Observations upon the Mast Cells of Rat Foetal Tissues

In 1953 RILEY and WEST[1] drew attention to a strong positive correlation between the histamine and mast cell content of a variety of normal and pathological tissues and noted that all their evidence suggested that tissue mast cells are rich in histamine. DIXON[2] also observed close correlation between the mast cell count and the histamine content in the skin of rats from $18^1/_2$ days gestation until the 7th day *post partum* but showed that histamine may be present in other tissues several days before the first appearance of mast cells in those tissues. For example, she found histamine in whole foetus extracts as early as the 11th day of gestation although HOLMGREN[3], to whom she referred, had found no mast cells in foetuses less than 14 mm in length (ca. 16th day of gestation). KAHLSON, ROSENGREN, and WHITE[4], who appear also to have relied upon HOLMGREN'S histological findings, state that histamine may first be detected on the 9th day of gestation and noted that foetal liver is a richer source of histamine than even a dog mastocytoma. They emphasized, however, that little histamine is found in foetal tissues, whose actual content of histamine is never very high.

A wider survey of the histological literature reveals a number of reports in which the first appearance of subcutaneous mast cells corresponds reasonably well with HOLMGREN'S findings; MAXIMOW[5] in 11 mm foetus; ALFEJEW[6] in 13 mm foetus: ARVY[7] in foetus of 16th day of gestation (for a comparison of foetal length and age, see DONALDSON[8]). LAGUESSE[9] and WEBB[10], however, first found subcutaneous mast cells a short time before birth and on the 19th day of gestation (although this was equated with 5 days before normal delivery) respectively.

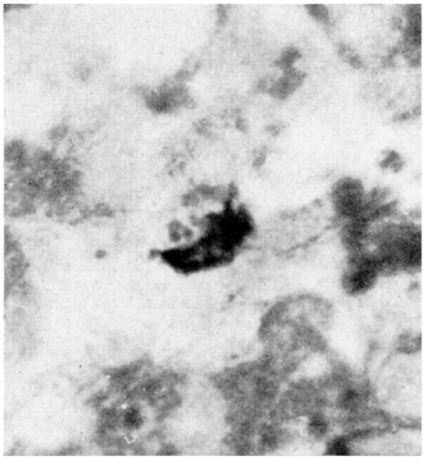
The first appearance of mast cells in more deeply situated connective tissue and in certain viscera is more variously reported. MAXIMOW[5] found them in the liver, the spleen and, indeed, in any organ which he studied in stages later than the 11 mm foetus; these stages were not specified. ALFEJEW[6] was more explicit in her report. By the 17 mm stage mast cells were present in the spleen and in deep connective tissue between muscles, around joints, in the mediastinum and between abdominal viscera. By the 28 mm stage they were present also in the mucosa of the stomach and in developing lymph nodes. URTUBEY[11] reported juvenilelike mast cells as first appearing 'en plein mesenchyme' of the 12 mm rat embryo. There are, then, discrepancies between these authors' reports and HOLMGREN'S; the latter found mast cells in tissues other than subcutaneous only in foetuses approaching 30 mm in length.

Despite the extensive evidence that the mast cells of the adult rat are rich in histamine it is not claimed that mast cells are the only source of histamine in the tissues[12]. It seems certain that they store histamine and can release it when suitably stimulated; their absence from histamine-rich foetal tissues might merely indicate that they have yet to assume their adult role. The discrepancies in the histological accounts of foetal tissues, however, remain and have seemed worthy of some investigation.

Foetuses for study were obtained by crossing Holtzman females with Wistar males. Mating was confirmed by vaginal smear and any pregnancy resulting was considered to begin in the middle of the night concerned. 26 foetuses were examined histologically for mast cells. 17 of these ($14^1/_2$, 15(X2), $15^1/_2$ (X4), $16^1/_2$ (X6), $18^1/_2$ (X2), and 20 (X2) days of gestation) were fixed in 10% formalin and stained with Mallory's methylene blue after paraffin sectioning. 9 ($14^1/_2$ (X5) and 15 (X4) days of gestation) were fixed and stained by the method of SMITH and ATKINSON[13]. Litter mates of these foetuses were measured for C. R. length and although small variations were noted between siblings, the lengths corresponded closely with the age/length table given by DONALDSON[8].

The only criterion for the identification of tissue mast cells appears to be the presence of metachromatically staining granules in the cytoplasm of otherwise undistinguished cells and this criterion was used. In some cells the granulation was sparse so that it was possible to make out a pale, central, granule-free zone which, presumably, corresponded with the nucleus. In most cells containing metachromatic granules, however, the granulation was so dense as to obscure the nuclear region.

