Relative number of mast cells (MC) in pinna of nude and normal Balb/c mice

nu/nu[a] Total count of MC	No. of scale projected	Average No. of MC per 1 scale unit (250 μm)	nu/+[b] Total count of MC	No. of scale projected	Average No. of MC per 1 scale unit (250 μm)
1115	96.0	11.60	864	88.8	9.72
641	56.2	13.20	423	43.7	9.67
789	52.0	15.17	796	69.8	11.40
638	53.0	12.78	435	43.0	10.10
1263	104.5	12.08	661	71.5	9.24
639	70.5	9.06			
638	64.0	9.96			

[a] Mean 11.9 ± 1.92; [b] mean 10.02 ± 0.84.

easier to perform. Contrary to earlier reports of elevated numbers of skin MC in 'nude' mice[10,11], we did not find any difference in the number of in the pinna of 'nude' and normal mice.

Materials and methods. 11-week-old Balb/c 'nude' (*nu/nu*) and litter-mate, normal heterozygotes (*nu/+*) mice of both sexes were used. The lower one-third of one pinna was removed, fixed 4–6 h in Bouin solution, embedded in paraffin and 7 μm sections were stained with 0.1% aqueous solution of toluidine blue, pH 7.6. Under 40 × 10 magnification, the total number of MC on both sides of elastic cartilage was counted alongside the projection of the Zeiss microplate (1 cm divided into 100 units) inserted in the ocular. Between 10 and 32 length of scale was projected into one section of pinna and, from each specimen, the number of MC was counted in 3–6 sections. Total number of MC from each section was pooled, divided by the number of scale projected, and the value obtained represented the average number of MC per length unit (250 μm). The mean values for both groups of mice were counted, and data obtained were statistically analyzed using Student's *t*-test at the level of $p = 0.05$.

Results and discussion. The table shows the number of animals used and the results obtained. The mean number of MC in pinna of nude mice was 11.98 ± 1.97, and, in those of normal heterozygotes, 10.02 ± 0.84. This difference is not significant at $p > 0.05$. The distribution of MC in both cases was similar. They were distributed homogenously in the skin of pinna.

The results are in contrast to those obtained by others[10,11] in skin taken from the back. KELLER et al.[11] reported nearly 4 times higher concentration of skin MC in 'nude' Balb/c mice than in normal mice. VIKLICKY et al.[10] found 3 times more MC in skin of 'nude' $B_{10}LP$ than in control mice.

The histology of pinnas in both 'nudes' and heterozygotes was similar, and the number of hair follicles and glands is equal. The only difference was lack of or retardation of hair development. The mitotic activity in the epidermis and glandular follicles, although not examined systematically, seemed to be identical in both cases.

We do not have an explanation for the discrepancy of skin MC content in regard to the site (back skin, pinna skin). The presence or absence of the 'nu' gene may be more important in determining the number of MC than skin site. The presence of this gene in both 'nude' and control mice may be responsible for similar MC content. KELLER et al.[11] used normal Balb/c (+/+) for their controls, while in the present study *nu/nu* were compared to *nu/+* animals.

It would be interesting to find whether the presence of large amounts of cartilage in pinna tissue exert some modifying effect on MC content. This possibility will be examined. At present, we are inclined to consider the local factor(s) or 'nu' gene in regulation of skin MC numbers more than the influence of the thymus itself. This assumption is based on the more pronounced histological difference of back 'nude' skin and hair-covered skin in contrast to pinnas, and on the lack of drastic differences in the absolute number of MC in lymph nodes.

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Foetal Blood Abnormality Associated with Hypodactyly in the hd Strain of Rat

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Summary. The homozygous foetuses of hypodactyl rats (hd strain) present an obvious red blood cell macrocytosis (day 14 of gestation). This blood abnormality could give rise to thrombosis leading to early necrosis of the extremities.

Some mutations are known to affect the number of digits in mouse[1], or to lead to limb amputations in rabbit[2], cat[3] and man[4]. Among them, the strain of br rabbit (brachydactylia), first described by GREENE and SAXTON[2] has been studied by several authors[5–8].

In that strain, a blood abnormality has been shown: the foetal primordial red cells are especially large and numerous, and could give rise spontaneously, between days 15 and 16 of gestation, to thrombosis, haemorrhages and necrosis of the extremities[7]. The lesions can be pre-

vented in utero by several treatments acting upon the foetal blood (phenylhydrazine, hyperoxia[7], folic acid, folinic acid and vitamin B_{12}[8]. The preventing action on macrocytosis of the last three treatments seems to indicate a failure in the folate metabolism, via alterations in the cell division. Moreover, pyrimethamine, a drug which inhibits the activity of the dihydrofolate reductase, and thus the formation of tetrahydrofolate, when administered to pregnant rats, induces foetal malformations, including brachydactylia and limb defects: syndactylia, oligodactylia and phocomelia[9]. It has been shown[9] that pyrimethamine could induce both foetal macrocytosis and necrosis of the extremities in the foetus.

