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ELEMENTAL LEVELS IN MAST CELL GRANULES DIFFER IN SECTIONS FROM NORMAL AND DIABETIC RATS: AN X-RAY MICROANALYSIS STUDY

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Abstract

Mast cells around the thymus of rats stain red with alcian blue and safranin indicating that the mast cells are probably of the peritoneal (connective tissue) type. After the onset of streptozotocin induced diabetes some cells contain both red and blue granules and blue staining cells may appear.

X-ray microanalysis of frozen freeze-dried sections from diabetic male CSE Wistar rats showed electron dense granules to have similar amounts of S to normal rat mast cell granules but reduced levels of Na, Mg, P, Cl and K. Two cells also had electron lucent granules with very high levels of Na, Cl, K and Ca and reduced concentrations of S.

The differences in elemental composition suggest that the mast cells from diabetic rats are not immature, but are related to the condition of induced diabetes, and that granules of very different composition can occur within a single cell.

X-ray microanalysis has given an insight into mast cell granule elemental content which was not possible by conventional biochemical methods.

Key Words: X-ray microanalysis, electron probe, frozen sections, thymus, mast cells, diabetes.

Introduction

A previous study has documented the elemental composition of normal rat mast cell granules from the thymus using freeze dried frozen sections and fully quantitative X-ray microanalysis (Kendall and Warley, 1986). Unlike granules extracted in non-ionic media which are said to be unstable in the presence of the mineral cations Na^+, K^+, Ca^{++} and Mg^{++} (Padawar, 1979), the in vivo granules contained all of these elements in addition to low levels of Fe, Zn and Ca. Differences in elemental composition between in vivo studies and organelles studied after separation can be expected and have been documented for nuclei (Jones et al., 1979). The examination of freeze dried frozen sections is also important as Yarom et al. (1975) did not mention K from work on conventionally fixed, dehydrated and Epon-embedded material.

The normal mast cell granule concentrations of S and K were high and in most cells the correlation between the elements was highly significant with an S:K ratio generally of < 2.2. A few cells had greater ratios due to lower K levels. One of these cells was judged immature (stage II, Combs, 1966). The high S content was anticipated from the known granule composition of mainly cationic proteins, acid mucopolysaccharides including the sulphated polysaccharide heparin, and bioamines (Padawar, 1979). Despite the identification and isolation of a binding protein (Lagunoff et al., 1964; Uvnas et al., 1970; Bergqvist et al., 1971; Uvnas and Aborg, 1977) the details of the binding of the granule components is not established. It was proposed therefore that the high K may play an important part in stabilising the granule components, and that the K levels may increase with maturity.

This paper presents data on mast cell granules in rats made diabetic by a single injection of streptozotocin and compares the results with normal mast cell granules. Whilst large numbers of animals were used for the light and conventional electron microscopic studies it was not possible to obtain data from many animals for X-ray microanalysis but the results are presented as the differences between the granules in normal and diabetic rats is large and very little is known about their elemental content.

Materials and Methods

For light and conventional electron microscopy thymus glands were excised from normal adult male CSE Wistar rats weighing between 280 - 350 gms after anaethetisation with sodium pentobarbitone.

Diabetes was induced in animals of a similar weight by treatment with 1 intraperitoneal injection of streptozotocin (55 mg/kg body wt in citrate buffer at pH 4.5). Glucose levels in the urine were monitored using Labstix (Ames). Full details of the methods used for handling the diabetic animals are given in Chatamra et al. (1985).

Tissue for light microscopy from 14 normal and 16 diabetic rats was fixed in 10% formal-saline, embedded in paraffin wax and cut at 10 µm. The sections were stained with alcian blue in magnesium chloride at 0.5 or 0.7M concentrations for the identification of mast cells or with haematoxylin and eosin. Sections were also stained with a combined alcian blue and safranin technique according to Csaba et al. (1969). For electron microscopy small pieces of tissue from 7 normal and 16 diabetic rats were immersed in Karnovsky's fixative for 1 hour at room temperature, washed in cacodylate buffer, pH 7.2, and post fixed in 1% osmium tetroxide for 1 hour at 4 °C before dehydration and embedding in araldite resin. Sections were stained with uranyl acetate and lead citrate (Venable & Coggeshall, 1965) and examined in a Hitachi H-300 microscope.

