

Table II. Uptake of  $^{28}\text{Mg}$  by different brain areas and pituitary gland in rats following intracarotid injection of 1  $\mu\text{Ci}$  of  $^{28}\text{Mg}$ 

Tissue	N	Control	Reserpine	Statistical significance ( $p$ )
Cortex	54	3,766 $\pm$ 649	4,215 $\pm$ 569	NS
Hippocampus	53	3,092 $\pm$ 837	5,323 $\pm$ 872	(< 0.06)
Thalamus	54	4,387 $\pm$ 2,200	3,546 $\pm$ 932	NS
Superior colliculus	27	3,835 $\pm$ 1,028	5,633 $\pm$ 1,333	NS
Cerebellum	51	5,059 $\pm$ 794	6,688 $\pm$ 872	NS
Medulla	27	9,028 $\pm$ 2,071	6,766 $\pm$ 1,554	NS
Pituitary gland	27	141,453 $\pm$ 39,065	239,794 $\pm$ 63,857	NS

The radioactivity is expressed as dpm/g of wet tissue. Values are expressed as means  $\pm$  S.E.M.

scintillation counter within 10 h following the start of the experimental procedure.

In order to separate the  $^{45}\text{Ca}$  and  $^{28}\text{Mg}$  counts, it was necessary to recount the tissue vials after the  $^{28}\text{Mg}$  radioactivity had, for all practical purposes, completely decayed. 8 half-lives (7 days) were considered adequate for  $^{28}\text{Mg}$  decay (less than 0.4% of the original  $^{28}\text{Mg}$  activity remained). Thus, the  $^{45}\text{Ca}$  was counted 7 days after the first count. The  $^{45}\text{Ca}$  counts (second count) were subtracted from the initial count ( $^{28}\text{Mg} + ^{45}\text{Ca}$ ) to obtain the  $^{28}\text{Mg}$  content of the samples. Appropriate corrections were made for the decay of both  $^{28}\text{Mg}$  and  $^{45}\text{Ca}$ . Standards containing equal amounts of  $^{45}\text{Ca}$  and  $^{28}\text{Mg}$  were used in the calculation of data and decay.

The amount of tissue radioactivity (dpm) of  $^{45}\text{Ca}$  and  $^{28}\text{Mg}$  for each tissue was divided by the tissue weight and expressed as dpm/g tissue. The data for each tissue was pooled for the animals within the control and test groups and subjected to statistical analysis using a two-tailed  $t$ -test.

Table I shows the  $^{45}\text{Ca}$  uptake in control and reserpine-tested animals. Statistically significant increases in  $^{45}\text{Ca}$  uptake due to reserpine were found in the cortex ( $p < 0.004$ ), hippocampus ( $p < 0.001$ ), thalamus ( $p < 0.038$ ) and pituitary gland ( $p < 0.018$ ). The relation between  $^{45}\text{Ca}$  in the cortex and hippocampus was the same as we reported previously<sup>4</sup> for saline controls (cortex-hippocampus,  $p < 0.040$ ) and the reserpine-tested animals (cortex-hippocampus,  $p < 0.005$ ). The higher statistical significance in the reserpine-tested group could be due to the marked increase in hippocampal  $^{45}\text{Ca}$  uptake.

Table II shows the  $^{28}\text{Mg}$  uptake in the various brain regions of the control and reserpine-tested animals. There was a definite trend, but not statistically significant, in the hippocampus showing an increase in  $^{28}\text{Mg}$  uptake due to reserpine. The ratio between the cortex and hippocampus uptake was greater than 1 in the control group (as previously reported<sup>5</sup>), but dropped to less than 1 in the reserpine-treated group.

In contrast to chronic administration, a single injection of reserpine (2 mg/kg, i.m.) did not modify the in vivo uptake of labelled calcium and magnesium.

