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[5], 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[6] 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.

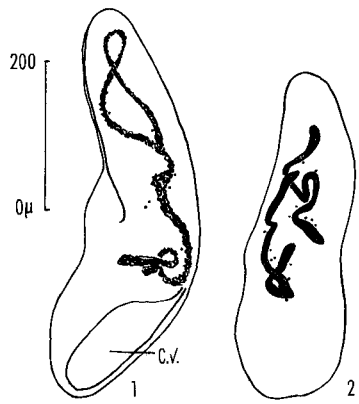

Fig. 1. A vegetative individual of *Spirostomum dharwarensis* n. sp. showing a cylindrical macronucleus and seven micronuclei distributed around it, × 200. (c.v. = Contractile vacuole.)

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, × 100.

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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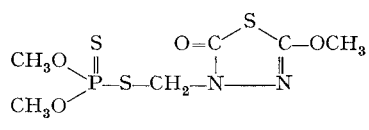
Department of Zoology, Karnatak Science College, Dharwar (India), May 31, 1965.

[5] P. B. PADMAVATHI, Proc. zool. Soc. Ind. *7*, 91 (1955).
[6] B. R. SESHACHAR and C. M. S. DAS, Proc. nat. Inst. Sci. Ind. *20*, 656 (1954).

Metabolism of GS 13005[1], a New Insecticide

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



Its potent biocidal activity and its toxicological behaviour[3] 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_2 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

[1] 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®.
[2] K. RÜFENACHT, J. R. Geigy S.A., Swiss Patents Nos. 3-92-521 and 3-95-637.
[3] 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.

in Table II with values representing means of two animals for each time interval.

In addition to the high rate of excretion, the results listed in Table II clearly demonstrate that no accumulation of GS 13005 takes place. A very fast distribution was established, the maximum of which does not exceed 8 h after application. The radioactive content of all organs is below the limits of determination 48 h after dosage, except for trace amounts in muscles. This rapid elimination of the radioactivity indicates that no essential part of the C 1-piece from the cleavage of the ring enters the general metabolism. It should be stressed that all radioactivity in the organs can be extracted and that no incorporation into high molecular compounds was observed.

Metabolism in plants. The metabolic breakdown of GS 13005 in plants was investigated when applied to the leaves of young bean plants (*Phaseolus vulgaris* L.) and to freshly harvested apples.

The ability of young bean leaves to split the heterocyclic moiety of the insecticide to CO_2 is demonstrated in Table III. Unchanged material shows up in the chloroform phase and, according to chromatographic examination, all radioactivity represents GS 13005 up to three days after treatment. Later on small amounts of metabolites are present.

After 14 days 5% of the applied insecticide is still unchanged. The water phase contains radioactivity in very polar form. An accumulation of this material does not occur. Its percentage reaches a maximum after 8 days and declines afterwards. The residue is considered mainly to include breakdown products used again by the plant in synthetic processes.

The studies with apples revealed an appreciable breakdown of GS 13005 to CO_2. The radioactivity which can be washed off the surface with acetone represents unchanged GS 13005. On the other hand, it is only during the first

Table I. Excretion of GS 13005 in the urine, faeces and expired air of the male rat

Time after application in h	% of recovered radioactivity			
	Urine	Faeces	Expired air	Day
0–24	39.7	0.63	27.8	68.13
24–48	2.2	0.60	7.8	10.60
48–72	0.7	0.05	0.5	1.25
72–96	0.2	0.02	0.3	0.52
Total excreted	42.8	1.3	36.4	80.5

Material used: GS 13005 C^{14}-labelled in the 5-position of the heterocycle[4]. Dose: 2.7 μc corresponding to 0.8 mg per animal of 180–200 g body weight per os. The animals were held in closed all-glass metabolism cages. Radioactive measurements were performed by liquid scintillation counting.

Table II. Distribution of GS 13005 in the organs of the male rat

Organ	% of recovered radioactivity Time after application in h				
	0.5	3	8	24	48
Stomach	41.5	28.4	1.66	0.58	< 0.05
Small intestine	2.66	2.33	2.67	0.87	< 0.05
Cecum + colon	1.75	1.68	3.96	3.21	0.05
Liver	1.84	1.15	0.75	0.22	< 0.05
Kidneys	0.40	0.42	0.35	0.13	< 0.05
Lung	0.32	0.34	0.24	0.10	< 0.05
Testis	0.28	0.69	0.59	0.14	< 0.05
Muscles	9.64	15.9	17.4	3.17	0.29
Body fat	3.48	3.26	2.54	0.65	< 0.05
Blood plasma	1.0	< 0.05	< 0.05	< 0.05	< 0.05
Blood cellular elements	0.51	< 0.05	< 0.05	< 0.05	< 0.05

Material and applied dose as in Table I. Procedure: Extraction of the isolated organs with acetone-methanol and dissolution of the evaporated extract with Hyamine. Liquid scintillation counting of the extracts was followed for time dependence for at least 24 h. The efficacy of the extractions was controlled by combustion of the residues. In no case were significant amounts of radioactivity found. (Limits of the determination: < 0.2% of the applied dose for combustions of the extracted residues of the first 3 organs and < 0.05% for the rest; < 0.05% for extracts.) Calculations: Total muscle as 39% of living body weight (sample collected from foreleg, hind leg and back), total body fat as 12% (sample collected from subcutaneous tissue, intestines and testis), blood volume as 6% with the relation of plasma to cellular elements as 1:1.

[4] The labelled GS 13005 was synthesized by Dr. D. E. Ryskievich, Geigy Research Laboratories, Ardsley (New York).

