

two positively migrating fractions, one at the albumin position and one on the positive side of the albumin position.

When the electrophoresed pattern is stained with PAS it may be observed that the PMS has separated into 3 glycoprotein fractions. One on the positive side of the albumin position, one at the albumin position, and one between the albumin and the  $\alpha$ -globulin position.

*Clinical studies.* Of the 8 mares originally selected for study 3 became pregnant. Using the HAI technique previously described, a positive result was recorded in all 3 at the 44th, 47th, and 53rd day following coitus.

Specimens of sera, taken from each mare were re-tested three days after the initial positive result. The result in each case remained positive.

Specimens of urine taken at this time were also positive, the concentration of gonadotrophin present being 7000, 21,000 and 29,000 IU/l.

No false positive results were recorded at any time in the five mares who were not pregnant.

*Discussion.* Antisera to PMS has been frequently produced by other workers (PIGEON, CLEGG, and COLE<sup>8</sup>; ZONDEK and SULMAN<sup>9</sup>; HAMBURGER, NIEMAN, and SORESENSEN<sup>10</sup>). WIDE and WIDE<sup>3</sup> used Freund's adjuvant combined with PMS to raise antisera to the hormone. There is the only other report to date on the use of such antisera for the diagnosis of pregnancy in the mare.

The disappearance of two of the precipitation lines (a and b) after absorption would indicate that the parent antigens of these two non-specific antibodies are present in normal horse sera. It is probable that more than one volume of normal horse serum is necessary to absorb each volume of antisera. Following these studies five volumes of normal horse sera were routinely used for each volume of antisera.

To date no reports of immunoelectrophoretic studies on PMS have been published. This study would indicate that the PMS used (Leo) contains two protein and three glycoprotein fractions. It is obvious that the use of the

relatively impure preparations of PMS which are currently available will produce a number of extraneous antibodies. It is possible, however, that these can be absorbed by the use of non-pregnant mares serum. It was hoped that the diagnosis of pregnancy in the mare could be achieved using an HAI method at an earlier stage than the methods currently available. From the positive results achieved in this preliminary study it would appear unlikely that this will prove possible, and that the 40th day following coitus is the earliest time at which chorionic gonadotrophin can be detected in the serum of the pregnant mare<sup>11</sup>.

*Résumé.* Une méthode pour la préparation d'un anti-sérum de haut titre contre le PMS est décrite. La spécificité a été établie par immunoelectrophorèse. On a mis au point une méthode à la HAI par laquelle le diagnostic de la grossesse peut être fait, chez la jument, entre le 42ème et le 54ème jour après le coït.

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<sup>8</sup> H. PIGEON, M. T. CLEGG, and H. H. COLE, *Acta endocr. (Kbh)* 35, 253 (1960).

<sup>9</sup> B. ZONDEK and F. SULMAN, *Proc. Soc. exp. Biol. Med.* 36, 708, 9368 (1937).

<sup>10</sup> C. HAMBURGER, A. NIEMAN, and A. SORENSON, *Acta endocr. (Kbh)* 26, 286 (1957).

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### Nuclear Apparatus and Binary Fission in *Spirostomum dharwarensis* n. sp.

*Spirostomum dharwarensis* n. sp. is a freshwater, moderate-sized ciliate about 600–800  $\mu$  long (Figure 1); it is named after the locality from which it was collected. The number of micronuclei is exactly seven. They are small bodies, each 2.0  $\mu$  in diameter. They are distributed along the macronuclear band. Sometimes one or two are found adhering to it. In vegetative phases both types of nuclei show a brilliantly positive Feulgen reaction, thereby revealing a considerable amount of DNA present in them.

*Spirostomum dharwarensis* has the following unique features: it has only seven micronuclei, the micronuclear division is of the synchronous type and the macronuclear division during binary fission is completed far in advance of the cytoplasmic division (Figure 2). In these features *S. dharwarensis* differs from all other species hitherto described under the genus *Spirostomum* (SESHACHAR and PADMAVATHI<sup>1</sup>). During binary fission, it is significant to

observe that the macronucleus shows increasingly poor reaction to the Feulgen dye during its condensing process, while the reaction shown by the micronuclei to this dye is not so poor. It may be inferred from this that in *S. dharwarensis* during the nuclear division a considerable amount of DNA disappears from the macronucleus while the micronuclei lose very little of this genetic material.

SESHACHAR and PADMAVATHI<sup>2</sup> regard the synchronous and selective types of micronuclear divisions in ciliates as some biochemical mechanisms acting under the external or internal stimuli. WADDINGTON'S<sup>3</sup> cytoplasmic chemodifferentiation hypothesis and HAMMERLING'S<sup>4</sup> physiological gradients hypothesis represent other ex-

<sup>1</sup> B. R. SESHACHAR and P. B. PADMAVATHI, *J. Protozool.* 3, 145 (1956).

<sup>2</sup> B. R. SESHACHAR and P. B. PADMAVATHI, *Curr. Sci.* 9, 281 (1956).

<sup>3</sup> C. H. WADDINGTON, *Symp. Soc. exp. Biol.* 2, 145 (1948).

