simplest organism in which it has yet been possible to demonstrate such constancy of pattern and position at the level of individual nerve cells. If, as in development of the nematode ventral cord, a nerve cell's lineage determines what cell contacts it will make[7,8], the pattern of central connections seen in the P. mucosa reticulum could be understood simply as a consequence of like-to-like contact between cells having equivalent positions in the cell lineage, as proximal cells presumably do. The close association and morphological similarity between unipolar neurones of the cerebral ganglion and 2 of the proximal cells raises the possibility that similar like-to-like patterns of contact involving nerve cell types other than reticular cells might be expected to occur in the CNS. Without functional information, it is not possible to say whether the morphological contacts between reticular cells are of any functional significance or even whether reticular cells are nerve cells at all. Reticular cells in Lopadorhynchus larvae are supposed to be neurones because of their morphology, staining properties and the fact that they send processes to roughly those areas of the larva that are contractile[3]. The last is also true for P. mucosa; contractions are initiated at the top of the larva and circumferentially at a point just posterior to the prototroch. The supposition that reticular cells are neurones is further supported by their location, in P. mucosa, in ganglionic rudiments.

7 J. E. Sulston, Phil. Trans. R. Soc. B *275*, 287 (1976).
8 J. G. White, E. Southgate, J. N. Thomson and S. Brenner, Phil. Trans. R. Soc. B *275*, 327 (1976).

Pigment in the spleen of C57BL/10ScSn and related mice[1]

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Summary. Pigment present in the spleen of C57BL and related mice, after the stress of experimental procedures, was shown to be melanin.

During experiments designed to examine the response of mice to inoculations of different species of mycoplasma (Hill, unpublished results), it was noticed that in an experiment with C57BL/10ScSn mice, at necropsy dark areas were visible in the spleens of some mice. This experiment was repeated with similar results. It was thought unlikely that the specific mycoplasma species used (strain 58B) should have caused this appearance. Other strains of mice, BALB/c, +nu, nu nu and A2Hr were unaffected when inoculated with the same species of mycoplasma. Therefore, it was decided to examine this anomaly.

Materials and methods. The animals used in these experiments were 4–6 weeks old mice from a specified pathogen free unit, category 4[2]. After inoculation the mice were housed in filter boxes[3] and kept in a conventional animal room. The medium used was as follows: 70 ml Difco PPLO broth, 1 ml penicillin G 100,000 units, 2 ml thallium acetate 2.5%, 10 ml yeast extract 25%, 20 ml horse serum, 1 ml glucose 10%, 1 ml phenol red 0.2%. Mycoplasma pulmonis, M. neurolyticum and mycoplasma strain 58B were freshly isolated from the lung of a rat, the conjunctiva of a mouse and the conjunctiva of a rat respectively. These isolates were subcultured in liquid media twice and then frozen in ampoules at –70 °C. 0.02 ml of each inoculum was given intranasally to each mouse as shown in table 1 and 2. Components of the media were diluted in PBS to give the same concentration as used in the medium. 6 uninoculated mice, housed under the same conditions, were kept as controls. The mice were kept for 3 weeks and then killed. A cut surface of spleen and nasopharyngeal swabs were rubbed over the surface of mycoplasma agar plates which were incubated at 37 °C in a humid atmosphere for 3 weeks. Portions of spleen were fixed in 10% formalin, and sections of the tissue stained with haematoxylin and eosin. Portions of liver, kidney and adrenals, from mice showing dark areas in the spleen, were also fixed and sections examined. Sections of spleen showing dark areas were bleached by the methods shown in table 3 and then stained with haematoxylin and eosin. Sections bleached by potassium chlorate and potassium permanganate methods were also stained with Schmorl and Masson-Fontana's silver stain. Further sections were stained (table 4).