The increase in the uptake of radiolabelled calcium is in agreement with the reports of a decrease in endogenous levels of calcium in the hippocampus and cortex of rats<sup>6</sup> and guinea-pig brain<sup>7</sup> following reserpine administration. RADOUCO-THOMAS<sup>8</sup> proposed that reserpine may stimulate catecholamine release by removing calcium from some functional site on the presynaptic membrane where its binding was inhibiting the catecholamine release. In our experiments the increased uptake of radiolabelled calcium by the pituitary gland, cortex, hippocampus and thalamus following administration of reserpine could indicate an attempt to replace this calcium. Further ultrastructural and biochemical investigations are needed to elucidate the mechanisms by which neurotransmitter releasing agents modify the blood-brain and blood-pituitary barriers<sup>9</sup>.

*Résumé.* L'administration chronique de réserpine augmente l'incorporation de  $^{45}\text{Ca}$  dans le cerveau et la glande pituitaire du rat.

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<sup>6</sup> D. H. ROSS, M. A. MEDINA and H. L. CARDENAS, *Science* 186, 63 (1974).

<sup>7</sup> S. RADOUCO-THOMAS, L. TESSIER and N. LAJEUNESSE, *Int. J. clin. Pharmac.* 5, 5 (1971).

<sup>8</sup> S. RADOUCO-THOMAS, *Int. J. clin. Pharmac.* 5, 271 (1971).

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## Effect of Subacute Poisoning by Cyolane<sup>1</sup> on Acetylcholine Esterase and Succinic Dehydrogenase in the Rat

It has been assumed that the toxicity of organophosphorus insecticides was not only due to their potency as anticholine esterase agents<sup>2-6</sup>. Thus the action of these insecticides on metabolic enzyme systems has drawn the attention of many investigators<sup>7-10</sup>.

The present investigation was undertaken to obtain information on the effect of Cyolane on rat acetylcholine esterase activity in brain and blood, and liver succinic

dehydrogenase activity. Cyolane, is widely used here in Egypt for control of cotton leaf worm, *Spodoptera littoralis*.

*Materials and methods.* Cyolane, 2-(diethoxy phosphanyl imino)- 1,3-dithiolane, was kindly supplied by American Cyanamide Company. Albino rats of both sexes, weighing 100-120 g and maintained on a stock diet, were used.

Cyolane was given orally in corn oil daily at doses

Percentage inhibition of brain serum, and erythrocytes acetylcholinesterase and liver succinic dehydrogenase activities after Cyolane administration<sup>a</sup>

Enzyme	Daily dose (mg/kg)	Percentage inhibition <sup>b</sup>		
		1 week	2 weeks	4 weeks
Brain acetylcholinesterase	0.9 <sup>c</sup>	54 ± 3.64	—	—
	0.18	40 ± 3.23	36 ± 3.3	35 ± 3.28
	0.09	28 ± 2.83	15 ± 2.88	16 ± 2.33
	0.045	0	0	0
Erythrocytes acetylcholinesterase	0.9 <sup>c</sup>	37 ± 3.75	—	—
	0.18	15 ± 3.28	40 ± 3.75	45 ± 2.33
	0.09	0	28 ± 2.31	30 ± 2.88
	0.045	0	10 ± 2.19	10 ± 1.87
Plasma acetylcholinesterase	0.9 <sup>c</sup>	28 ± 1.04	—	—
	0.18	5 ± 1.87	44 ± 3.3	42 ± 3.64
	0.09	0	30 ± 1.9	30 ± 2.48
	0.045	0	15 ± 2.54	17 ± 1.87
Liver succinic dehydrogenase	0.9 <sup>c</sup>	0	—	—
	0.18	0	37 ± 1.73	40 ± 1.27
	0.09	0	30 ± 2.71	35 ± 1.38
	0.045	0	12 ± 2.54	15 ± 1.78

<sup>a</sup> Data are means of 4–6 rats. <sup>b</sup> Mean ± S.D. <sup>c</sup> Rats treated with 0.9 mg/kg died within 10 days.

corresponding to 0.9, 0.18, 0.09, and 0.045 mg/kg (1/10, 1/50, 1/100 and 1/200 of the LD<sub>50</sub> respectively, for 1, 2 and 4 weeks. Rats were sacrificed 24 h after the last dose. Blood was collected in centrifuge tubes, and brain and liver were quickly removed for enzymatic assay.