<sup>4</sup> J. HAMMERLING, *Arch. Entmech. Org.* 131, 1 (1934).

planations. From the reaction shown by the nuclei in *S. dharwarensis* to the Feulgen dye, I feel that it is possible to offer one more probable explanation. During the process of division of the nuclei in ciliates much of the DNA in them is converted into the RNA required, thereby increasing the rate of metabolic processes within the cell.

In ciliates showing a selective micronuclear division, the macronucleus gives throughout a bright Feulgen reaction as in *S. ambiguum* Padmavathi<sup>5</sup>, indicating that it does not part with much of its DNA. So a number of micronuclei contribute their DNA material for the build-up of RNA. Now, as the DNA material in them falls below the optimum level, the majority of the micronuclei degenerates, while only a few maintain themselves through

the process of mitosis and complete their division. On the other hand, in ciliates showing a synchronous division, the condensing macronucleus shows a poor reaction to the Feulgen dye, as in *S. dharwarensis*, thereby suggesting that it is parting with a considerable amount of DNA during the process of condensation. Hence there is no need for the micronuclei to contribute their DNA material towards the RNA build-up. For this reason all the micronuclei maintain themselves successfully through the mitotic division – a synchronous division. In *Epistylis articulata* SESHACHAR and DAS<sup>6</sup> have conclusively demonstrated such a conversion of the macronuclear DNA into RNA in the exconjugants. Attempts are being made in our laboratory to show experimentally this conversion of DNA into RNA during the predivisional stages in *Spirostomum* too. Until the experimental data provide support for the present interpretation, this view may be taken as merely one of the possible explanations of the events underlying such complicated phenomena as the nuclear divisions in ciliates.

*Résumé.* Dans cet article on a décrit l'appareil nucléaire et la fission binaire dans le cilié d'eau douce *Spirostomum dharwarensis*. La division micronucléaire est de type synchrone. En ce qui concerne le mode de division micronucléaire, on a étudié également le déplacement graduel du type sélectif au type synchrone dans le genre *Spirostomum*.

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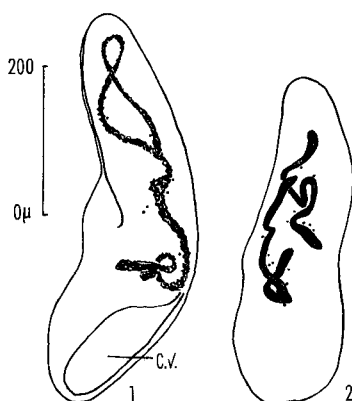


Fig. 1. A vegetative individual of *Spirostomum dharwarensis* n. sp. showing a cylindrical macronucleus and seven micronuclei distributed around it,  $\times 200$ . (c.v. = Contractile vacuole.)

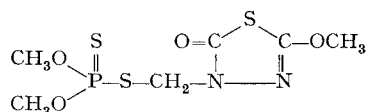
Fig. 2. Two daughter macronuclei have grown to a considerable size within the parent ciliate body. Still there is no sign of any cytoplasmic fission,  $\times 100$ .

<sup>5</sup> P. B. PADMAVATHI, Proc. zool. Soc. Ind. 7, 91 (1955).

<sup>6</sup> B. R. SESHACHAR and C. M. S. DAS, Proc. nat. Inst. Sci. Ind. 20, 656 (1954).

### Metabolism of GS 13005<sup>1</sup>, a New Insecticide

GS 13005<sup>1</sup> is a new organophosphorus insecticide with the structure O,O-dimethyl-S-[2-methoxy-1,3,4-thiadiazol-5-(4H)onyl-(4)methyl]-dithiophosphate<sup>2</sup>:



Its potent biocidal activity and its toxicological behaviour<sup>3</sup> have been described. In this paper, the results of metabolism studies on the distribution and excretion of the substance in animals and plants are reported. Details on the metabolic fate of the molecule in vivo and in vitro will be published elsewhere.

*Metabolism in animals.* In a series of balance studies, the excretion of orally applied labelled GS 13005 in the urine, faeces and expired air of the rat was examined. The mean values of four experiments are summarized in Table I.

Table I shows a high degree of recovery of the radioactivity applied. The distribution pattern indicates a complete absorption of the substance and an intense metabolism with concomitant cleavage of the heterocyclic moiety to CO<sub>2</sub> as the main result. The excretion of polar metabolites in the urine is apparently of the same importance as the expiration.

The distribution of GS 13005 in the organs of the rat was studied in order to support these results and to exclude specific retention or accumulation of the insecticide or its metabolites in any organ. The results are reported

<sup>1</sup> GS 13005 is the active ingredient of a new insecticide of J. R. Geigy S.A., Basle, Switzerland, registered under the Trade mark of Supracide®.

<sup>2</sup> K. RÜFENACHT, J. R. Geigy S.A., Swiss Patents Nos. 3-92-521 and 3-95-637.

<sup>3</sup> H. GROB, R. GASSER, and M. A. RUZETTE, *Further Investigations with GS 13005 for Use in Orchards and Vineyards*. 3rd British Insecticide and Fungicide Conference in Brighton (1965), in press.