tained a potent vasoconstrictor substance, probably angiotensin, which was also present in arterial blood but not in renal vein blood from the untouched right kidney. After 3 weeks the left kidney ceased to release a pressor agent and in addition no such agent was demonstrable in the general circulation by bioassay. Hence the chronic stage of unilateral renal hypertension could not be attributed to the direct vasoconstructive effect of a humoral pressor agent.

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Mast Cell Population Density in Rat Skin. (31296)

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The relationship of the mast cell, its presence and distribution within the skin, to the general vitality of skin has yet to be defined. Part of this difficulty is due to insufficient information about the distribution of mast cells within skin. Some investigators have determined the distribution as a function of surface area of tissue examined, and others have used subjective and nonparametric systems(2,5,8,9,12). It is obvious that in order properly to appreciate the role of the mast cell in skin, distribution within the tissue should be realistically determined. Using a statistical cell counting method of Floderus (7), we previously(3) were able to study mast cell distribution in the liver. Applying this method to the problem of determining mast cell distribution in the skin of rats, a series of interesting observations has been collected and statistically analyzed for this report.

Materials and methods. Eight Sprague-Dawley rats, males and females, 3 to 4 months of age and *ca.* 350 g were used. The animals were noninfected, untreated, and were considered normal. Tissues were fixed in 10% neutral formalin and prepared by the paraffin technique. Five micron sections were stained in toluidine blue (0.05% in 70% ethanol, pH 4.4) for one minute followed by extraction in 4 changes of 95% ethanol for 15 seconds each. Microscopic examination was by bright field at 640×. In each rat 10 different skin regions of dermis were studied; *viz.*, lip, cheek, distal ear (*i.e.*, free end of pinna), dorsum, ventrum, footpad, and tail. In 3 of these regions, lip, dorsum, and footpad, the counts were extended to include superficial and deep portions of the connective tissue. A total of 20 fields were counted for each specimen, *i.e.*, 1600 fields in all.

The method developed by Floderus(7) for cell counting was used to make the mast cell population counts. The formula is:

$$N = \frac{n \cdot 1000}{a + d - 2h}$$

The notation is defined as:

N = total number of nuclei present in one cubic millimeter of tissue
n = number of nuclei, and nuclear fragments present in one square millimeter of tissue
a = thickness of the section in micra
d = diameter of the cell nucleus in micra
h = height in micra of the thinnest nuclear fragment included in the count

To make the count as accurate as possible, tissue shrinkage during histological processing was taken into account. Shrinkage increased the mast cell count slightly. Wichman(13) estimated that rat skin in paraffin sections would have 15% shrinkage, therefore all counts were corrected by this factor per unit volume.

The mast cells were measured with a Bausch and Lomb mechanical ocular micrometer, and with a size range of 10-20 μ for the longest axis of the cell, and 3-5 μ for the diameter of the nucleus.

The population counts were subjected to an analysis of variance to determine if there were significant differences between tissues. Then the Student-Neuman-Keuls' test(10) was applied to ascertain which of the mean counts were significantly different from each other. For this test the following formula was used:

$$W_p = q_\alpha(p_1 N_2) S_{\bar{x}}$$

$S_{\bar{x}} = \sqrt{(\text{error mean square})/r}$; the error mean square was obtained during the analysis of variance and was the sum of squares within the columns divided by the degrees of freedom;

r = number of replications (in this case 8);

Substituting in the formula:

$S_{\bar{x}}$ = 23,341,722/8
$S_{\bar{x}}$ = 1,707
p_1 = number of means
N_2 = degrees of freedom
q = index of probability
Wp = range; a measure of dispersion
F = ratio of variance of two populations
F_{01} = predicted F ratio which must be exceeded to indicate significant difference between populations at 99% probability.

By use of this formula the populations of each tissue were ranked, and it was possible to determine if significant differences existed between any of the various ranking positions.

Results. Table I summarizes all mast cell counts, corrected for tissue shrinkage. The analysis of variance showed there were significant differences among the various tissues. at F_{01} (7,79) = 2.87, the calculated F value was 28.19. Since this latter value exceeded that required for statistical significance, this meant that the mast cell populations were different in different tissues. In order to compare these differences tissue for tissue, the Student-Neuman-Keuls' test was employed. The results of this test permitted a graphic comparison of the population of each tissue with the populations of 9 other tissues. In Table II, the mast cell population density for each tissue examined is ranked from lowest to highest order. Based upon the W_p value for p ranking from 2 to

TABLE I. Mast Cell Population Density Corrected for Specimen Shrinkage.

