Es ist möglich, dass die Strahlenschutzwirkung des Libriums ursächlich auf die Senkung der Körpertemperatur[4] und die Hemmung des Zellstoffwechsels[6] und Gesamtstoffwechsels (unveröffentlicht) zurückgeht. Soweit bisher ersichtlich, hat Librium auf den Abfall des Körpergewichts nach der Bestrahlung keinen Einfluss[7]. Eine ausführliche Darstellung (Probit-Transformation der Dosis-Mortalitätsbeziehungen ohne und mit Librium) erfolgt an anderer Stelle[8].

*Summary.* Librium 'Roche' (7-Chlor-2-methylamino-5-phenyl-3 H-1,4-benzodiazepin-4-oxyd-hydrochlorid) increases significantly the percentage of survival of lethally X-irradiated male mice in the dose range between 7.5 and 12.5 mg/kg.

A. LOCKER und H. ELLEGAST

*Österreichische Studiengesellschaft für Atomenergie (Institut für Biologie und Landwirtschaft des Reaktorzentrums Seibersdorf-Wien) und I. Medizinische Klinik der Universität Wien (Österreich), 11. Mai 1962.*

[6] A. LOCKER, Z. ges. exp. Med., im Druck.
[7] A. LOCKER, Physikal. Verhdlg. *4*, 175 (1962).
[8] Librium-Reinsubstanz wurde uns von der Fa. Hoffmann-La Roche, Wien, zur Verfügung gestellt.

## Leucine Aminopeptase in the Spleen

Leucine aminopeptidase (LAP) has been demonstrated by quantitative and histochemical methods in many tissues in which it participates in the disintegration and also, to some extent, the synthesis of proteins. The enzyme reacts with certain peptides and amides containing an N-terminal amino acid with an aliphatic sidechain[1]. LAP has been demonstrated in the spleen by quantitative methods, and NACHLAS reported that he demonstrated it in the spleen, also, by histochemical methods[2]. He did not, however, describe its localisation more accurately. In the present work the occurrence of LAP in mouse and rat spleen was studied histochemically.

The material comprised 40 white rats and mice of both sexes. The animals ranged in age from 1 day to 10 months. They were killed by rapid decapitation and 20 $\mu$ sections were cut immediately with a freezing microtome from the spleen which was removed intact. Some of the sections were stained with toluidine[3], or by a modification of Wolbach's Giemsa staining technique[4], for the control study. In the other sections LAP was determined histochemically by the method of NACHLAS et al.[2]. Acetate buffer (pH 6.5) or Sörensen's phosphate buffer (pH 6.6) was used in the incubation fluid. The preparations were incubated for 15 min to 4 h at 37° or 24°C.

The positive LAP reaction in the spleen of adult animals corresponded in intensity to the reaction produced by splenic tissue found in the same sections. According to the quantitative determination performed by GREEN[5], the LAP activity of the spleen is 12 and that of the pancreas 11 if the activity of the kidney is denoted by the value 100. The most pronounced positive reaction in mouse was established in the walls of the central arteries of the spleen, and a slightly weaker reaction in the reticulum cells of the lymphatic tissue around these arteries. The nature of the lymph nodes encountered in mouse spleen varies from one individual to another, obviously according to the functional status of the spleen[6]. Large pale cells containing chromatin debris occurred in the middle of some of the nodes. Disintegration of lymphocytes obviously occurs in these reaction centres[6]. The reaction was relatively pronounced in these places. There were basophilic lymphocytes displaying numerous mitotic figures in the middle of another type of node. Formation of new lymphocytes occurred in these germinal centres[6]. These nodules gave a weaker reaction. The two lymph node types could be confused at times. The 'perifollicular collar' is generally poorly developed in the mouse[6,7]. Some preparations showed a partly open 'collar' formed by a few reticulum cell layers giving a positive reaction. The lymphocytes are negative, as is also the case elsewhere in the organism[8,9].