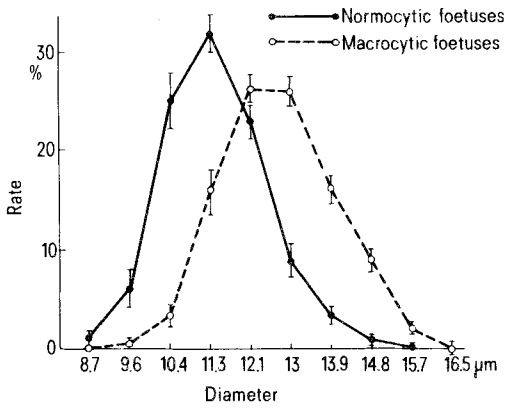

Fig. 1. Distribution of nucleated primordial cell diameters in hd rat foetus (day 14 of gestation). Two populations are visible. (Mean ± S. E. M.).

Thus, in many cases, there is apparently a relationship between the existence of a foetal blood macrocytosis and the formation of thrombosis followed by necrosis and amputations.

A strain of rats exists, the hd strain, first described by SABOURDY and BOZIC[10], in which the homozygous foetuses present digit abnormalities manifested both in their number and form. The hd gene is also responsible for sterility observed in the homozygous male. Hypodactyly is inherited as a recessive character in both sexes.

In this study, we tried to find out whether a blood abnormality, similar to that observed in the br rabbit foetus, could be discovered in the hd rat strain, what could permit an explanation of these abnormalities of the extremities.

Hypodactyl mutation in the hd rat occurred in a Wistar strain of rats in which a female carrier has been mated with a male of the Long Evans strain. All the animals have the black-hooded pattern. Hypodactyl rats

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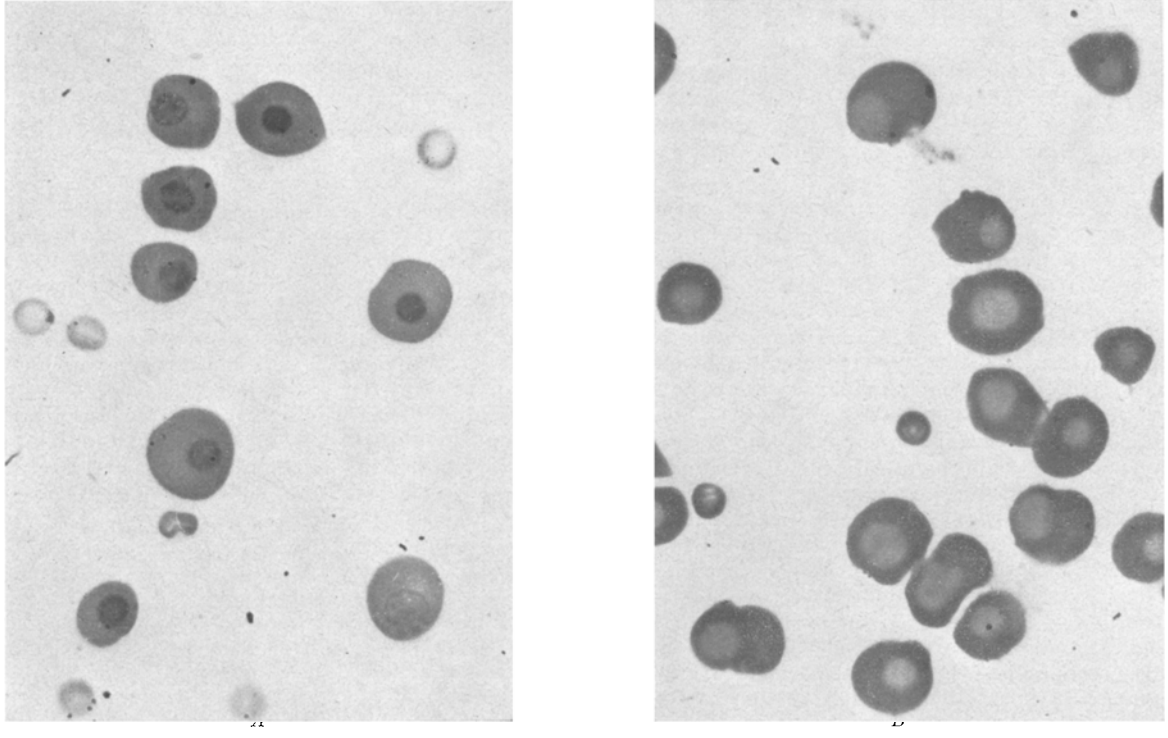

Fig. 2. Nucleated primordial cells in hd rat foetuses. (X 1100), (Day 14 of gestation). *A* Normocytic primordial cells. *B* Macrocytic primordial cells.

show a reduction in the number of digits of both forefeet and hindfeet. The forefeet are generally tridactyl and the hindfeet tetradactyl.

