alone. The potentiated anomaly rates may possibly be explained by inhibited catecholamine uptake with the use of cocaine. These results agree with a study of catecholamine uptake in embryonic chicks using tritiated norepinephrine and epinephrine[4]. Two enzymes which metabolize catecholamines (catechol-*O*-methyl transferase – COMT and monoamine oxidase – MAO) are present in the chick embryo on the 4th day of incubation[12]. However, MAO is intracellular and probably does not metabolize the exogenous norepinephrine and epinephrine. The extent to which COMT metabolizes catecholamines is most likely insignificant in the termination of overall catecholamine activity[4].

Cocaine does not increase the anomaly rate among isoproterenol treated embryos. This result may possibly be explained by the fact that isoproterenol is apparently not taken up by post-ganglionic sympathetic neurons[13]. However, cocaine in combination with isoproterenol induces different types of anomalies from those frequently induced by isoproterenol alone, that is, less cases of aortic hypoplasia and interrupted aortic arch complexes were observed.

Furthermore, cocaine does not increase the anomaly rate among those embryos treated with phenylephrine. Assuming that cardiovascular anomalies can be induced in embryonic chicks by β-adrenoreceptor stimulation, this result is expected. Phenylephrine acts primarily on α-adrenergic receptors.

This study has also shown that, using as a criterion for potency the frequency of aortic arch anomaly type IA-1 (formerly described as absence of the 3rd right aortic arch with an anomalous origin of the right common carotid artery from the right ductus caroticus)[2], I is more potent than E which in turn is more potent than N or P at 4×10^{-9} *M*/embryo. This relation of I $\gg$ E $>$ N or P correlates with a β-adrenoreceptor response to the drugs and confirms previous findings that a β-receptor mechanism might possibly be involved in the induction of cardiovascular anomalies[2].

Furthermore, cocaine specifically potentiates the effects of epinephrine and norepinephrine in the formation of aortic arch anomaly type IA-1; from 0 to 14% in the case of epinephrine (considering IA-1 anomalies induced spontaneously and with cocaine) and from 1 to 10% in the case of norepinephrine (computed from Table). Cocaine does not potentiate the IA-1 anomaly rate when administered with isoproterenol or phenylephrine. Cocaine in all cases did not potentiate aortic hypoplasia or interrupted aortic arch complexes.

Cocaine in small doses (5×10^{-9} to 1×10^{-7} *M*) does not induce aortic arch or cardiac anomalies in embryonic chicks. However, type IA-1 is occasionally induced when 5×10^{-7} *M* is administered (see Table). Furthermore, several cases were observed which demonstrated premature closure of the right ductus arteriosus and persistence of the left ductus caroticus. This may be explained if cocaine also affects the reuptake of endogenous catecholamines into nerve endings. In effect large amounts of circulating endogenous catecholamines might possibly cause cardiovascular anomalies in this system. Since it has been demonstrated that cocaine in a dose $^1/_7$ to $^1/_6$ that used in this study inhibits more than 80% of norepinephrine uptake by the whole chick embryo and more than 50% by the embryonic heart on the 5th day of development[4], this hypothesis seems possible.

In conclusion, whereas cocaine was administered to embryos 3 h prior to catecholamine injection in a previous study[4], this study demonstrated potentiation with a 5 min pretreatment period. This finding suggests that cocaine rapidly affects the uptake mechanism of nerve endings in embryonic chicks.

[12] L. J. Ignarro and F. E. Shideman, J. Pharmac. exp. Ther. *159*, 29 (1968).

[13] G. Hertting, Biochem. Pharmac. *13*, 1119 (1964).

A New Quinoline-Carbamate Aphicide[1]

W. J. Esser[2], W. J. Kloft[2] and A. S. Tahori[3]

Institut für Angewandte Zoologie der Universität Bonn, An der Immenburg 1, D-5300 Bonn (German Federal Republic, BRD), 14 January 1976.

Summary. We found that it was nearly impossible to apply the quinoline-carbamate aphicide per os by means of synthetic diets, owing to its high feeding-deterrent-effect. After application via the roots of the host plant, this systemic compound is deposited on the leaf surface. The results suggest that the toxic effect is not the result of the oral uptake of phloem sap, but of the tarsal contact with the toxicant. Sensitivity of aphids to this compound and LD_{50}-values were determined after topical applications.

The aphicidal effect and the symptoms and phases of the intoxication of a new aphicide were investigated. Earlier experiments from the producer had shown that the compound was much more effective against aphids than against other insects. The following aphid species were used: *Aulacorthum circumflexum* (Buckton), *Aphis craccivora* Koch and two strains of *Myzus persicae* (Sulzer), one susceptible to insecticides and the second resistant to organophosphorus compounds. All aphids were reared parthenogenetically on suitable host plants in the laboratory. The experiments with oral application were carried out only with *A. circumflexum* since it has a spontaneous and even nutrient uptake from artificial media[4,5].

The aphicide, (concentration 99.9%) a quinoline-carbamate[6], 4 (N.N-dimethyl-carbamoyloxy-)-2-methyl-5.6.7.8-tetrahydro-quinoline, also known as Hoe 25 682,

[1] We are grateful to the Deutsche Forschungsgemeinschaft for offering a grant.