Results and discussion. 3 weeks after inoculation all animals appeared healthy. At necropsy 20 out of 93 spleens showed dark areas partially banding the spleens (one on each) transversely but not exceeding $^1/_2$ of the whole spleen (figure 1). The size of these spleens were within normal limits. The other organs appeared normal, and no histological abnormality was seen on any of the livers, kidneys or adrenals examined. No deposits were seen in

Table 1. Strains of mice and inocula

Inoculate	Strain	Site	No. of mice	No. affected
M. pulmonis	C57BL/10ScSn	Nasal	6	0
M. neurolyticum	C57BL/10ScSn	Nasal	6	2
M. 58B	C57BL/10ScSn	Nasal	6	2
Whole media	C57BL/10ScSn	Nasal	6	1
$^1/_{100}$ phenol red 0.2%	C57BL/10ScSn	Nasal	5	2
$^1/_{50}$ thallium acetate 2.5%	C57BL/10ScSn	Nasal	5	0
$^1/_{100}$ penicillin G 100,000 units	C57BL/10ScSn	Nasal	5	1
$^1/_{10}$ yeast extract 25%	C57BL/10ScSn	Nasal	5	1
$^1/_5$ horse serum	C57BL/10ScSn	Nasal	5	3
M. 58B	C57BL/10ScSn	Nasal	6	3
M. 58B	C57BR	Nasal	6	0
M. 58B	C57BL	Nasal	6	1
M. 58B	C57BL	Conjuctiva	6	2
Distilled water	C57BL	Nasal	20	2
Total			93	20

the spleens of C57BR or NZB mice (table 2) or the uninoculated control mice. All 93 spleen sections were stained with haematoxylin (Ehrlich's) and eosin (H & E) stain. 20 sections showed a dark deposit in varying sizes and shapes scattered over the parenchymal tissue with no special distribution pattern. These deposits were also seen with Giemsa stain. The splenic tissue appeared normal. Sections treated with alcoholic picric acid excluded formalin pigment. The deposit did not dissolve in Gooding Stewarts decalcifying fluid so calcium was also excluded. The deposit was washed out and removed from the tissue by Mayer's technique ($KC10_3$ and HCl) and potassium permanganate solution ($KMnO_4$) which indicated the presence of melanin pigment (table 3). Of the stains used in table 4 those for bile, chromaffin, haemosiderin, calcium, haemoglobin and lipofuscin were negative, however the stains for melanin were positive (figure 2).

A fatal dermatitis[4] has been described in C57BL/10 and related strains. This is believed to be due to an endocrine inbalance. In this syndrome spleens are usually grossly enlarged but melanin has not been recorded. Nieberle and Cohrs[5] state that in melanosis, of unknown aetiology, melanin is deposited in various organs and especially in the lung and liver, but the spleen is not mentioned. In these experiments thallous acetate had no effect on the mice and only 10% were affected by an inoculum of distilled water. The brown coated related strain, C57BR mice, were unaffected, possibly because black mice have more melanin in their skin. However, the NZB mice, a black strain unrelated to the C57BL mice, were also unaffected. The deposition of melanin in the spleen is thought to be due to a mild stress factor such as the inoculation procedure, combined with foreign material inoculated into the black strains related to C57BL mice.

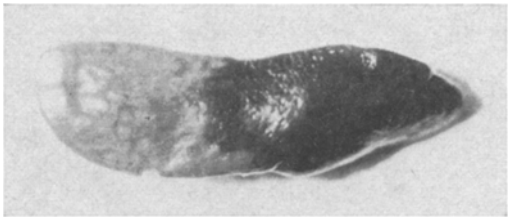

Fig. 1. Dark area partially banding the spleen of C57BL/ScSn mouse 3 weeks after inoculation. Magnification × 6.