	Lip papillary	Lip reticular	Cheek	Distal ear	Dorsum papillary	Dorsum reticular	Ventrum	Tail	Footpad papillary	Footpad reticular
1.	6,933	48,396	17,649	16,206	4,773	8,214	3,552	2,331	4,329	19,647
2.	7,659	45,621	19,758	10,212	5,994	7,215	2,553	2,300	3,400	20,424
3.	6,105	35,520	21,756	15,730	5,100	7,548	1,776	2,442	7,215	10,434
4.	6,150	14,940	28,749	18,900	4,550	2,664	2,550	2,553	4,440	13,330
5.	5,040	21,060	12,321	13,860	6,993	9,002	3,010	2,710	6,756	11,050
6.	8,280	16,020	16,380	11,340	5,106	6,060	2,650	2,570	3,060	12,600
7.	9,500	18,000	13,680	18,360	4,100	5,580	3,150	3,663	6,180	18,000
8.	10,212	17,880	18,981	11,700	5,100	3,600	5,800	2,070	7,420	11,700
$\bar{x}$	7,450	27,137	18,500	14,512	4,537	6,200	3,300	2,537	5,312	14,625
$\bar{\bar{x}}$ = 10,421										

TABLE II. Relation of Significance of Mast Cell Population Density in Different Tissues.

Tail	Ventrum	Dorsum pap.	Foot-pad pap.	Dorsum retic.	Lip pap.	Distal ear	Footpad retic.	Cheek	Lip retic.	
8	7	5	9	6	1	4	10	3	2	
2,537	3,300	4,537	5,312	6,200	7,450	14,572	14,625	18,600	27,137	
										P
					•———	———•		•———	———•	2
				•———	———————	———•				3
					•———	———•				
							•———	———————	———•	3
			•———	———————	———•					4
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					•———	———————	———————	———•		4
						•———	———————	———————	———•	4
		•———	———————	———•						
			•———	———————	———————	———•				5
				•———	———————	———————	———————	———•		
					•———	———————	———————	———————	———•	5
	•———	———————	———————	———————	———•					6
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•———	———————	———————	———————	———————	———•					7
	•———	———————	———————	———————	———————	———•				
		•———	———————	———————	———————	———————	———————	———•		7
			•———	———————	———————	———————	———————	———————	———•	7
•———	———————	———————	———————	———————	———————	———•				8
	•———	———————	———————	———————	———————	———————	———————	———•		8
		•———	———————	———————	———————	———————	———————	———————	———•	8
•———	———————	———————	———————	———————	———————	———————	———————	———•		9
	•———	———————	———————	———————	———————	———————	———————	———————	———•	9
•———	———————	———————	———————	———————	———————	———————	———————	———————	———•	10

10, significant differences between tissues are indicated by the lines extending between the columns for the particular tissues involved. For example, at p = 2, lip papillary level was significantly different from distal ear and at p = 5, lip papillary level was significantly different from lip reticular level. Thus, an overall comparison of significant differences was possible.

In 3 tissues there was an obvious difference in mast cell populations in the superficial and deep dermis; in order to make more meaningful comparisons, these regions were counted separately and were labeled as papillary and reticular. Fig. 1 demonstrates how such an area of skin appeared and delineates the two regions.

The Student-Neuman-Keuls' test gave results as summarized in Table II. These results indicated that the mast cell populations of the papillary regions of lip, dorsum, and footpad were the same. In these same 3 tissues the reticular populations were significantly different from each other. Also, in footpad and lip the papillary and reticular populations were significantly different, while in dorsum they were the same.

In other tissues, it appeared that ventrum and tail had populations similar to that found in the papillary regions noted above. Cheek and ear had similar populations, and were both similar to the populations of the footpad reticular region.

There were similar populations in tail, ventrum, papillary regions (dorsum, ventrum, footpad), and reticular dorsum. That is, these 6 skin regions had essentially the same mast cell populations.

