significant difference was noted. The shell ratio in the high altitude treated F_1 cocoons was 14.8% higher ($p < 0.01$) than that of the normal.

The induction of quiescence in the phaenerocephalic pupa has great significance to the muga silk industry. The hazardous rearing during summer and winter for seed purpose may be eliminated by this process. The surplus cocoons obtained from the spring and autumn generations can be safely preserved at low temperature which will serve as buffer stock between the two commercial generations. This may reduce the scarcity of seed cocoons thereby rejuvenating the muga silk industry.

7 February 1985; Revised 20 May 1985

1. Thangavelu, K. and Subba Rao, G., *Curr. Sci.*, 1982, **51**, 1023.
2. Subba Rao, G., *Natl. Sem. Silk Res. Dev.*, 1983, p. 11.
3. Anon., *Govt. of India Tariff Commission Report*, Govt. Press, Nasik, 1964, p. 63.
4. Anon., *Statistical Biennial*, Central Silk Board, Bangalore, 1984, p. 54.
5. Wigglesworth, V. B., *The principles of insect physiology*, 7th edn., Chapman and Hall Ltd., U.K., 1973, p. 746
6. Chapman, R. F., *The insects: Structure and function*, The English Language Book Society, London, 1973, p. 882.
7. Yagi, S., *The juvenile hormones*, Plenum Press, New York, 1976, p. 288.
8. Schneiderman, H. A. and Williams, C. M., *Biol. Bull.*, 1953, **105**, 320
9. Jolly, M. S., Sen, S. K. and Ahsan, M. M., *Tasar culture*, Ambika Publishers, Bombay, 1974, p. 176.
10. Sahey, D. N., Akhter, M. N. and Sinha, S. S., *Proc. 1st. Int. Sem. on Nonmulberry silk*, Ranchi, 1974, p. 170.
11. Jolly, M. S., Sonwalker, S. K. and Prasad, G. K., *Nonmulberry silks*, F. A. O., Rome, 1979, p. 164.
12. Bailey, N. T. T., *Statistical methods in biology*, English University Press Ltd., U.K., 1965, p. 152.
13. Denlinger, D. L., *Nature (London)*, 1974, **252**, 223.

HAEMOGRAM CHANGES DURING SENESCENCE PROCESSES IN *SCHIZODACTYLUS MONSTROSUS* D (ORTHOPTERA: SCHIZODACTYLIDAE).

SANJAY MANDAL and ANUPAMA MANDAL

Department of Zoology, A M College, Jhalda, Purulia 723 202, India.

**Department of Zoology, University of Burdwan, Burdwan 713 104, India.*

ALTERATION in the haemogram as an index of various physiological conditions in insects has been well studied[1-3]. Similarly, storage of different nutrients (carbohydrate, protein, lipid and amino acids) by haemocytes and their role in maintaining the normal nutrient balance during different stress conditions are also well documented[4,5]. Mandal[6] advocated that during the ageing process in *Schizodactylus monstrosus*, the volume of haemolymph and the number of haemocytes circulating in the haemolymph gradually decrease. He showed that some of the oxidative enzymes in haemolymph and fatbodies increase gradually with age. The present investigation reports the effect of a reductive agent on the changes of haemogram, total sugar, lipid, free amino acids and proteins in haemolymph with ageing process.

The source material used has been described elsewhere[7]. The stock was maintained in the laboratory at $28° \pm 1°C$ with 12:12 photoperiod and 90% relative humidity in sandy beds[8]. Adult insects were used for the experiment 2 hr after moulting. The insects were 5, 10, 20, 40, and 80 days old when sacrificed for haemocyte count and biochemical estimation. A 2% solution of the reduced glutathione in distilled water was injected at a dose of 40 μl insect after 2 hr of adult emergence. Control insects received only equivalent quantities of distilled water. Each insect was then labelled individually and separately kept in glass jars as the insects have cannibalic habits. Small nymphs of cockroach and grass-hopper were given as food[7].

Haemolymph was collected from both control and treated insects by puncturing the femur of the hind leg using a graduated glass capillary tube and kept in a test tube at $4\ C \pm 0.5\ C$ until ready for use. Total sugar in the haemolymph was determined following the anthrone method[9] using glucose as the standard. Total lipid was estimated by vanilline reactive method[10]. Free amino acid was estimated spectrophotometrically[11]. The total protein content was estimated by standard technique[12]. The pH of the haemolymph was determined using microindicator pH paper. The specific gravity was measured using the microspecific gravity bottle. To observe the total free haemocyte count (THC) and differential haemocyte count (DHC), haemolymph samples were allowed to fill the Neubauer haemocytometric chambers by capillary action. No anticoagulant was used to avoid the errors in counting. Plasma coagulation in *S. monstrosus* occurred at about 200–210 sec after the blood was shed. DHC was made by staining air-dried blood films with Leishman's stain.

