Although this hypothesis could account for the increased ammonia production in L15, it does not explain why the levels of activity of the urea cycle enzymes were lower in *A. means* liver cultured in L15 than in MEM (assuming that the level of urea cycle enzyme activity is related to the amount of urea excreted)[4]. The high concentrations of amino acids may themselves produce inhibitory effects. For example, MAAS and CLARK[15] showed that all the enzymes (including OTC) involved in arginine synthesis in *E. coli* were repressed in the presence of arginine by a repressor protein synthesized by a regulator gene. It is possible that a similar type of control mechanism could operate in *A. means* hepatocytes.

On the basis of the hypothesis outlined above, it could be predicted that a high concentration of glutamic acid in the culture medium would result in increased ammonia release from cultured *A. means* liver fragments. However, during the 9-day experimental period total ammonia production was much lower in MEM + 10 m*M* glutamic acid (86 ± 6 µmoles/g wet weight). This finding may also be related to transaminase activity in the cultured tissue. Glutamic acid may be deaminated by glutamate dehydrogenase and the amino groups fed into excretory pathways, or it may be transaminated to other amino acids by transaminase enzymes such as GOT and GPT. The equilibrium constants for transamination reactions are low, so that the reactions are freely reversible, depending on relative substrate concentrations. It is therefore possible that high concentrations of glutamic acid in culture medium stimulate transaminase reactions in the direction: glutamic acid → other amino acids. This implies that, in cultured *A. means* liver, large quantities of glutamic acid are preferentially converted by transaminase enzymes, but that glutamate dehydrogenase activity is sufficient to maintain a maximum level of urea excretion without the production of excess free amino groups which would be released as ammonia[16].

Thus, it is proposed that in *A. means* liver cultures, urea excretion proceeds at the maximum rate under culture conditions; in high concentrations, alanine and glycine are deaminated, either directly or via glutamic acid, resulting in an increase in ammonia production; a high concentration of glutamic acid stimulates its transamination to other amino acids with a corresponding reduction in ammonia production.

[15] W. K. MAAS and A. J. CLARKE, J. molec. Biol. *8*, 365 (1964).
[16] D. BROWN, N. FLEMING and M. BALLS, Gen. comp. Endocr., in press (1976).

Mast Cells in the Skin of Normal, Hairless and Athymic Mice[1]

R. KELLER[2], M. W. HESS and J. F. RILEY

Institut für Medizinische Mikrobiologie, Arbeitsgruppe für Immunbiologie, Schönleinstrasse 22, CH-8032 Zürich (Switzerland); Pathologisches Institut der Universität Bern, CH-3000 Bern (Switzerland); and Radiotherapy Department, Ninewells Hospital, Dundee DD2 1UB (Scotland), 15 October 1975.

Summary. The skin of congenitally athymic *nu/nu* mice is rich in mast cells which stain metachromatically, contain histamine and 5-hydroxytryptamine, and participate in the PCA reaction. Mast cells of athymic mice have thus the attributes of normal mast cells.

BURNET[3,4] has recently reiterated his view that the tissue mast cell represents an end cell of the T lymphocyte. Congenitally thymus-deprived (*nu/nu*) mice thus provide a convenient test system for analyzing this hypothesis by comparing the mast cell content in the skin of *nu/nu* and normal mice. Since chemical carcinogenesis in normal mouse skin is accompanied by a local accumulation of mast cells[5], skin reactions and mast cell responses following topical application of a chemical carcinogen were also examined in normal Balb/c, in athymic *nu/nu* Balb/c, and in 'hairless' (*hr/hr*) mice.

Material and methods. Normal pathogen-free Balb/c mice, hairless (*hr/hr*) mice (Institut für Biologisch-Medizinische Forschung AG, Füllinsdorf, Switzerland) and congenitally athymic nude mice (*nu/nu*, third backcross generation with Balb/c; Bomholdgård, Ry, Denmark) were painted twice, one week apart, along the centre of the back with 0.2 ml of an 0.25% solution of 7,12,dimethylbenz(a)anthracene (DMBA) in acetone containing no promoting agent; untreated or acetone-treated animals served as controls. Mice had neither been shaved nor epilated before topical application of DMBA. In a few athymic mice, Balb/c thymus was implanted subcutaneously 3 weeks before first DMBA painting.

For histology, samples of skin taken from controls or 3 months after DMBA painting were fixed in Baker's calciumformol, cleared in methylbenzoate, embedded in paraffin, sectioned at 3 to 4 µm, and stained with toluidine blue (pH 3.0) for mast cells. Mast cell counts are expressed per linear cm of sectioned skin. Fixed, unstained sections were examined for 5-hydroxytryptamine (5-HT) fluorescence (excitation 380–415 nm; emission 520 to 530 nm).

For the estimation of histamine, portions of freshly excised dorsal skin were rapidly weighed and extracted with 10% trichloroacetic acid for subsequent assay on the standard guinea-pig ileum preparation.

The capacity to bind reaginic antibody was tested by passive cutaneous anaphylaxis (PCA). Normal Balb/c mice were immunized by an i.p. injection of 100 µg bovine serum albumin (BSA) and *Bordetella pertussis* vaccine[6]. Dilutions of sera with a PCA titer of 1:80 to 1:320 were injected into the skin of normal and *nu/nu* Balb/c mice. 48 h later, the binding of reaginic antibody was assessed by i.v. injection of 1 mg BSA in 0.5 ml 0.25% Evans blue in saline.