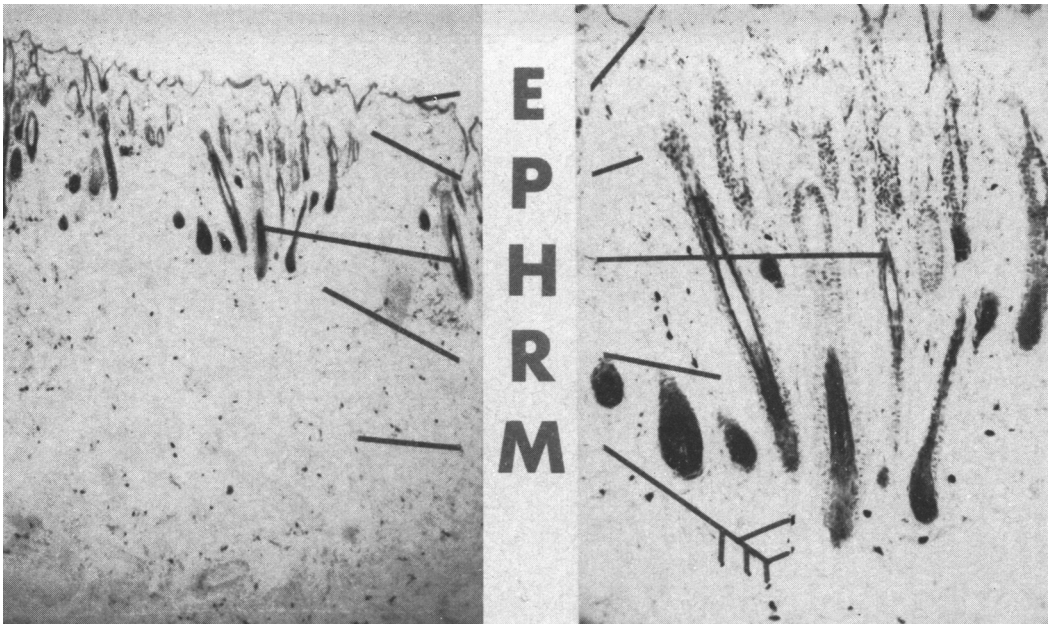

FIG. 1. Section of rat skin from dorsum, left: 35 ×, right: 160 ×. E = epidermis; P = papillary dermis; H = hair follicles; R = reticular dermis; M = mast cells.

The largest populations were in the head region of the animals, with the exception of the reticular footpad which also had a large population.

When the populations of all tissues were ranked according to increasing numbers, there was an increase in mast cell population generally from tail to head.

Discussion. Table I indicates that the largest mast cell population occurred in those skin regions which received most movement, for example the lip, which is subject to movement in several planes; and the cheek, with the movement of mastication. It might be expected that the highly movable tail would show a large mast cell population, but counts of the mast cells in tail skin were the lowest of all regions studied. This appeared to be an exception, but may be explained by the relatively immovable scale-like arrangement of rat tail skin.

The lips contained a large mast cell population. This may be related to the position of the mouth as portal of entry for infection, since the mast cell is involved in the inflammatory reaction, and lip is one of the fastest healing skin areas. Or, the population here may be related to special blood structures found in the rat lip-cheek region *viz.*, the sinus hairs. These are tactile hairs whose follicles are surrounded by blood-filled sinuses. Such a condition of relative blood stasis might lead to local thrombosis. Since part of the mast cell's granule content is heparin, the mast cell may function here to produce a local or tissue anticoagulant.

It was noted that the deeper layers of connective tissue in lip and footpad contained significantly greater numbers of mast cells than the more superficial layers. Emerson and Cross(6) found the same relationship in normal canine skin. This may be related to the more extensive vascularity of the deeper layers, and to the fact that mast cells are more numerous in perivascular areas.

In regard to the smaller number of mast cells in the superficial dermis there may still remain some error in these counts. The counts in Table I are based upon the staining techniques as outlined above. Toluidine blue clearly demonstrated mast cell metachromasia and the morphology of well-granulated cells. But observation of extended staining duration indicated an interesting possibility of mast cell tinctorial behavior.

Sections of rat skin were stained for 24

hours in toluidine blue. These sections showed heavy overstaining, but also demonstrated metachromatic granules in cells in and near the lower epidermal border. Simpson stated unequivocally that new mast cells are formed in this region of dermis(11). The counts in Table I showed lesser numbers of recognizable mast cells in this region. We may theorize that there are equally great numbers of mast cells here also, but containing very few or poorly demonstrable metachromatic granules. Csaba(4) found mast cells among normal epidermal cells; and Williams(14) and Mikail *et al*(8) also noted this fact.

If this is actually the case, then it remains to be determined why the mast cells of the superficial dermis are so poorly granulated. It may be that the chemical substances causing metachromasia, (*viz.*, acid mucopolysaccharides) are utilized rapidly for epidermal proliferation. Or, as some have suggested(4), the mast cell may originate from transformed epidermal cells and manufacture metachromatic granule materials as they migrate deeper into the dermal connective tissue. However, the present study appears to cast doubt on this hypothesis since in 2 tissues having papillary and reticular zones there existed significantly different numbers of mast cells; much greater numbers being present in the deeper tissue.

Considering another aspect of the results, where increasing numbers of mast cells were found going from tail to head, we can only speculate. Whether this indicates some manner of migration from a progenitor tissue, or a metabolic requirement of the more specialized head tissues for mast cell products, is not clear.

In conclusion, it appears that the proposed method of counting mast cells in skin has been found to be superior to simple area counts. It is more accurate since it is based upon the number of cells per unit volume of tissue, and makes provision for the different size ranges of mast cells encountered. This method has been used by others(1,12) using similar small numbers of animals. Our mast cell count results were in essential agreement with results from area counts done by other investigators on large numbers of animals(2,5,8,9) when all numerical results were prorated to cells per cubic millimeter basis.

Summary. Mast cell population counts were made in 8 normal rats of 10 skin regions. Counting was by the method of Floderus, which gives mast cell numbers per cubic millimeter of tissue. The results showed significant differences in the mast cell populations for various skin regions. Possible explanations of the population differences are discussed. The procedure for Floderus' counting method is given.

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