THC and DHC of the haemolymph of the control insects showed that the total number of free haemocytes significantly decreased with the advancing age of the insect (table 1). Treatment of the reduced glutathione to the insects caused a rapid and significant increase in the total free haemocyte population (table 1). This gradual decline in total haemocyte population was not observed in glutathione-treated insects (table 1). The THC of the treated insects showed that the number of total free haemocyte/mm^3 haemolymph remained more or less constant with the age of the insects. DHC studies of the haemolymph of control insects showed five distinct categories of the haemocyte population *viz* prohaemocyte, plasmatocyte, granular haemocyte, adipohaemocyte and spherule cells. This is also corroborated by the studies of Jones[13]. The pH of the haemolymph in both the treated and control insects is 7.0. The specific gravity of haemolymph in the control was 1.15, while after glutathione treatment the specific gravity increased to 1.25. The individual population of a haemocyte type in haemolymph of control insects greatly varied with the advancing age of the insects (table 1). Application of glutathione caused changes in the population of each selective haemocyte in the haemolymph. The population of each of the selective haemocyte of glutathione-treated insects remained more or less constant with advancing age, while the percentage of each haemocyte population in the haemolymph of control insects greatly fluctuated with age (table 1). Total sugar, lipid, protein and free amino acid levels in the haemolymph of control insects exhibited declining trends with advancing age while these remain more or less steady in glutathione-treated insects (table 2). Application of glutathione also significantly increased the levels of total sugar, lipid and free amino acids in the haemolymph in comparison to the respective age group of the control insects excepting the total protein content which remained more or less same (table 2).

Alteration in the number of haemocyte in relation to age of the control insects demonstrates their strong response during the physiological senescence stress condition. During physiological stress the homeostatic balance is actually maintained by the circulat-

Table 1 *Total haemocyte count (cells/mm^3) and differential haemocyte count (expressed as %) in control and reduced glutathione treated S. monstrosus in relation to different age groups.*

Haemocyte type	Treatment	Age of the insects (days)					
		0	5	10	20	40	80
Total haemocyte count	Control	16.20 (0.12)	12.90* (0.15)	12.10 (0.12)	8.30* (0.18)	8.00 (0.11)	6.20* (0.17)
	Glutathione treated	18.5:** (0.13)	18.00** (0.22)	24.29** (1.21)	19.59** (0.89)	18.23** (0.12)	17.52** (0.29)
Prohaemocyte	Control (C)	20	18	16	10	12	11
	Glutathione treated (Gt)	25	22	21	22	20	20
Plasmatocyte	(C)	15	14	12	15	15	15
	(Gt)	18	18	16	17	18	17
Granular haemocyte	(C)	43	46	50	50	48	40
	(Gt)	32	32	34	32	32	32
Adipohaemocyte	(C)	18	20	20	23	24	25
	(Gt)	22	23	25	23	23	22
Spherule cells	(C)	4	2	2	2	1	0
	(Gt)	2	2	2	2	2	2

Number within the parentheses indicates the ± S. E. M.
* = $p < 0.01$ in comparison to each age group of same treatment, ** = $p < 0.01$ in comparison to the respective age group of control cases with treated cases.

Table 2 *Total sugar, lipid, protein (mg/100 ml) and free amino acid (μg/100 ml) content of haemolymph of S. monstrosus with age in control and glutathione treated cases. Data are the mean ± S. E. M. of 9 replications.*

Treatment	Contents	Age of the insects (days)					
		0	5	10	20	40	80
Control	Total sugar	1.14	1.04	1.03	0.89	0.80	0.66
		(0.01)	(0.01)	(0.02)	(0.02)	(0.03)	(0.01)
	Total lipid	2.61	2.56	2.41	2.32	2.30	2.05
		(0.03)	(0.02)	(0.03)	(0.01)	(0.01)	(0.01)
	Total amino acid	1881	1275	954	840	813	705
		(21)	(19)	(16)	(20)	(23)	(16)
	Total protein	2.98	2.40	2 05	2.19	1.95	2.04
		(0.12)	(0.16)	(0.31)	(0.45)	(0.05)	(0.12)
Glutathione treated	Total sugar	1.18	1.15	1.10	1.08	1.10	1.09
		(0.02)	(0.01)	(0.01)	(0.02)	(0.01)	(0.01)
	Total lipid	2.65	2.72	2.62	2.60	2.58	2.57
		(0.02)	(0.20)	(0.01)	(0.20)	(0.10)	(0.20)
	Total amino acid	1881	1891	1890	1871	1875	1881
		(15)	(10)	(8)	(20)	(15)	(10)
	Total protein	2.85	3.25	2.81	2.75	2.90	2.98
		(0.02)	(0.08)	(0.12)	(0.14)	(0.15)	(0.16)