[2] Institut für Angewandte Zoologie der Universität Bonn (BRD).

[3] Israel Institute for Biological Research, Ness-Ziona, visiting professor (Oct. 1974–Sep. 1975), Institut für Angewandte Zoologie der Universität Bonn (BRD).

[4] P. Ehrhardt, Habilitationsschrift, Universität Bonn (1968).

[5] D. Holtgräwe and W. Kloft, Oecologia *14*, 229 (1974).

[6] W. Knauf, G. Salbeck and F. Schwerdtle, Meded. Landbhoogesch. Opzoek Stns Gent *27*, 427 (1975).

was kindly supplied by Hoechst AG, Frankfurt/Main, Germany. It is soluble in organic solvents and at 6.5% concentration in water.

The oral application method to test the systemic effect of an insecticide has been suggested by KLOFT and KUNKEL[7]. This test was carried out with the help of a parafilm-membrane®[8], held between the aphids and an aqueous solution of insecticide. The solution, containing 18% sucrose[9], was made radioactive by adding ^{32}P orthophosphate at a specific activity of 0.1 mCi/ml. The uptake was measured with a Philips counter, combined with an automatic sample changer, (voltage of the GM tube: 600).

Topical application was carried out by means of a microsyringe. An amount of 0.2 µl of the aphicide in butanone solution was applied dorsally to adult apterous virgines, earlier anaesthetized with 20 sec dosage of CO_2. The aphids were kept on host plants during application, and then in small aphid cages, held on the plants. Mortality was checked after 20 h. Apparently moribund aphids, unable to recover, were considered dead.

In the case where 18% sucrose solution contained Hoe 25 682, the uptake was strongly reduced (Table I), and no peroral toxicity could be obtained. However, Hoe 25 682 did penetrate the parafilm-membrane, killing aphids sitting on it (Table II). The presence of penetrated Hoe 25 682 on the other side of the membrane was proven, using the technique described by ELLMAN et al.[10], and the values obtained were considerably above threshold. In the preliminary experiments, such feeding-deterrent-effects were also observed in the ant *Lasius niger* L. and the large milkweed bug *Oncopeltus fasciatus* (Dallas).

Hoe 25 682 showed systemic effects on bean plants raised on Knop's nutrient solution[11]. Within 2 days, the

Table I. Oral uptake of Hoe 25682 by 4th stage larvae of *A. circumflexum* from an 18% sucrose solution containing ^{32}P orthophosphate at a specific activity of 0.1 mCi/ml

Concentration of Hoe 25682 (%)	Time of exposure (h)	Total number of aphids	No. of radioactive aphids at the end of the experiment	Average counts per 1 radioactive aphid/h of exposure (counts/100 sec)	Weighted average
0	3.5	8	1	10.14	6
0	24.5	16	14	1.64	
0	53	8	8	7.03	
0	67	3	3	15.26	
0.01	8	47	2	0	
0.001	52.5	26	4	0.09	
0.0007	29.5	15	2	0.15	0.083
0.0007	51	31	8	0.05	
0.0001	28	8	8	1.48	0.61
0.0001	77.5	15	15	0.63	
0.0001	98.5	24	24	0.35	

Table II. Contact toxicity of Hoe 25682 to 4th stage larvae of *A. circumflexum*. Aphids kept on parafilm-membrane-sachets with 18% sucrose solution containing the aphicide and ^{32}P orthophosphate

No. of aphids	Concentration of Hoe 25682 (%)	Time of exposure (h)													
		1	2	3	4	5	6	7	8	10	15	20	25	30	40	50
		Mortality (%)														
46	0.1	12.7	80.5	96	100											
47	0.01	4.2	14.5	42	69	85	93	100								
26	0.001						2.3		2.3	13	23	41	59	64	84	92
46	0.0007							4	5	6	8	9	11		19	35

Only 6 out of 133 of the dead aphids showed a radioactive count.

Table III. Systemic effect of Hoe 25682 in *Vicia faba*

Height of plant (cm)	Tested leaf square (cm^2)	Amount of Hoe 25682 per cm^2 leaf square ($\mu g \cdot 10^{-3}$)
11	9	0.33
13	32	0.43
16	20	0.50

Aphicide applied at a concentration of 0.5 mg in 50 ml of Knop's nutrient solution[11] per plant. The presence of the aphicide on leaf surface was verified by the method of ELLMAN et al.[10].

Table IV. Aphicidal effect of Hoe 25682 by topical application to adult apterous virgines

Aphid strain	Amount of Hoe 25682 per aphid ($\mu g \cdot 10^{-3}$)	No. of aphids	Kill corrected after Abbott (%)	Weighted average of % kill
Aphis craccivora	0.01	97	24.74	
	0.015	124	57.25	
	0.02	103	48.22	
	0.025	84	78	
	0.04	71	92.95	
Aulacorthum circumflexum	0.025	84	43.9	42.26
	0.025	88	40.7	
	0.033	40	57.76	
	0.04	74	33.89	60.2
	0.04	110	77.99	
	0.05	84	79.35	
Myzus persicae (susceptible)	0.02	81	8.64	
	0.05	87	45.08	34.52
	0.05	99	22.87	
	0.05	47	39.53	
	0.1	105	62.67	68.75
	0.1	63	78.89	
Myzus persicae (OP-resistant)	0.05	86	31.89	
	0.07	86	56.1	
	0.1	102	73.6	

0.2 μl of butanone solution applied with a microsyringe. Mortality was checked after 20 h.
Mortality in the control never exceeded 14%, otherwise experiments were discarded.