A positive LAP reaction was generally not demonstrable in the area of red pulp. However, when phosphatase buffer was used a very faint reaction was elicited in the reticulum cells even outside the lymph nodes, but it was probably due to diffusion. Mast cells, which are encountered in the spleen of some mouse strains[6], were not found at all in the preparations examined. Extramedullary hematopoiesis is


Fig. 1. In the black and white pictures the contrast does not correspond fully to the degrees of intensity of the colour reaction. The spleen of a rat ♀, aged 8 months, phosphate buffer, incubation for 90 min. Positive reaction in lymphatic tissue (a) around which is seen a more faintly reacting 'perifollicular halo', (b) at the outer margin of which there is a circle, (c) formed by reticulum cells which give a stronger reaction. The area (d) of red pulp is negative ( × 50).

[1] M. DIXON and E. G. WEBB, *Enzymes* (Longmans, London 1958), p. 642.
[2] M. M. NACHLAS, D. T. CRAWFORD, and A. M. SELIGMAN, J. Histochem. Cytochem. *5*, 264 (1957).
[3] C. F. A. CULLING, *Handbook of Histopathological and Museum Technique* (Butterworth & Co., London 1957), p. 179.
[4] J. BRONTE-GATENBY and H. W. BEAMS, *The Microtomist's Vade-Mecum* (11. Edit. J. & A. Churchill, London 1950), p. 865.
[5] M. N. GREEN, K.-C. TSOU, R. BRESSLER, and A. M. SELIGMAN, Arch. Biochem. Biophys. *57*, 458 (1958).
[6] T. B. DUNN, J. of Nat. Canc. Inst. *14*, 1281 (1954).
[7] D. SNELL, *Biology of the Laboratory Mouse* (Dover Publ. Inc., New York 1941), p. 96.
[8] G. A. ACKERMAN, J. Histochem. Cytochem. *8*, 386 (1960).
[9] L. K. KORHONEN and S. RUPONEN, Acta anat., in press.

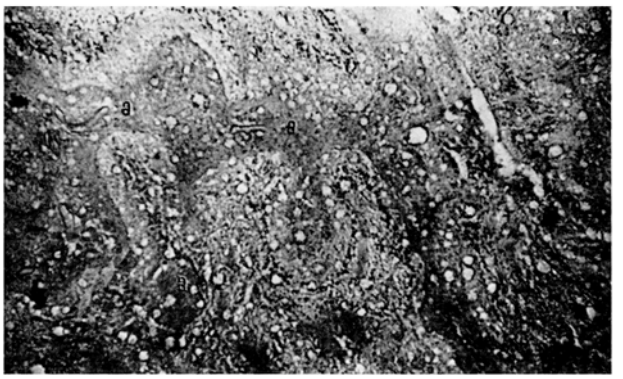

Fig. 2. The spleen of a rat ♀ aged 3 weeks, phosphate buffer, incubation for 90 min. Positive reaction in the lymphatic bands (a), the perifollicular parts react more faintly than in Figure 1 ( × 33).

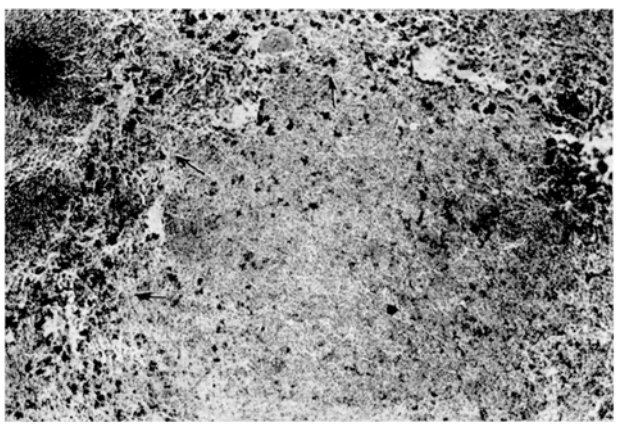

Fig. 3. The spleen of a mouse ♀ of 9 months, acetate buffer, incubation for 120 min. Positive reaction in the lymph node around which is seen an incomplete, strongly positive 'perifollicular halo' (arrows) ( × 100).

established regularly in mouse spleen[3,6,10] and these islets produce a positive reaction in the area of red pulp.