In the six foetuses of $14^1/_2$ days gestation, treated by either of the histological techniques, no mast cells were seen. Nor were any seen in the formalin/methylene blue foetuses of 15 days gestation. In three of the four 15 days gestation foetuses treated by the method of SMITH and ATKINSON, and in all the older foetuses ($15^1/_2$–20 days gestation) mast cells were present in the subcutaneous connective tissue. In the 15 days specimens, mast cell granulation was slight and the cells rather sparsely scattered but by $15^1/_2$ days of gestation typically granule-rich mast cells were present and, in places, quite numerous. In one $15^1/_2$ days gestation specimen a few typical mast cells were found in the liver (Figure), and liver mast cells were found in all the older foetuses. The number of mast cells in the liver was not large at any stage, but was perhaps constant relative to the bulk of the liver itself. By $16^1/_2$ days of gestation mast cells were constantly found in the deep connective tissues, including the mesen-



Mast cell in liver of $15^1/_2$ day rat foetus (× 1700).

[1] J. F. RILEY and G. B. WEST, J. Physiol. *120*, 528 (1953).
[2] JOAN B. DIXON, J. Physiol. *147*, 144 (1959).
[3] H. J. HOLMGREN, Acta anat. *2*, 40 (1946).
[4] G. KAHLSON, ELSA ROSENGREN, and T. WHITE, J. Physiol. *151*, 131 (1960).
[5] A. MAXIMOW, Folia haematol. *4*, 611 (1907).
[6] SOPHIA ALFEJEW, Folia haematol. (Lpz.) *30*, 111 (1924).
[7] L. ARVY, C. R. Ass. Anat. XLIII Réunion, 165 (1956).
[8] H. H. DONALDSON, *The Rat*, 2nd Ed., American Anatomical Memoirs, Wistar Institute (Philadelphia 1924).
[9] E. LAGUESSE, C. R. Soc. Biol. *82*, 1415 (1919).
[10] R. L. WEBB, Anat. Rec. *64* (Suppl. 4), 55 (1936).
[11] L. URTUBEY, Bull. Histol. appl. A, La Physiol. *25*, 151 (1948).
[12] J. F. RILEY, *The Mast Cells* (E. & S. Livingstone Ltd., Edinburgh and London 1959).
[13] E. W. SMITH and W. B. ATKINSON, Science *123*, 941 (1956).

teries of three of the six specimens studied. By $18^1/_2$ days of gestation mast cells were more numerous in all the tissues mentioned and a few were also present in the substance of somatic muscles. In the oldest foetuses studied ($20^1/_2$ days of gestation) mast cells were very numerous in the mesenteries, especially in relation to blood vessels, and in all connective tissue. A few were also found in the endoneurium of the nerves to the extrinsic ocular muscles.

Blood mast cells were not seen in any of the foetuses studied (unless those found constantly in the liver fall into that category), and a tendency for mast cells to lie close to blood vessels was noted only in relation to the mesenteric mast cells of the oldest ($20^1/_2$ day) foetuses.

The present observations approximate quite closely to those of ALFEJEW[6] and do not contradict MAXIMOW'S[5]. It is clear that mast cells are much more widely distributed in rat foetal tissues in specimens between 17 and 28 mm long (say $15^1/_2$–$20^1/_2$ days gestation) than HOLMGREN has reported, or than DIXON[2] and KAHLSON et al.[4] (relying only upon HOLMGREN'S report) had supposed. It is equally clear, however, that histamine is first present in rat foetal tissues some four or five days before mast cells may be recognized there and that the source of this histamine, while still unknown, is in cells other than mast cells.

In view of the report by BENDITT, WONG, ARASE, and ROEPER[14] that serotonin occurs in mast cells obtained from rat peritoneal washings there might seem to be some correlation between DIXON'S report that 5-Hydroxytryptamine is first detectable in the foetus of $15^1/_2$ days gestation and our own finding of the earliest mast cells in the subcutaneous connective tissues of foetuses of the same age. However, since PARRAT and WEST[15] have apparently been unable to confirm that serotonin occurs in the mast cells of adult rat skin, the possibility of morphologically identical mast cells having various biochemical potentials must be considered, as well as the idea that the developing mast cells may shift their roles as the surrounding tissue alters with maturation. In spite of these considerations, the interrelationship between foetal serotonin and mast cells may be more apparent than real.

Résumé. Dans les études physiologiques récentes, il est dit que l'histamine foetale se montre plusieurs jours avant l'apparition des mastocytes dans les tissus foetaux. Le présent travail démontre que les mastocytes apparaissent plus tôt et sont plus répandus qu'on ne le supposait. Ces constatations confirment d'anciennes observations apparemment oubliées.

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[14] E. P. BENDITT, R. L. WONG, M. ARASE, and E. ROEPER, Proc. Soc. exp. Biol. Med. *90*, 303 (1955).

[15] J. R. PARRAT and G. B. WEST, J. Physiol. *134*, 169 (1954).

