is ascribed to mast cell–eosinophil associations [8]. In the present investigation an attempt was made to determine the role of mast cells in the mechanism of increased permeability of the mesenteric microvessels of parathyroidectomized rats.

Counting the number of mast cells in the mesentery showed a decrease in their total number and an increase in the percentage of degranulated forms (Table 2). This process was most marked on the 9th day of the experiment.

Quantitative fluorescence analysis of the mean histamine and serotonin content in the mesenteric mast cells revealed a significant decrease in the content of biogenic amines ($P < 0.001$) at all times of investigation. At the same time the serum histamine concentration was raised. For instance, on the 5th day the histamine concentration was 16.6% above normal ($P < 0.01$), and on the 9th-15th day it was 33.3% above normal ($P < 0.001$). Counting the eosinophils showed that in the early stages of the experiment (5th and 9th days) there was a sharp decrease in their number in the mesentery, by the 15th day it showed a tendency to increase, and on the 30th day the number of eosinophils did not differ significantly from that in intact rats.

The results indicate that in experimental hypoparathyroidism a marked increase in vascular permeability is observed at all times of the experiment, accompanied by increased degranulation of the mast cells and liberation of biogenic amines into the intercellular ground substance, with a simultaneous rise in the serum histamine concentration. At the same time a sharp decrease was found in the number of tissue eosinophils which, as we know, possess histaminase activity, in the early stages of the experiment [8].

An important role in the mechanism of increased vascular permeability and of the ultrastrucutral changes in the vessel walls in hypoparathyroidism is thus played by the biogenic amines of the mast cells, increased liberation of which takes place, in the writers' view, because of a decrease in the Ca^{++} concentration in the blood serum and, correspondingly, in the tissue fluid [9]. A fall of the Ca^{++} ion concentration is known to lead to increased permeability of cell membranes for Na^{+} ions [5]. As a result of penetration of Na^{+} ions inside the cell biogenic amines are liberated from the labrocytes by a cation exchange mecha-

nism [6]. This hypothesis is also confirmed by the results of the writers' previous investigations, which showed a marked fall in the Ca^{++} content in the mast cells and endothelium of the mesenteric microvessels in experimental hypoparathyroidism [2].

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INHIBITION OF PLATELET AGGREGATION BY IMMUNE COMPLEXES.

I. CLINICAL STUDIES

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There is as yet no general agreement regarding physiological interaction of immune complexes (IC) and platelets. Precipitates of IC are known to induce platelet aggregation (PA) [14]. This lay at the basis of a method of detecting IC in blood serum by their ability to induce PA *in vitro* [12]. At the same time it was noted that human platelets, unlike platelets of laboratory animals, are not aggregated by IC [4], and it was also suggested that the presence of F_c-receptors for IgG on the surface of the platelets determines their aggregation only under the influence of IC formed by class G antibodies [9].

The physiological mechanism determining interaction between platelets and circulating IC assume special significance in atherosclerosis and its clinical manifestations. In the pathogenesis of atherosclerosis, hypotheses postulating the primary nature of the lesion in the endothelial layer of blood vessels and/or proliferation of smooth-muscle cells of the arterial wall are being increasingly accepted [2, 13]. One of the possible mechanisms of damage to the endothelium may be the pathological action of antigen–antibody complexes, the level of which rises in ischemic heart disease (IHD) and myocardial infarction [5, 10, 16]. We also know that in atherosclerosis platelet dysfunction is observed, with an increased tendency toward aggregation [3]. Finally, it has been shown that smooth-muscle cells are powerful stimulators of PA [1]. Consequently, in the process of atherogenesis, injury to the

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