Acetylcholinesterase in brain was estimated after the method of HESTRIN<sup>11</sup>, using 10% rat brain homogenate and an incubation period of 15 min. Acetylcholinesterase in erythrocytes and plasma were assayed according to MICHEL<sup>12</sup>. Succinic dehydrogenase activity was measured colorimetrically using 0.5% solution of 2-3-5-triphenyl-tetrazolium chloride according to the method of FAHMY<sup>13</sup>.

**Results and discussion.** In the present experiments, repeated short-term administration of Cyolane to rats resulted in a decrease in acetylcholinesterase activity of brain, erythrocytes and plasma, (Table). This inhibition was comparable to other organophosphorus pesticides<sup>2–6</sup>.

The inhibition of the enzymes under investigation remained almost constant from 2 up to 4 weeks. It seems that the cumulative effects with repeated administration of low doses were compensated physiologically<sup>2</sup>.

The extent of inhibition of acetylcholinesterase and succinic dehydrogenase was proportional to the Cyolane dose. This proportionality does not seem to exist between the percentage inhibition and the administration period<sup>14</sup>.

**Zusammenfassung.** Das Phosphorsäureester-Insektizid Cyolan hemmt nicht nur die Cholinesterasen, sondern auch die Succinodehydrogenase der Leber.

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- 2-(Diethoxy phosphanyl imino)-1,3-dithiolane.
- W. B. STAVINOGA, J. A. RIEGER JR., L. C. RYAN and P. W. SMITH, *Adv. Chem. Ser.* 60, 79 (1966).
- D. MILOSAVLJEVIC-ANDJELKOVIC, M. P. MILOSEVIC, *Jugoslav. physiol. pharmacol. Acta* 4, 253 (1968).
- G. V. LAVRINEKO, *Zdravookhr. Beloruss.* 6, 38 (1968).
- V. I. SVATKOV, *Cig. Primen; Toksik. Pestits. klin. Otravlenii* 8, 311 (1970).
- D. D. POLOZ, *Sel'Skokhoz. Biol.* 8, 219 (1973).
- YU S. KAGAN, G. A. RADINOV, L. YA. VORONINA and L. S. VELICHKO, *Cig. Toksik. Pestits. klin. Otravlenii* 5, 283 (1967).
- T. SYROWATKA, *Poez. Panstw. Zakl. Hig.* 20, 557 (1969).
- T. L. PROKLINA-KAMINSKAYA, *Cig. Tr.* 1, 91 (1969).
- H. NAKAKITA, Y. KATSUMATA and T. OZAWA, *J. Biochem., Tokyo* 69, 589 (1971).
- S. HESTRIN, *J. biol. Chem.* 180, 249 (1949).
- H. O. MICHEL, *J. Lab. clin. Med.* 34, 1564 (1949).
- A. R. FAHMY and E. O. F. WALSH, *Biochem. J.* 58, 231 (1954).
- C. H. WILLIAMS, *Toxic. appl. Pharmac.* 16, 533 (1970).

## An Autoradiographic Demonstration of Blood Cell Renewal in *Styela clava* (Urochordata: Ascidiacea)

The blood cells of ascidians circulate in the blood channels and wander throughout the tissues and the tunic. Although some blood cell types are common to all ascidians, other blood cell types often differ from species to species. The number of blood cells described in any one species also varies with the morphological criteria of the authors. With light microscopy, 8 types have been described in *Styela clava*<sup>1</sup> while 5 types have been described in *Styela plicata*<sup>2</sup>.

The origin and renewal of ascidian blood cells have been the subject of controversy. The neural gland<sup>3</sup> and haemoblasts in the connective tissue<sup>4</sup> have been reported as sites of blood cell formation. Several authors have

- W. C. GEORGE, *Q. Jl. microsc. Sci.* 87, 391 (1939).
- T. OHUYE, *Sci. Rep. Res. Insts Tohoku Univ., Biol.* 11, 191 (1936).
- L. CUÉNOT, *Archs Zool. exp. gén.* 9, 13 (1891).
- J. M. PÉRÈS, *Annl. Inst. océanogr., Monaco* 21, 229 (1943).