Separate animals were used for X-ray microanalysis. These were treated as described above but the animals were bled through the heart before the thymus was rapidly dissected out. Sections were examined from 5 diabetic rats but mast cells suitable for analysis were only found in the connective tissue of the capsule in animals diabetic for 16 days (1 cell) and 42 days (7 cells). The results were compared with data from a previous study of the elemental content of granules in 19 mast cells from 5 normal adult male rats weighing approximately 250 gms (Kendall and Warley, 1986). The thymuses analysed are part of a series of diabetic animals under study, and they are considered typical of others rendered diabetic for similar periods of time (Warley, in press).

Small blocks of tissue were frozen onto stubs in Freon 22 cooled in liquid nitrogen. Sectioning was performed at -65 to -70°C with a Slee cryoultramicrotome, and sections approximately 300 nm thick were collected onto Formvar or pioloform coated Ni grids (150 or 300 x 75 mesh). The sections were allowed to freeze dry at the cutting temperature for 1 h before being transferred to a desiccator of the same temperature that contained a molecular sieve. The sections were then allowed to warm up overnight to room temperature when they were carbon coated before analysis in an AEI EMMA-4 fitted with a Link Systems 860 series 2 energy dispersive detection system.

Analysis proceeded for 100s live time at 60kV accelerating voltage and 4 nA beam current measured with a Faraday cage. The specimen area was cooled with gaseous nitrogen to reduce contamination. The analyses were conducted at x 10,000 with a probe diameter of 0.5 cm (0.8 μm^2). Spectra were processed using the Quantem-FLS software (Link Systems Ltd., High Wycombe, Bucks, England) as described in Hall and Gupta (1982). For quantitation, reference was made to standards in gelatin, the preparation of which is fully described in Kendall et al., (1985).

Results

Alcian blue with 0.7M magnesium chloride enabled mast cells to be identified in the capsule around the thymus glands (Fig. 1), along septa carrying blood vessels into the gland and occasionally within the cortex. The mast cells were spaced about 0.5 - 1 mm apart around the gland in normal rats, but were closer in diabetic rats as the thymus glands were greatly atrophied. All of the mast cells in the normal glands, and most in diabetic glands stained red with alcian blue and safranin. Some of the long term diabetic rats had mast cells with both red and blue granules and also some blue staining cells were observed.

Under the electron microscope sections of the membrane - bound, homogeneous, electron dense granules were observed closely packed together with rarely more than 0.2 µm between adjacent granules. The maximum granule diameter was about 1 µm in all the normal and many of the diabetic rats (Fig. 2). However, in rats diabetic for at least 1 month, there were some cells which had 2 forms of granule. Up to half of the granules were as described above but the remainder appeared larger, particulate and more electron lucent with an electron light region under the granule membrane (Fig. 3).

Frozen freeze-dried sections (Fig. 4) revealed little cytoplasm between the granules so it was not possible to analyse the cytoplasm in any of the mast cells of diabetic rats. Three cells had sectioned nuclei visible from which 12 observations indicated mean nuclear concentrations for K of 330±61 mmol per kg dry wt and for S, 186±33 mmol per kg dry wt.

The elemental concentrations of the granules from 2 diabetic rats are compared in Table 1 with the value for granules obtained from a previous study of 5 normal rats (Kendall and Warley, 1986). When compared with the normals, the 42 day diabetic rat had similar levels of S and Fe, significantly less Na, Mg, P, Cl and K and raised levels of Zn and Ca (although for these 2 elements the levels observed were very low so that the results should be treated with caution). The S:K ratio was 17.5±3.7 (n = 50) compared with a ratio of 3.0±3.7 for normal rat mast cells (n = 158) and the correlation coefficient for S:K in this diabetic rat was 0.74. The 16 day diabetic rat had 1 cell that was analysed, and the concentrations, apart from low S, were intermediate between the normal and long term diabetic values.