Zur Nahrungsaufnahme von *Megoura viciae* Buckt., einer phloemsaugenden Aphide (*Homoptera, Rhynchota*)

Die Tracer-Methode ist vorzüglich geeignet, Untersuchungen am Saugakt und zur Nahrungsaufnahme pflanzensaugender Insekten durchzuführen. So liegen speziell über phloemsaugende Aphiden mehrere Arbeiten vor[1–7]. In allen Fällen wurde die Wirtspflanze mit P^{32} markiert und nach dem Saugen der Läuse deren Radioaktivität gemessen. Auf diese Weise wurden Probleme des wechselseitigen Verhältnisses Wirtspflanze und Insekt bearbeitet, vor allem auch im Hinblick auf den Übertragungsmechanismus phytopathogener Viren.

Vorliegende Untersuchungen befassen sich speziell mit dem Problem der Nahrungsaufnahme bei einem Phloemsauger. Dabei stehen drei Fragen im Vordergrund: (1) Wann erreichen die Läuse mit ihren Stechborsten das Phloem? (2) Nehmen die Tiere auch ausserhalb des Phloems Nahrung auf? (3) Wie verläuft die Nahrungsaufnahme in Abhängigkeit von der Zeit? Daneben ergaben sich beim Experimentieren noch andere interessante Fragen, wie im folgenden noch näher ausgeführt wird.

Junge, etwa 15 cm hohe *Vicia faba*-Pflanzen wurden mit den Wurzeln in phosphatfreie Knoopsche Nährlösung gestellt, der markiertes Phosphat zugesetzt war. Die spezifische Aktivität der Lösung betrug 0,3 mC/ml. Die apteren Virgines von *Megoura viciae* erwiesen sich besonders wegen ihrer Grösse für die Versuche als gut geeignet, da die Läuse jeweils einzeln gut beobachtet und nach Aufnahme des Tracers gemessen werden konnten. Es wurde hier also nicht, wie meist üblich, mit Gruppen von Versuchstieren gearbeitet, denn diese Methode kann, wie aus den Ergebnissen deutlich wird, sehr leicht zu fehlerhaften Resultaten führen. Die zu den Versuchen verwendeten Tiere waren stets «Saugtiere», das heisst, sie wurden unmittelbar, ohne Hungerperiode, von der nicht radioaktiven Pflanze auf die markierte Versuchspflanze gebracht. Diese Tiere stechen nämlich sehr bald nach der Übertragung wieder an und wechseln ihren Saugort viel seltener als «Hungertiere», die vor dem endgültigen Anstich erst zahlreiche kurzdauernde Probestiche ausführen. Die von den Tieren aufgenommene Aktivität wurde unter Korrektur des Nullwertes und physikalischen Abfalls genügend lange gemessen.

Allen *Aphididae* ist ein typisches Saugverhalten gemeinsam. Der Anstich erfolgt durch senkrechtes Aufsetzen des Labiums auf die pflanzliche Epidermis und daran anschliessendes, äusserlich nicht mehr sichtbares Vordringen der Stechborsten in das Innere der Pflanze. Dabei werden die Antennen der Laus langsam nach hinten geklappt, so dass sie schliesslich fast dem Abdomen anliegen[8,9]. Der Beginn des Anstichs ist auch bei *Megoura viciae* äusserlich gut sichtbar. Je 30 bis 60 Läuse wurden in dieser Saughaltung über 1, 3, 5, 7, 10, 20 min usw. auf der markierten Pflanze einzeln beobachtet, nach der entsprechenden Zeit entfernt und ihre Aktivität gemessen.

In Übereinstimmung mit Untersuchungen an *Aphis fabae* Scop.[4] ergab sich, dass in den ersten Minuten nach Beginn des Anstichs keinerlei messbare Aktivität aufgenommen wird. Erst nach 7 min können die Läuse radioaktiv werden, und danach steigt die von ihnen inkorporierte Aktivität sprunghaft an. Es darf also mit Sicherheit angenommen werden, dass die Läuse bereits nach 7 min

[1] M. F. DAY und H. IRZYKIEWICZ, Austr. J. Biol. Sci. B *6*, 98 (1953).

[2] M. A. WATSON und H. L. NIXON, Ann. appl. Biol. *40*, 537 (1953).

[3] C. J. BANKS und H. L. NIXON, Entomol. exp. appl. *2*, 77 (1959).

[4] E. HENNIG, Naturw. *46*, 410 (1959).

[5] W. KLOFT, Z. angew. Ent. *45*, 337; *46*, 42 (1960).

[6] W. KLOFT und H. KUNKEL, Proc. XI. Intern. Congr. Ent. (Wien 1960).

[7] W. KLOFT und P. EHRHARDT, Proc. Symp. *Radioisotopes and Radiation in Entomology* of the IAEA (Bombay 1960), im Druck.

[8] H. A. VAN HOOF, Med. no. 161 Lab. Ent. Wageningen (1958).

[9] J. MAREK, Diss. (Würzburg 1959).