Material and methods. We used matings between hypodactyl females (hd/hd) and normal heterozygous males (hd/+). In that type of mating, the ratio of normal to hypodactyl is not significantly different from 1:1[11].

Normal heterozygous males (hd/+) were introduced to the cage of females to mate, and 12 days later, the pregnant females were recognized by palpation.

In order to study the foetal blood, day 14 of gestation was chosen, since the nucleated red cells are still numerous at that stage. Five pregnant hd/hd rats were operated and the foetuses removed on day 14, blood smears were made and stained by the panoptic method. The microscopic image of the blood cells was projected on paper with a projection microscope. For each foetus, the outline of 200 nucleated red cells was drawn and the diameters directly measured. The frequency of each diameter was determined (36 foetuses from 5 mothers).

Results and discussion. Although it seems almost impossible to distinguish the abnormal foetuses macroscopically at that stage (the abnormalities become apparent on day 15), the study of the foetal blood cells permit one to observe two populations in the ratio 1:1 (Figure 1): in 17 foetuses, a very obvious macrocytosis is seen: about 80% of the cells have a diameter > 12 µm, while in 19 foetuses, only 36% are > 12 µm. The difference between the two percentages is statistically significant (method of χ^2: $p < 0.001$). Moreover, the aspect of the nucleated red blood cells is very different in the macrocytic cells to normals; their nucleus is generally large and irregular and paler than in normocytic cells (Figure 2).

The macrocytosis observed during the early foetal development of hd/hd rat resembles both that of br/br rabbit and the macrocytosis due to pyrimethamine. In the latter cases an impairment in the folate or vitamin B_{12} metabolism seems to be implicated. Moreover, in the hd/hd strain, foetal red cell macrocytosis is associated with sterility in the homozygous males, as has already been observed in man with such a metabolic failure[12].

The hd gene could be responsible for a disturbance of cell division which leads to macrocytosis. The abnormal nucleated red cells could then be destroyed too massively and could block up the smallest arteries of the extremities, which could give rise very soon to necrosis and amputation of the digits. Further work with vitamin treatment to the pregnant rat should determine the truth of this hypothesis.

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Rosette Formation by Human T and B Lymphocytes in the Presence of Adrenergic and Cholinergic Drugs[1]

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Summary. It was shown that adrenergic drugs, which increase the intracellular levels of cAMP, inhibit the rosette formation by T-lymphocytes, but stimulate the rosettes produced by B-lymphocytes. Cholinergic drugs, which increase the levels of cGMP, on the contrary, stimulate the formation of rosettes by T-lymphocytes but inhibit those produced by B-lymphocytes.

It was recently demonstrated that E-rosette formation by human T lymphocytes is either inhibited by drugs which increase the intracellular levels of cyclic adenosine monophosphate (cAMP)[2–4], or stimulated by drugs which raise the levels of cyclic guanosine monophosphate (cGMP)[5,6]. In this paper we present evidence suggesting that human T and B lymphocytes bear adrenergic and cholinergic receptors whose stimulation results in antagonistic effects on rosette formation.

Material and methods. Lymphocytes. Lymphocyte suspensions obtained from peripheral blood of normal subjects by Ficoll-Hypaque were adjusted to contain $2 \cdot 10^6$ cells/ml. *Red blood cells*: Unsensitized (E) sheep red blood cells (SRBC) were prepared weekly in Hank's solution. Sensitized (EAC) SRBC were prepared according to LAY and NUSSENZWEIG[7], using rabbit antiserum and human complement.

Drugs. The following drugs (Sigma Chemical Co.) were assayed: theophilline 10^{-3} *M*, papaverine 10^{-3} *M*, isoproterenol $2 \cdot 10^{-4}$ *M*, dibutyryl cAMP $2 \cdot 10^{-4}$ *M*, carbamylcholine 10^{-6} *M*, pilocarpine 10^{-3} *M*, dibutyryl cGMP $2 \cdot 10^{-4}$ *M*, atropine sulfate 10^{-6} *M*. Solutions of the drugs were prepared just before use in Hanks' solution with final pH adjusted to 7.4.

Rosette assays. Drug effects on rosette formation were assayed by incubation 0.45 ml of the lymphocyte suspension containing $2 \cdot 10^6$ viable cells/ml with 0.05 ml of the drug dilution, for 60 min at 37 °C. The mixture was separated in two tubes, each receiving, respectively, 0.5 ml of a 0.25% suspension of unsensitized (E) and sensitized (EAC) SRBC. Control tubes received no drugs. The tubes were then centrifuged at room temperature for 5 min at 200 *g* and incubated at 37 °C for 30 min (for B-lymphocyte rosettes) or at 4 °C for 60 min (for T-lymphocytes rosettes). The resulting pellets were gently resuspended and counted in a hemocytometer. The viability

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