In the rat diabetic for 42 days, 2 additional cells were analysed each with a mixture of electron dense and electron lucent

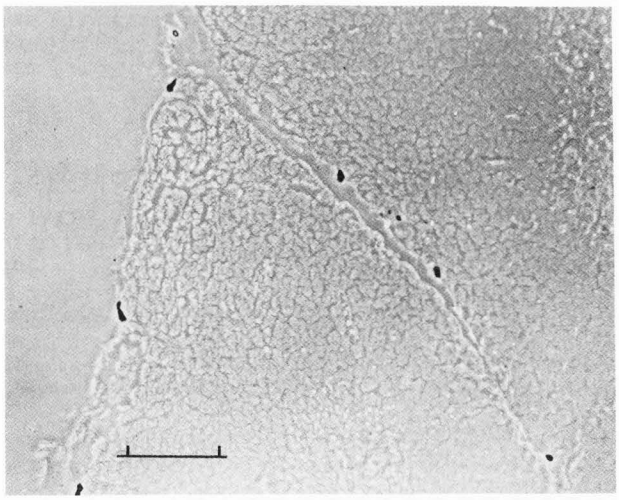

Figure 1. Low power view of a rat thymus stained with alcian blue and 0.7 $MgCl_2$ to show the mast cells in the capsule. Bar = 100 μm.

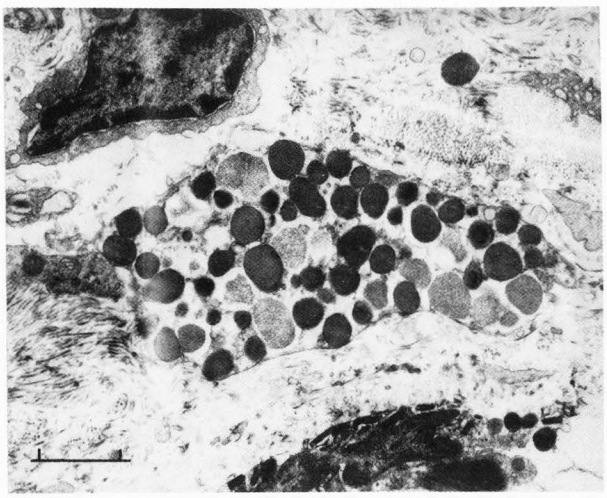

Figure 3. EM of a mast cell from a rat diabetic for 45 days to show electron lucent and dense granules within the same cell. Bar = 10 μm.

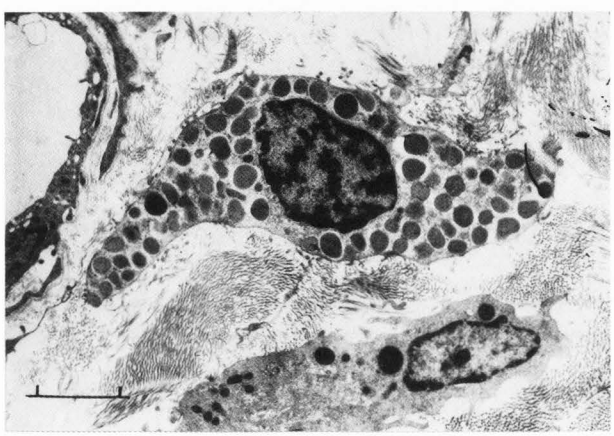

Figure 2. EM of a typical mast cell from a rat diabetic for 3 days to show the size and distribution of the electron dense granules. Bar = 10 μm.

