agreement with data obtained by Italian workers [5], who showed that the number of H2c Purkinje cells begins to increase between the 9th and 12th passages after birth.

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MORPHOLOGICAL DATA ON INCREASED FUNCTIONAL ACTIVITY OF MAST CELLS IN SPONTANEOUSLY HYPERTENSIVE RATS

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The system of mast cells (MC), capable of secreting biologically active substances (histamine, serotonin, heparin, etc.) which are deposited in their cytoplasm, is a highly important close-range peripheral regulator of microcirculatory permeability [1, 4]. Through their influence on the tissue blood flow, MC in chronic arterial hypertension ought evidently to reflect changes in circulatory conditions.

The object of this investigation was to study the general morphological characteristics of MC in spontaneously hypertensive rats (SHR) before the development of hypertension and during the period of stable elevation of the arterial pressure. The test object was the dura mater, which is a convenient object with which to study the state of the connective tissue MC system.

EXPERIMENTAL METHOD

Female Kyoto—Wistar rats aged 2-22 weeks, with blood pressure of 170-190 mm Hg were used. The control consisted of inbred female NKWR (Kyoto—Wistar) rats with blood pressure of 70-120 mm Hg (Table 1). Allowing for the circadian rhythm of MC activity in the dura [2] the rats were decapitated at 10-11 a.m. The dura was stretched on a slide, dried, and treated by the method in [3]. Preliminary tests showed that luminescence microscopy revealed all MC but does not allow their functional state (stage of the secretory process or type of MC) to be identified. To determine these parameters sections were stained with 0.1% toluidine blue solution and the number of MC in the different stages was counted and expressed as a percentage. The ratio between these values gave the secretory formula for the whole MC population of the dura. For these counts and determination of the total number of MC in the dura the whole area of the latter was "scanned" by means of a square ocular diaphragm, corresponding to one field of vision of the preparation with an area of 1 mm^2. The total number of MC counted in each specimen of dura ranged from 800 to 1000 in 500 fields of vision, with a ×25 objective. The following criteria were used for guidance when determining the secretory formula of MC. MC with a mean diameter of 10.8 ± 0.3 μ and with marked metachromasia, with readily distinguishable separate granules and outline of nucleus, and also very dark MC whose cytoplasm was

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TABLE 1. Age and Weight of Animals Used in Experiment

Group of animals	Number of animals	Age, weeks	Body weight, g	BP, mm Hg
Spontaneously hypertensive rats	18	2	21,3±0,7	—
	12	4	47,0±2,0	106±3,6
	12	8	113,0±5,1	157±2,9
	6	22	213,0±5,2	190±6,1
Normotensive rats	12	2	21,3±0,6	—
	12	4	68,0±3,6	89±3,8
	12	8	112,0±4,9	89±6,2
	8	22	204,0±2,0	127±1,7

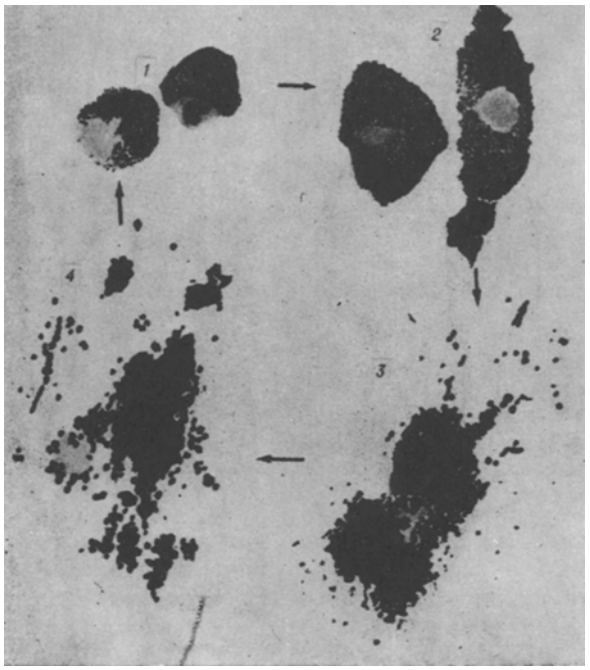

Fig. 1. Principal types of MC. 1) Young forms, 2) mature nonsecreting, 3) mature secreting, 4) degranulating. Stained with toluidine blue, 672 ×.