Number within the parentheses indicates ± S. E. M.

ing haemocyte[14,15]. Rockstein and Miquel[16] advocated that the amount of haemolymph and force of the circulation of haemolymph varied with increasing age and haemocyte population. The present experimental result also showed that the total free haemocyte population gradually decreased with advancing age in control insects. The changes in the frequencies of a selective haemocyte population might also be a possible cause for the senescence processes, as these changes in the haemocytes might create some abnormalities in the physiology which actually triggers the senescence process. In a detailed study Mandal[6] and Kanungo[17] showed that the ageing processes in insects and mice are actually initiated by the shifting of the total metabolic status from reductive state to the oxidative state. They also showed several oxidative enzymes which significantly increased with age. The present results corroborate their findings, because the declining trend of the total sugar, protein, lipid and free amino acid is inhibited by treating the reductive agent and the glutathione, which is again confirmed by the increased specific gravity of the haemolymph after glutathione treatment. Similarly the haemocyte population, (both THC and DHC) also showed marked variation after glutathione treatment as against the control ones.

AM is thankful to Burdwan University for financial help.

10 June 1985

1. Meyer, H., Preiss, B. and Bauer, S., *Biochem. J.*, 1960, **76**, 27.
2. Clegg, J. S. and Evans, D. R., *J. Exp. Biol.*, 1961, **38**, 77.
3. Bursell, E., *J. Insect Physiol.*, 1963, **9**, 439.
4. Arnold, J. W., In: *The physiology of Insecta*, Academic Press, New York and London, 1974, p. 202.
6. Mandal, S., *Zool. Jb. Physiol.*, 1985, **89**, (in press).
7. Mandal, S. and Choudhuri, D. K., *Zool. Jb. Physiol.*, 1982, **86**, 489.
8. Mandal, S., *Ph.D. thesis*, University of Burdwan, Burdwan, 1983.
9. Roe, J. H., *J. Biol. Chem.*, 1955, **212**, 335.
10. Goldsworthy, G. J., Mordue, W. and Guthkelch, J., *Gen. Comp. Endocrinol.*, 1972, **18**, 545.
11. Rosen, H., *Arch. Biochem. Biophys.*, 1957, **67**, 115.
12. Lowry, O. H., Rosebrough, N. J., Farr, A. L. and Randall, R. J., *J. Biol. Chem.*, 1951, **193**, 265.
13. Jones, J. C., *Am. Zool.*, 1962, **2**, 209.
14. Wigglesworth, V. B., *J. Micros. Sci.*, 1956, **97**, 89.
15. Staurenge de Cunha, M. A. and Morussig, A. C., *Naturalia*, 1981, **6**, 43.
16. Rockstein, M. and Miquel, J., In: *The physiology*

of Insecta, Academic Press, New York and London, 1973, **1**, 371.
17. Kanungo, M. S., In: *Biochemistry of ageing*, Academic Press, London, 1980.