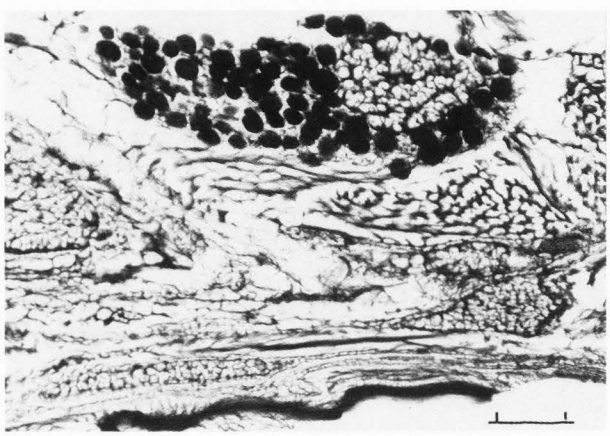

Figure 4. Frozen freeze-dried section of a mast cell from a rat diabetic for 45 days. Bar = 10 μm.

granules; the results are compared in Table 2 with the 5 cells in which all the granules were electron dense. All the electron dense granules were of a similar elemental content despite some differences (S ($P = < 0.01$); Cl ($P = < 0.1$); and K ($P = < 0.01$)), but the electron lucent granules were highly significantly higher in Na, Cl, K and Ca, had less S and Zn, an S:K ratio of 1.8±0.03 and a correlation coefficient for S and K of 0.96.

Discussion

Previous studies have documented the changes in body weight and thymic weight that occur with the onset of diabetes caused by a single injection of streptozotocin (Chatamra et al., 1983; 1985) and the animals used in this study were typical of those previously described.

Alcian blue with defined molar concentrations of magnesium chloride can be used to stain all mast cells in tissues, whereas alcian blue and safranin together can be used to differentiate mast cell populations. Mast cells in the rat, as in other animals, are heterogeneous (Lee et al., 1985; Katz et al., 1985) especially with respect to their proteoglycan content. Peritoneal mast cells (connective tissue mast cells) contain heparin and usually stain blue with the combined alcian blue and safranin stains but mucosal mast cells contain chondroitin sulphate di-B and usually stain red. Rat thymic mast cells show some characteristics of peritoneal mast cells when grown in culture (Ishizaka et al., 1976, 1977) so that the red staining found in this study is consistent with those studies and probably indicates a peritoneal type of mast cell with heparin in the granules. However, of interest is the finding of red and blue granules in the

same cell, and the appearance of blue stained cells in the diabetic animals. It appears that the same cell can have granules of different composition under certain conditions.

There are several factors that make mast cells in tissue sections difficult to find and analyse, and account for the small numbers of cells and animals considered here for the X-ray microanalysis studies: mast cells are well separated in the tissues; microanalysis blocks are smaller than those required for conventional electron microscopy; and only cells in the central areas of the grid and not close to a grid bar were analysed. Thus any conclusions can only be tentative. However, the order of changes observed is great, and it is expected that further work should consolidate most of the findings.

An earlier study (Kendall and Warley, 1986) considered in detail the elemental content of normal rat thymic mast cell granules including the sources of error and factors affecting the results. The results were compared with a few analyses of mast cell cytoplasm, and with red blood cells analysed from the same tissue sections. It was concluded that K and S were both greatly elevated in the granules, and that the K must be bound. The elevated K levels were surprising as other X-ray microanalysis studies of the highly proteinaceous exocrine pancreatic granules (Nakagaki et al., 1984; Koomans and Wei, 1985) had reported low levels of K. The granule composition is however completely different in mast cells as the granules contain a great excess of heparin, a sulphated mucopolysaccharide, over the amines and cationic protein (West, 1959). The high K levels observed in normal mast cell granules have a precedent in other cells usually where there are mucopolysaccharides (Appleton et al., 1979; and studies quoted in Gupta and Hall, 1981). Also Scott (1978) considered the preferences of various polyanions for Na and K and found that ester sulphates strongly prefer K. Thus different levels of K in pancreatic and mast cell granules is explicable.

Potassium could be participating in the binding of the granule contents (mainly heparin, histamine or other amines, and a basic binding protein). This is an attractive idea as the binding of the contents is not fully explained, the presence of high K was not previously suspected and there is a close relationship between the S and K concentrations in the majority of cells as seen in the very high correlation coefficients between S and K and the low S:K ratios (maximum frequency was 0.8-0.9).