Mouse spleen represents phylogenetically an older type of spleen dominated by lymphatic tissue[10]. In rat spleen the proportion of red pulp is greater and the lymphatic tissue is accumulated in bands around the central arteries. These lymphatic nodules showed a similar positive LAP reaction to that of mouse spleen. The 'perifollicular collar' is distinctly formed in rats and there are seen in it reticulum cells arranged in a circle and giving a positive reaction. Around these cells are other reticulum cells

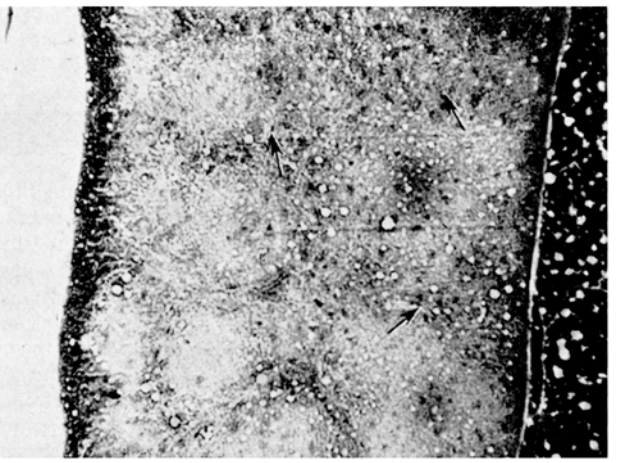

Fig. 4. The spleen of a mouse ♀ aged 2 weeks, acetate buffer, incubation for 120 min. Positive reaction only in the central arteries and close to them in the lymphatic tissue (arrows). The reaction is considerably fainter than in the pancreatic tissue seen on the right ( × 33).

arranged in a loose circle forming a 'perifollicular halo' which reacts more weakly. Schweigger-Seidel's sheathed arteries were not encountered in the spleen of mouse and rat.

Comparison of the spleens of animals of different age showed that the positive LAP reaction was fainter in younger animals and, especially in mice, occurred in a narrower zone around the central arteries. Compared with the older animals, the difference was especially clear in the spleens of mice under 2–3 weeks of age and of rats less than 3 weeks old. This observation concurs with findings concerning the phases of development of the lymphatic tissue of the spleen[10].

*Zusammenfassung.* Leucinaminopeptidase wird histochemisch in der Milz von Mäusen und Ratten untersucht. Eine besonders ausgeprägte Reaktion zeigte sich in der Zentralarterienwandung und in den umgebenden Reticulumzellen der weissen Pulpa. Im Bereich der roten Pulpa verhalten sich nur die Inseln positiv, während eine extramedulläre Hämatopoese vorkommt.

L. K. Korhonen and S. Ruponen

*Department of Anatomy, University of Turku (Finland), May 10, 1962.*

[10] P. Cohrs, R. Jaffe, and H. Meessen, *Pathologie der Laboratoriumstiere* (Springer Verlag, Berlin 1958), I. Bd., p. 330.

## Microchromosomes in the Embryonic Mitosis of the Domestic Fowl

Although recently there have been a number of publications on the somatic chromosomes of higher vertebrates, yet curiously enough only a few reports are available on the somatic chromosomes of the domestic fowl. Earlier workers like Lecaillon[1] and Hance[2,3], probably handicapped by the inadequate techniques and inherent refractory nature of the bird chromosomes, recorded a comparatively smaller and variable number of chromosomes than the later workers who counted as many as 66 (White[4]) and 78 (Ohno[5]) chromosomes in the somatic cells of the domestic fowl.

[1] A. Lecaillon, C. R. Soc. Biol. *69*, 31 (1910).
[2] R. T. Hance, J. Morph. Physiol. *43*, 119 (1926).
[3] R. T. Hance, Biol. Bull. *51*, 143 (1926).
[4] M. J. D. White, J. Genet. *26*, 345 (1932).
[5] S. Ohno, Chromosoma *11*, 484 (1961).