[1] This work was supported by grants from the Swiss National Science Foundation (No. 3.516.71 and 3.234.74) and the Fritz Hoffmann-La-Roche-Stiftung.
[2] The skilful technical assistance of Miss R. KEIST is gratefully acknowledged.
[3] F. M. BURNET, J. Path. Bact. *89*, 271 (1965).
[4] F. M. BURNET, Med. Hypothesis *1*, 3 (1975).
[5] J. F. RILEY, Experientia *24*, 1237 (1968).
[6] I. MOTA and J. M. PEIXOTO, Life Sci. *5*, 1723 (1966).

	Normal Balb/c		*nu/nu* Balb/c			'Hairless' mice	
	Controls	DMBA treated	Controls	DMBA treated	Thymus implant DMBA treated	Controls	DMBA treated
Initial No. of animals	6	12	10	16	6	6	10
No. of animals surviving 3 months	6	12	7	12	6	6	9
Animals with papillomas	none	10	none	none	none	none	none
Number of mast cells	160 (± 10)	420 (± 50)	610 (± 110)	557 (± 50)	470 (± 45)	380 (± 50)	355 (± 20)
Mast cell metachromasia	+++	+++	+++	+++	n.d.	+++	+++
Mast cell fluorescence	+++	+++	++	++	n.d.	+++	+++
Skin histamine	0.0139 (± 0.0035)	n.d.	0.0421 (± 0.0144)	n.d.	n.d.	0.0996 (± 0.0295)	n.d.
Passive cutaneous anaphylaxis	+		+			n.d.	

n.d. = not done.

Results. The first and striking observation on painting the 3 groups with DMBA was a complete lack of response of *nu/nu* and *hr/hr* skin to the surface application of the carcinogen. Normal Balb/c mice developed a local inflammatory skin reaction and epilation beginning at the end of the first week. Neither *nu/nu* nor *hr/hr* mice displayed these skin changes. By 3 months, 20 of 22 surviving Balb/c mice bore papillomatous skin tumors whereas exposure to DMBA was not followed by detectable changes in the skin of the other two groups (Table).

Histologically, the findings were equally unexpected for by far the largest numbers of mast cells in untreated skin were seen in athymic *nu/nu* mice (Table). Here, small elongated cells with characteristic granules lay parallel to the epidermis, and larger, more rounded cells were scattered throughout the deeper dermis. In the skin of the few *nu/nu* mice which had received a thymus implant, the number of mast cells and their variations in size were as in athymic *nu/nu* mice. The skin of normal Balb/c mice, rather poor in mast cells, reacted to DMBA by a marked increase in the number of mast cells under the now thickened epidermis, and their accumulation was most pronounced around the base of the skin tumors. Skin of *hr/hr* mice contained large numbers of well developed mast cells (Table).

Pharmacologically, the findings for histamine paralleled the mast cell content observed in the 3 groups of mice, taking into account the wide variations in size of the mast cells in *nu/nu* skin. Mast cells in all 3 groups displayed a golden-brown fluorescence in UVL, characteristic of 5-HT[5]. This was most obvious in mast cells clustered around the base of a papilloma[7].

Finally, the skin of *nu/nu* mice bound reaginic antibody to a comparable extent, as did normal Balb/c mice, thus indicating that the number of receptors for reaginic antibody is not diminished.

Discussion. Two points of interest emerge. The first concerns the striking lack of response to the surface application of DMBA in *nu/nu* and *hr/hr* mice. The carcinogen was effective, as shown by the development of skin tumors in the majority of Balb/c mice. Hair follicles are thought to provide the preferential portal of entry for topically applied hydrocarbons in mice[8]. The almost total lack of follicles in adult *hr/hr* mice, and their scarcity in *nu/nu* mice, together with a thickening of the interfollicular epidermis, may thus account for the early failure in both groups to show erythema and the subsequent development of papillomas.

The second point to be emphasized is the rich content of mast cells in the skin of congenitally athymic *nu/nu* mice. By generally accepted criteria, these are normal mast cells: they stain metachromatically, they contain histamine and 5-HT, and they participate normally in the PCA reaction. Whatever the origin and function of such cells may be, they can hardly be derived from the thymus. This fails to support the premise of BURNET[3,4] that the tissue mast cell is an end-stage of the T lymphocyte.

[7] R. E. COUPLAND and J. F. RILEY, Nature, Lond. *187*, 1128 (1960).
[8] B. C. GIOVANELLA, J. LIEGEL and C. HEIDELBERGER, Cancer Res. *30*, 2590 (1970).

Biological Activity of Vernoflexuoside on the Basis of *Allium* Test

S. KOHLMÜNZER, J. GRZYBEK and W. KISIEL

Department of Pharmaceutical Botany, Medical Academy, 16 Krupnicza Street, Kraków (Poland), and Institute of Pharmacology PAN, Kraków (Poland), 18 August 1975.

Summary. Biological activity of vernoflexuoside (Vf) a new sesquiterpene lactone glucoside, isolated from *Vernonia flexuosa* Sims was investigated by means of *Allium* test. Vf showed cytostatic activity and produced mitotic disturbances as well as symptoms of nuclear structure degeneration.

In the course of phytochemical investigation of *Vernonia flexuosa* Sims (*Compositae*), cultivated in the experimental plots of the Pharmacological Institute of Polish Academy of Sciences in Cracow, a guaianolide sesquiterpene lactone glucoside, so far unknown, was isolated by KISIEL[1] as the major constituent of the roots. This compound was named vernoflexuoside (Vf) by the author, who also established its structure (Figure 1).

The cytostatic activity of containing α-methylene-γ-lactone grouping was suggested, since the other representatives of this chemical group are known to be active[2]. Thus, an evaluation of the effect of this compound on the