In this study whilst granule S levels generally remained similar to those of normal animals, K (also Cl, P and Na) levels were low in cells which had entirely electron dense granules. This is reflected in the very high S:K ratios observed in these granules. High S:K ratios (also due to low K, but with no correlation between the elements) were found in 6 cells in the previous study, and one of those cells had an immature morphology. Thus it was previously proposed that the high ratios might be related to maturation events. In the diabetic rats, cells filled with electron dense granules did not show golgi, rough endoplasmic reticulum nor extensive cytoplasm between granules and were not morphologically like the immature cells previously found in normal rats. Also the S:K ratios were higher and there was some degree of correlation between S and K concentrations. It is more likely that the mast cells in diabetic rats were affected either by streptozotocin or the diabetic state.

Streptozotocin is considered to have a specific effect on pancreatic B cells secreting insulin, and Chatamra et al. (1985) consider that the effects on the thymus are related to diabetes, not the streptozotocin. Warley (1987) has reported that K is reduced in diabetic rat thymocytes. Furthermore there is no reason to suspect a loss of K from these sections during preparation as high K levels were found in electron lucent granules adjacent to electron dense granules with low K in 2 cells (analyses performed less than 0.4 μm apart).

The 2 cells with both electron lucent and electron dense granules are difficult to interpret. From conventional EMs it was thought that the cells may have partially degranulated as there are similarities with the observations of Csaba and Olah (1968) and Csaba et al. (1969), but if they are the same cells as those which stain red and blue with alcian blue and safranin, other possibilities may exist. Csaba (1969) found that in a cell free system the colours of the reaction changed with amine heparin content and considered this a reflection of maturity changes. The electron lucent granules may have a different biochemical composition from the electron dense granules: they certainly have a very distinct elemental composition (less S, more Na, Cl, K and Ca). This makes them very different from the possible immature cells of normal rats. The effect of the diabetic state on the mast cells is unknown and interpretation of the status of these cells will have to await further research.

Conclusions

The mast cells of the normal rat thymus are probably of the peritoneal (connective tissue) type. Their elemental content of high S and K is distinctive. After a single injection of streptozotocin to induce diabetes mast cells examined 16 days or 42 days later had granules with similar levels of S but greatly reduced concentrations of Na, Mg, P, Cl and K although morphologically they were similar to normal rat mast cells. Some cells had granules of 2 forms - electron lucent and dense. The electron lucent granules differed from the rest in having reduced levels of S, and raised concentrations of Na, Cl, K and Ca. Since these results are based on small numbers of animals and cells it is only possible to suggest that these changes are not related to maturity changes in the granules, but to the diabetic state.

Acknowledgements

The author wishes to thank the Sir Jules Thorn Charitable Trust for financial support, Dr Warley for invaluable discussions and Mr Ian Morris for technical help.

Table 1. Mean (± SE) concentrations of elements (mmol/kg dry wt) in mast cell granules from 19 cells from 5 normal rats, 1 cell from a rat diabetic for 16 days and 5 cells from a rat diabetic for 42 days. Student's t tests were applied to the results for granules from normal and rats diabetic for 42 days and *** = P <0.001; ** = P <0.01; ns = not significant; n = number of granules.

	NORMALS	DIABETICS		
		16 days	42 days	t-values
	n = 158	n = 13	n = 50	
Na	278±16	151±25	111±10	8.9***
Mg	20±2	13±3	12±2	2.6**
P	142±6	154±24	62±4	11.2***
S	1418±56	833±73	1567±72	ns
Cl	371±18	236±21	123±7	13.0***
K	944±57	221±19	104±8	14.6***
Fe	3±1	5±1	4±1	ns
Zn	10±1	6±1	13±1	-2.4**
Ca	-8±1	-1±1	1±1	-6.1***

Table 2. Mean (± SE) concentrations of elements (mmol/kg dry wt) in 2 mast cells which had both electron dense and lucent granules compared with cells from a rat diabetic for 42 days. The t-values are derived from Student's t tests applied to cells in 42 day diabetic rats and to electron-lucent granules. *** = P <0.001; ** = P <0.01; * = P <0.1 and ns = not significant; n = number of granules.