densely packed with granules were classed in type 1. Type 2 consisted of mature, nonsecreting MC with a mean diameter of 18.1 ± 0.5 μ, abundant metachromatic granules, and hardly distinguishable nuclear outlines, and also MC with single granules. Type 3 included MC in a state of apocrine secretion, 20.5 ± 0.5 μ in diameter, whose nucleus was either completely masked by granules, or was difficult to distinguish among them. Type 4 consisted of MC with a holocrine type of secretion, with the appearance of aggregation of many scattered granules with no distinct cell boundaries (Fig. 1).

EXPERIMENTAL RESULTS

A characteristic arrangement of MC mainly along the course of blood vessels and nerves was observed in the dura of rats of all ages. In the transverse sinus was an aggregation of small (7.0-10.0 μ) MC, up to 40 in number in one field of vision. The number of MC per unit area of dura varied only a little in different age periods. This topography of MC distribution also was characteristic of SHR of all age groups tested. However, rats with hypertension differed from the control group by having significantly more MC. Whereas at the age of 2 weeks this difference was very small and was not statistically significant, once the blood pressure had started to rise (4 weeks) and later, corresponding to the stage of stable, high hypertension (8 weeks), the number of MC in these animals was 23% greater than the number in rats of the control group.

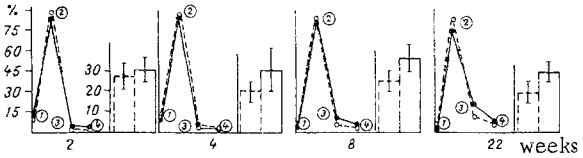

Fig. 2. Morphometric criteria of functional activity of MC. Columns bounded by broken line — number of MC/mm^2 dura of NKWR rats; columns bounded by continuous line — number of MC/mm^2 dura of SHR. 1-4) Principal types of MC.

Differences between the two groups also were found on analysis of the secretory formula of MC. It will be clear from Fig. 2 that the cell population in the experimental and control animals consisted mainly of mature, nonsecreting MC. In rats aged 2 and 4 weeks 9-12% of the cells were young forms, and only single cells were in the phase of secretion (and in a state of degranulation). Starting with 8 weeks, the proportion of secreting cells, including degranulating cells, increased in rats of both groups, and this was accompanied by some decrease in the relative proportion of young forms of cells (Fig. 2). In rats which had developed stable hypertension the increase in the number of mature secreting cells was more marked than in the control. Both an increase in the number of MC (hyperplasia) and the character of the change in ratio between the different types of cells on determination of the secretory formula thus point to strengthening of the functional activity of the MC system in SHR, coinciding in time with the appearance of a stable rise of arterial pressure.

The concrete causes of intensification of secretory activity of the MC system in rats with spontaneous hypertension are not yet sufficiently clear. We know that the secretion process itself in MC is controlled by the intracellular concentration of hydrated Ca^{++} [6]. Since in spontaneous hypertension, just as in essential hypertension in man, membrane regulation of cytoplasmic calcium is disturbed in a number of cell objects [5], it can be tentatively suggested that increased secretory activity in the MC system also reflects disturbances in the calcium-transport system of the MC plasma membrane, to the same degree as is observed in other types of cells. In this connection strengthening of functional activity of the MC system in SHR can be regarded as a component of functional disturbances of the same order as hyperactivity of adrenergic terminals and increased contractility of vascular smooth muscles, the cause of which has been reliably shown to be a disturbance of the mechanism of membrane regulation of the intracellular calcium concentration. There is no doubt that qualitative changes in the secretory function of cells releasing heparin sulfate, serotonin, histamine, and other biologically active substances into the tissue require study.

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