Table V. The toxicity of Hoe 25682 to adult apterous aphids

Species	LD_{50} (10^{-3} μg/mg weight)	Slope of the regression line
A. craccivora	0.02	2.05
A. circumflexum	0.04	1.86
M. persicae (susceptible)	0.20	1.76
M. persicae (OP-resistant)	0.21	1.98

insecticide appeared on the leaf surface (Table III). Most of the aphids were killed or were affected and dropped on the ground. Results of the topical application experiment are shown in Tables IV and V in terms of LD_{50}.

The symptoms of intoxication by Hoe 25 682 in aphids were multifold: 1. knockdown was followed by excitation and motor hyperactivity. 2. The aphids showed stumbling movements. 3. Aphids responded only feebly to stimuli (adynamic phase[12]). 4. They culminated in death.

These results were similar to those observed by HOLTGRÄWE[13] for the organophosphorus compound triazophos, 1-phenyl-1.2.4-triazolyl-3-(0.0 diethylthionophosphate). The only exception was the observation of repeated short-term recovery in aphids, especially in *A. craccivora*, which were exposed to Hoe 25 682. This can be explained by the rapid metabolic destruction of carbamates[14]; however, recurring penetration of toxicant will finally kill the insects.

[7] W. KLOFT and H. KUNKEL, Z. Pflkrankh. PflPath. PflSchutz *76*, 1 (1969).
[8] T. E. MITTLER and R. H. DADD, Ann. ent. Soc. Am. *57*, 139 (1964).
[9] I. DANNEEL, Z. angew. Zool. *56*, 229 (1969).
[10] G. L. ELLMAN, K. D. COURTNEY, V. ANDRES and R. M. FEATHERSTONE, Biochem. Pharmac. *7*, 88 (1961).
[11] W. SCHROPP, *Der Vegetationsversuch* (Neumann Verlag, Radebeul und Berlin 1951).
[12] E.-F. SCHULZE, Dissertation, Universität Würzburg (1965).
[13] D. HOLTGRÄWE, Dissertation, Universität Bonn (1974).
[14] R. D. O'BRIEN, *Insecticides, Action and Metabolisms* (Academic Press, New York 1967).

Metabolically Regulated Cyclical Contractures in Microinjected *Spirostomum:* a Pharmacological Study

S. DIKSTEIN and R. B. HAWKES[1]

Department of Applied Pharmacology, Hebrew University, P. O. Box 12065, Jerusalem (Israel), 8 March 1976.

Summary. Spirostomum was treated extracellularly and intracellularly with a range of metabolites to investigate the intracellular regulation of cyclic calcium movements. The results indicate close links between calcium movements and mitochondrial metabolism.

Externally applied pharmacodynamic stimuli may trigger cyclic myonemal contractures in the heterotrich ciliate *Spirostomum*[2]. Studies with detergent-extracted cell models[3], intracellular aequorin luminescence[4] and CaEGTA/EGTA buffer microinjection[5] concur that stimulus-contracture coupling is through an increase in the cytoplasmic free Ca^{2+} concentration. Although contraction and re-extension of extracted cells does not require exogenous metabolites[3], and so resembles other myonemal contractile mechanisms[6], the role of nucleotide phosphate in vivo remains unclear[7]. Because Ca^{2+} to trigger contracture is released from intracellular stores, not derived extracellularly[7], and because the release is not mediated by cell surface membrane depolarization[5,8],

[1] We are grateful to Dr. A. GAUDEMAR, Institut de Chimie des Substances Naturelle, Gif-sur-Yvette, France for a gift of bongkrekic acid, to Dr. D. C. ALDRIDGE, Biochemistry Dept., I. C. I. Ltd., Macclesfield, U. K. for a gift of avenceolide and to E. M. ETTIENNE for many hours of discussion. Dr. R. B. HAWKES is a Visiting Research Fellow at the Hebrew University of Jerusalem.

[2] M. A. SLEIGH, Acta protozool. *7*, 335 (1970).
[3] R. B. HAWKES, D. V. HOLBERTON and W. S. OGLE, Proc. 3rd Int. Congr. Biophys., in press (1976).
[4] E. M. ETTIENNE, J. gen. Physiol. *56*, 168 (1970).
[5] R. B. HAWKES and D. V. HOLBERTON, J. cell. Physiol. *84*, 225 (1974).
[6] W. B. AMOS, Nature, Lond. *229*, 127 (1971).
[7] R. B. HAWKES, in preparation.
[8] E. M. ETTIENNE and S. DIKSTEIN, Nature, Lond. *250*, 782 (1974).