	DIABETICS			
	42 days	E-dense	E-lucent	t-values
	n = 50	n = 10	n = 27	
Na	111±10	119±15	825±52	-13.5***
Mg	11±7	18±8	27±7	- 2.1*
P	62±4	52±16	64±6	ns
S	1567±72	1269±72	770±54	8.9***
Cl	123±7	168±15	436±29	-10.4***
K	104±8	78±5	437±31	-10.5***
Fe	4±1	6±2	8±2	-1.8*
Zn	13±1	10±2	4±2	5.4**
Ca	1±1	0±2	40±4	-8.4***

Note: The mean Na value for the normal rat granules was incorrectly given in the previous publication Kendall and Warley (1986).

References

Appleton TC, Newell PF, Machin J (1979) Ionic gradients within mantle-collar epithelial cells of the land snail Otala lactea. Cell Tissue Res. 199, 83-97.

Bergqvist U, Samuelsson G, Uvnas B (1971) Chemical composition of basophil granules from isolated rat mast cells. Acta Physiol. Scand. 83, 362-372.

Chatamra K, Daniel PM, Kendall MD, Lam DKC (1983) A preliminary report on the histological changes in the thymus found in rats rendered diabetic with streptozotocin. J. Anat. 137, 413-414.

Chatamra K, Daniel PM, Kendall MD, Lam DKC (1985) Atrophy of the thymus in rats rendered diabetic by streptozotocin. Horm. Metab. Res. 17, 630-632.

Combs JW (1966) Maturation of rat mast cells. An electron microscope study. J. Cell Biol. 31, 563-575.

Csaba G (1969) Mechanism of the formation of mast-cell granules. II. Cell free model. Acta biol. Acad. Sci. Hung. 20, 205-210.

Csaba G, Olah J (1968) Mechanism of the formation of mast cell granules. I. Ultrastructural and histochemical study. Acta biol. Acad. Sci. Hung. 19, 347-62.

Csaba G, Surjan LJr, Fischer J, Kiss J, Toro IJr (1969) On the mechanism of mast cell formation. Effect of glucocorticoids on the mast cells of normal and thymectomized rats. Acta biol. Acad. Sci. Hung., 20, 57-74.

Gupta BL, Hall TA (1981) Ions and water in cells and tissues studied by microprobe analysis of frozen-hydrated sections in the SEM. 39th Ann. Proc. Electron Microscopy Soc. Amer. Atlanta, Georgia. Claitor's Publishing Division, Baton Rouge, Louisiana, 654-656.

Hall TA, Gupta BL (1982) Quantification for the X-ray microanalysis of cryosections. J. Microsc., 126, 333-345.

Ishizaka T, Okudaira H, Mauser LE, Ishizaka K (1976) Development of rat mast cells in vitro. I. Differentiation of mast cells from thymus cells. J. Immunol. 116, 747-754.

Ishizaka T, Adachi T, Chang TH, Ishizaka K (1977) Development of rat mast cells in vitro. II. Biologic function of cultured mast cells. J. Immunol. 118, 211-217.

Jones RT, Johnson RT, Gupta BL, Hall TA (1979) The quantitative measurement of electrolyte elements in nuclei of maturing erythrocytes of chick embryo using electron-probe X-ray microanalysis. J. Cell Sci. 35, 67-85.

Katz HR, Stevens RL, Austen KF (1985) Heterogeneity of mammalian mast cells differentiated in vivo and in vitro. J. Allergy Clin. Immunol. 76, 250-259.

Kendall MD, Warley A (1986) Elemental content of mast cell granules measured by X-ray microanalysis of rat thymic tissue sections. J. Cell Sci. 83, 77-87.

Kendall MD, Warley A, Morris IW (1985)

Differences in apparent elemental composition of tissues and cells using a fully quantitative X-ray microanalysis system. J. Micr. 138, 35-42.

Lagunoff D, Phillips MT, Iseri, OA, Benditt EP (1964) Isolation and preliminary characterization of rat mast cell granules. Lab. Invest. 13, 1331-1344.

Lee TDG, Swieter M, Bienenstock J, Befus AD (1985) Heterogeneity in mast cell populations. Clin. Immunol. Rev. 4, 143-199.