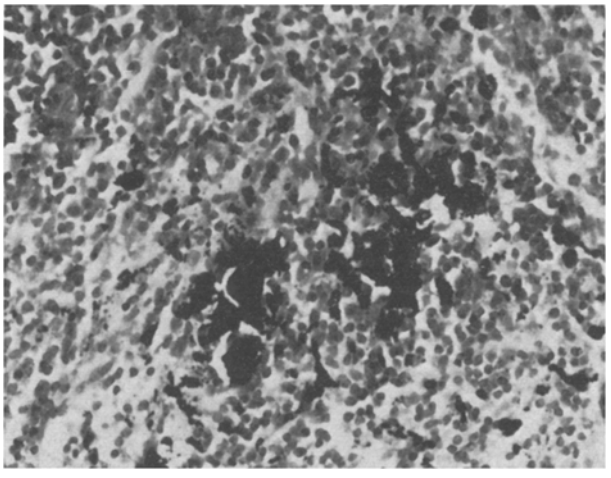

Fig. 2. Spleen section showing melanin deposits over the paranchymal tissue. Masson-Fontana stain. Magnification × 600.

Table 2. Strains of mice and inocula

Inoculate	Strain	Site	No of mice	No. affected
Whole media	C57BR	Nasal	20	0
Whole media	NZB	Nasal	20	0
$^1/_{50}$ thallium acetate 2.5%	C57BL	Nasal	20	0
Total			60	0

Table 3. Solvents used to bleach pigment on section of spleen

Solvent	Pigment identified	Result
Alcoholic picric acid[6]	Formalin	Negative
Formic acid[6]	Calcium	Negative
Potassium chlorate and hydrochloric acid[7]	Melanin	Positive
Potassium permanganate and oxalic acid[7]	Melanin	Positive

Table 4. Stains used to exclude and identify pigment on sections of spleen

Staining technique	Identification	Reaction
Haematoxylin and eosin[8]	General bile	Dark deposits negative
Giemsa[6]	General chromaffin	Dark deposits negative
Perls' prussian[9]	Haemosiderin	Negative
Von Kossa[10]	Calcium	Negative
Schmorl[11]	Melanin	Positive
Masson-Fontana[12]	Melanin	Positive
Gmelin[13]	Bile	Negative
Periodic acid Schiff[14]	Lipofuscin	Negative
Sudan black B[15]	Lipofuscin	Negative
Long Ziehl-Neelsen[6]	Lipofuscin	Negative

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2 G. H. Townsend, Vet. Rec. *83*, 225 (1969).
3 G. H. Townsend and M. H. Robinson. The UFAW Handbook on the Care and Management of Laboratory Animals, chapter 8, 4th ed. Edited by the staff of UFAW. Churchill Livingstone, Edinburgh and London 1972.
4 H. D. Stowe, J. L. Wagner and J. R. Pick, Lab. Ann. Sci. *21*, 892 (1971).
5 Nieberle and Cohrs, in: Special Pathological Anatomy of Domestic Animals, p. 495. Ed. P. Cohrs. Pergamon Press, Oxford 1966.
6 J. D. Bancroft and A. Stevens, in: Histological stains and their diagnostic uses, p. 92. Churchill Livingstone, Edinburgh 1975.
7 R. A. B. Drury and E. A. Wallington, in: Carleton's histological techniques, p. 230. Oxford University Press, New York and Toronto 1967.
8 P. Ehrlich, Z. wiss. Mikrosk. *3*, 150 (1886).
9 N. Perls, Virchows Arch. path. Anat. Physiol. *39*, 42 (1867).
10 J. von Kossa, Beitr. path. Anat. *29*, 163 (1901).
11 G. Schmorl, Die Pathologisch-histologischen Untersuchungsmethoden, 16th edition. Ed. P. Geipel. Vogel, Berlin 1934.
12 P. Masson, Am. J. Path. *4*, 181 (1928).
13 F. Tiedemann and L. Gmelin, Die Verdauung nach Versuchen. Heidelberg 1826.
14 A. G. E. Pearse, The Periodic Acid Schiff Reaction. Ass. clin. Path., broadsheet No. 26, 1959.
15 L. Lison and J. Dagnelie, Bull. Histol. appl. Physiol. Path. *12*, 85 (1935).