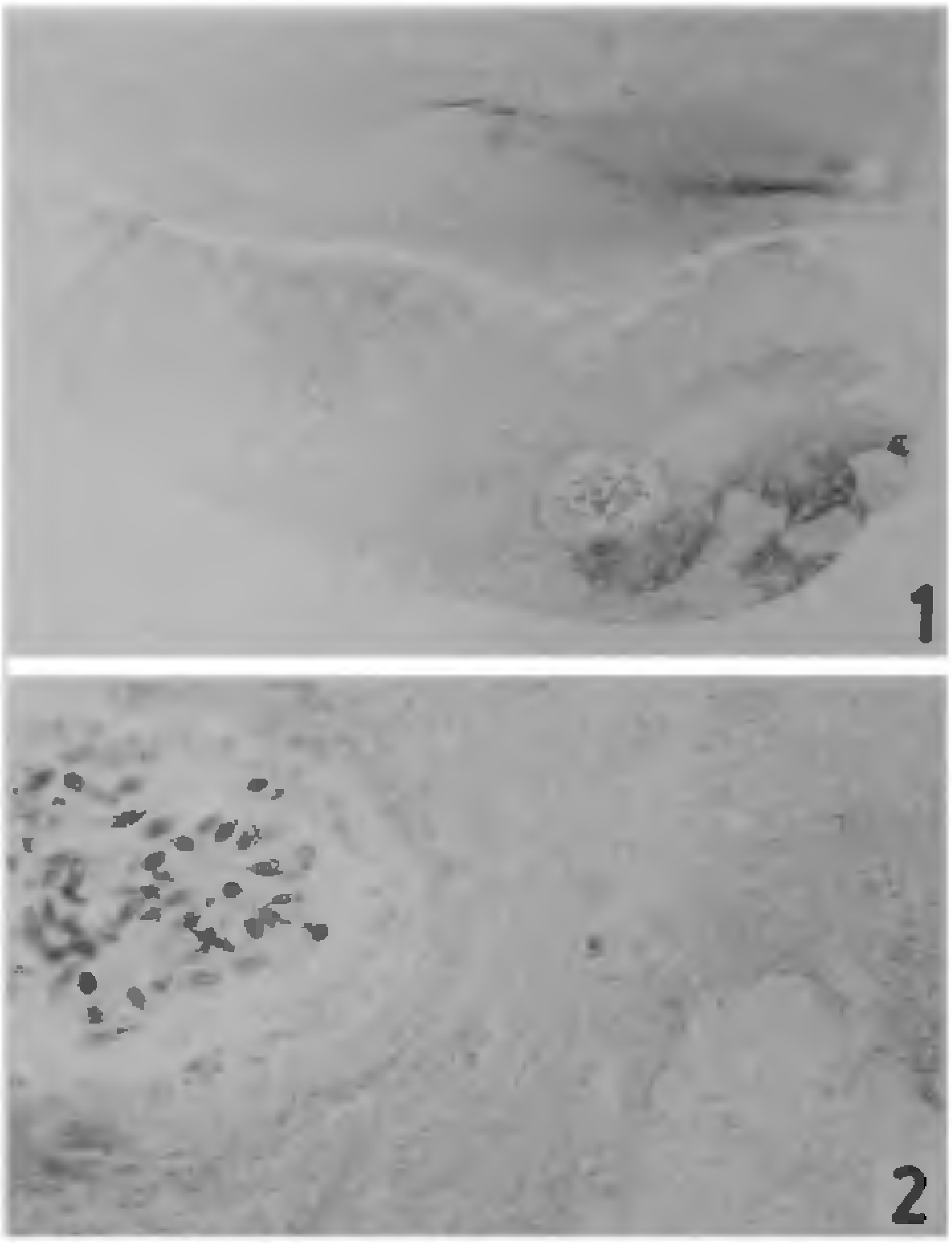
OCCURRENCE OF MYXOSPORIDIAN CYST IN THE PITUITARY OF THE TELEOST *CHANNA PUNCTATUS* (BLOCH)

RAJ NARAYAN RAM and A. G. SATHYANESAN
Department of Zoology, Banaras Hindu University, Varanasi 221005, India

OCCURRENCE of parasitisation in the pituitary is very rare[1,2]. Over 500 brains with intact pituitary used in this study were fixed in bouin's fluid. Paraffin sections were cut at 5 μm thick and stained in Alcian blue (AB)-Periodic acid-Schiff (PAS)-Orange G (OG) and Lead haematoxylin (PbH)-PAS-OG.

While examining the serial sections of the brains of *C. punctatus* with intact pituitary in relation to various experiments, a large myxosporidian parasitic cyst was seen in one of them occupying a part of the posterior neurohypophysis, pars intermedia (PI) and proximal pars distalis (PPD) (figure 1). The cyst wall is fibrillar and multilayered (figure 2). Despite this large cyst the surrounding pituitary cells appear to be normal. The fish also did not show any external signs of the presence of the infection.

Kerr[3] reported that the presence of plerocercoid stage of the tapeworm (*Ligula intestinalis*) in the body cavity of the Roach (*Leuciscus rutilus*) caused reduction in size and granulation of the basophils in the PPD of the pituitary. Trematode parasite was reported in the third ventricle, and close to the nucleus preopticus and pituitary of *Ophiocephalus punctatus*[1,2]; and myxosporidian parasites were noticed in the neurohypophysis of *Barbus stigma*[1]. However, no marked change was noticed[1,2] in the pituitary cell types in response to parasitisation. In the present study also, despite the presence of the large myxosporidian cyst, the pituitary cytology was not affected.



Figures 1, 2. 1. A part of the brain with intact pituitary showing myxosporidian cyst in the neurohypophysis between PI and PPD. × 75. **2.** Higher magnification of the part of the cyst. AB-PAS-OG. × 400.

RNR is grateful to the CSIR for a fellowship.

23 May 1985

1. Sathyanesan, A. G., *Nature* (*London*), 1957, **180**, 95.
2. Joshi, B. N. and Sathyanesan, A. G., *Mikroskopie* (*Wein*), 1981, **38**, 253.
3. Kerr, T., *Q. J. Micro. Sci.*, 1948, **89**, 129.