Nakagaki I, Sasaki S, Shiguma M, Imai Y (1984) Distribution of elements in the pancreatic exocrine cells determined by electron probe X-ray microanalysis. Pflugers Arch. 401, 340-345.

Padawar J (1979) Mast cell structure: implications for normal physiology and degranulation. In: The Mast Cell. (eds J. Pepys & A.M. Edwards). London: Pitman Medical.

Roomans GM, Wei X (1985) X-ray microanalysis of resting and stimulated rat pancreas. Acta Physiol. Scand. 124, 353-359.

Scott JE (1978) Some comments on specific ionic interactions. In: Ions in Macromolecular and Biological Systems. (eds D.H. Everett & B. Vincent), 132-133. Proc. 29th Symp. Colston Res. Soc. Bristol: Scientechnica.

Uvnas B, Aborg H-C (1977) On the cation exchanger properties of rat mast cell granules and their storage of histamine. Acta Physiol. Scand. 100, 309-314.

Uvnas B, Aborg H-C, Bergendorff A (1970) Storage of histamine in mast cells. Evidence for an ionic binding of histamine to carboxyls in the granule heparin-protein complex. Acta Physiol. Scand. 336 (Suppl.) 3-26.

Venable J, Coggeshall R (1965) A simplified lead citrate stain for use in electron microscopy. J. Cell Biol. 25, 407-408.

Warley A (1987) Alterations in the metabolism of potassium in thymocytes of diabetic rats. J. Physiol. (in press).

West GB (1959) Tissue mast cells and tissue amines. J. Pharmac. Pharmacol. 11, 513-534.

Yarom R, Maunder CA, Hall TA, Dubowitz V (1975) Microanalysis of mast cells in rat muscle. Experientia 31, 1339-1340.

Discussion with Reviewers

G.A. Kuijpers: Do you have an explanation for the fact that K is less in the granules of diabetic animals? For instance does the change in K indicate a change in the binding capacity for K causing K to leak out of the granules? Or does it possibly suggest a change in K transport or granule membrane potential leading to a decreased concentration of non-bound thermodynamically active K in the granule? Is there any way of distinguishing between the possibilities?

Author: In diabetic patients with insulin lack the increased amounts of acetyl co-A in fat metabolism are converted into ketone bodies because of the intracellular depletion of the carbohydrate components of the cycle. The dissociated ketone bodies yield hydrogen ions causing a metabolic acidosis which results in acidification of the urine and an eventual loss of Na^+ and K^+ in addition to the cationic loss accompanying glucose induced osmotic diuresis. Total body Na^+ and K^+ are depleted and whereas serum Na^+ falls, that of K^+ does not at the expense of the intracellular K^+ (partly due to acidosis and also because the movement of K^+ into cells is dependent on insulin and glucose). Insulin effects on adipose tissue, muscle and liver are mainly studied in diabetes, and whilst stimulated peripheral blood lymphocytes have insulin receptors the situation in the thymus is unclear. However, the thymus does have a very high mitotic rate and insulin receptors could be expressed in this population of cells. The rapid response of the thymus to a single injection of streptozotocin suggests that the gland is responsive to insulin so that cation loss could occur there and I would suspect that changes in K transport may be basic to the observations of this paper. However, there is no experimental evidence that can aid an explanation of the observations recorded in this paper and since K (and other cations) were not previously suspected of being important components of the granules other factors may be responsible for the loss of K from mast cell granules in the diabetic state.

G. Hook: What is the author's speculation linking the low K concentration in the granules and the increased number of mast cells around the thymus gland in diabetic animals?

Author: Firstly it may be only an apparent increase in the number of mast cells around the gland as the thymus atrophies rapidly after the induction of diabetes but the connective tissue around does not appear to be mobilised (at least in the short term). A separate stereological study needs to be undertaken to judge numbers relative to thymus cortex and medulla and to connective tissue mass. If an increase of mast cell numbers is substantiated, then it is important to know if the physiological abilities of the mast cell are compromised so that the production of mast cells may go up to compensate, or whether more mast cells in general are needed by the body for some aspect of the diabetic state. Both are fascinating problems but beyond the scope of this paper.