Table III. Distribution of radioactivity after foliar application of C^{14}-GS 13005 to beans

Fractions	% of recovered radioactivity Days after treatment								
	2	3	4	5	7	8	10	12	14
CO_2	2.3	8.9	12.5	15.3	20.4	22.2	24.4	26.0	27.4
Chloroform phase	71.5	41.2	32.0	15.4	17.6	10.3	11.3	10.2	6.9
Water phase	7.1	22.6	16.2	27.8	24.4	37.7	27.7	17.9	14.9
Residue	2.1	6.1	6.0	8.8	7.3	9.1	8.6	8.4	12.4
Total	83.0	78.8	66.7	67.3	69.7	79.3	72.0	62.5	61.6

Material as in Table I. Dose: 0.464 μc per plant, dissolved in 0.1 ml of acetone. Procedure: the plants were kept in a closed system and air drawn through it; after harvest of the plants homogenization in acetone; partitioning of the acetone extract between chloroform and water; determination of the radioactivity not extractable from the residue by combustion.

Table IV. Balance studies with apples treated with C^{14}-GS 13005

Fractions	% of recovered radioactivity								
	Days after treatment								
	0	2	6	10	15	21	30	42	56
CO_2	0	1.1	5.1	11.2	17.9	23.6	26.6	30.5	31.6
Acetone wash	98.7	59.8	38.8	30.0	18.3	5.0	4.6	1.1	2.2
Acetone extract	0.6	33.1	52.2	48.7	34.6	48.4	38.8	40.7	35.6
Residue	0.2	2.1	4.6	7.4	15.1	11.9	11.4	20.2	12.7
Total	99.5	96.1	100.7	97.3	85.9	88.9	81.4	92.5	82.1

Material as in Table I. Dose: 0.369 μc per apple, topically applied in 0.1 ml of acetone. Storage of the apples in a dessicator connected to an air pump; the apple was rinsed with acetone prior to homogenization in acetone; the activity not extractable from the residue was determined by combustion.

ten days that the acetone extract of the total fruit contains some unchanged material.

Résumé. Le métabolisme de l'insecticide GS 13005 (marqué par le C^{14} dans l'hétérocycle) fut étudié chez l'animal et dans la plante. Une conversion considérable de cette molécule en CO_2 fut constatée dans tous les deux. En plus aucune accumulation de l'insecticide intact ou de ses produits métaboliques n'a pu être observée, ni dans la plante, ni dans l'animal.

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Departement Forschung Schädlingsbekämpfung, J. R. Geigy AG, Basel (Switzerland), October 25, 1965.

Some Relations Between the Structure and the Antibacterial Activity of Natural Coumarins

It is a well-known fact that dicoumarol and anticoagulants in general retard the growth of Gram-positive microorganisms. Little, however, is known about the antibacterial effects of natural coumarins[1,2]. As was ascertained in our previous experiments[3], out of eighteen natural coumarins and furanocoumarins, only ostruthin (6-geranyl-7-hydroxycoumarin) shows a strong effect specifically on Gram-positive organisms. The present report gives the results of our investigation on the effect of further related coumarins and furanocoumarins on Gram-positive and Gram-negative organisms. We have tried to ascertain the influence of the side isoprenic chain and of phenolic hydroxyl on the antibacterial activity.

Coumarins used[4]: (1) coumarin, (2) umbelliferone (7-hydroxycoumarin), (3) osthol (7-methoxy-8-isopentenylcoumarin), (4) ostruthin (6-geranyl-7-hydroxycoumarin), (5) ostruthin-methylether, (6) umbelliprenin (7-farnesoxycoumarin), (7) ammoresinol (3-farnesyl-4:7-dihydroxycoumarin), (8) diacetylammoresinol, (9) ammoresinoldimethylether, (10) dicoumarol (3:3'-methylene-bis-(4-hydroxycoumarin).

Furanocoumarins used[4]: peucedanin (11-methoxy-10-isopropylpsoralene), imperatorin (8-isopentenyloxypsoralene), isoimperatorin (5-isopentenyloxypsoralene), oxypeucedanin (5-(2':3'-epoxy)-isopentyloxypsoralene), ostruthol (5-(3'-hydroxy-2'-angelicyloxy)-isopentyloxypsoralene).

Alcoholic solutions of coumarins and furanocoumarins were tested on agar plates in a concentration of $5 \cdot 10^{-3} M$ by means of the paper disc method[3].

Microorganisms used: of the Gram-positive microorganisms, members of the genera Staphylococcus, Micrococcus, and Bacillus. Gram-negative organisms were represented by *Escherichia coli*, *Aerobacter aerogenes*, and *Serratia marcescens*.

The results are given in the Table. With the exception of the slight effect of peucedanin on Gram-positive organisms, all furanocoumarins were ineffective and are therefore not mentioned in the Table. Similarly, we do not mention in the Table Gram-negative organisms which are not affected by any of the preparations used.

It follows from the results obtained that all effective derivatives, similarly dicoumarol, act selectively on Gram-positive microorganisms. The greatest effect was shown by ostruthin (4) and ammoresinol (7). In order to produce the effect of ostruthin, the presence of the side chain is necessary. Umbelliferone (2) has no effect at all. Methylation of the –OH group in ostruthin results in a weakening of the inhibitory effect (5), but not in its nullification. The presence of phenolic hydroxyl is not the

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[3] V. DADÁK, Československ. farm. *7*, 394 (1958).
[4] The derivatives characteristic of the family Daucaceae were obtained thanks to Prof. Dr. F. WESSELY, Universität Wien, peucedanin thanks to Prof. Dr. H. SCHMID, Universität Zürich, the methylated and acetylated derivatives were prepared according to SPÄTH[5].
[5] E. SPÄTH, A. J. SIMON, and J. LINTNER, Ber. dtsch. chem. Ges. *69*, 1656 (1936).