exposing the animals to virus dispersed in an aerosol. Rabies transmission through inhalation evidently occurred in two persons who entered Frio Cave, where sentinel animals were subsequently infected by a similar route (*4*) and where the virus was later isolated from air (*5*). Little effort has been made, however, to establish the actual tissue sites of viral exit or entry or to determine the subsequent pathogenesis of infection by the respiratory route.

Hronovský and Benda (*6*) exposed guinea pigs to an aerosol of rabies virus and found evidence that the virus invaded the central nervous system after first replicating in the olfactory epithelium of the nasal mucosa, and Hronovský (*7*) obtained similar results after exposing suckling mice to the virus by intranasal instillation. Presumably the virus progressed centripetally from the nasal tissues to the brain via the olfactory nerves. Fischman and Schaeffer (*8*) reported similar studies. However, they did not examine nasal tissues for viral antigen until the animals had become moribund, at which time centrifugal infection from the central nervous system could have contributed to the nasal tissue infection.

Schaaf and Schaal (*9*) found rabies virus in tissues of nasal mucosa of naturally infected animals. In this instance, bovines were presumably infected by the bite route, and the virus advanced to the central nervous system and from there centrifugally to the nasal mucosa and other peripheral tissues. We observed rabies virus antigen in olfactory receptor cells of moribund mice that had been inoculated with the virus by the intracerebral route; evidently a similar centrifugal spread of virus had occurred in these animals.

Our demonstration of intracytoplasmic rabies antigen in the olfactory receptor cells of naturally infected bats indicates that viral replication had occurred in these tissues and that the virus was not merely present from saliva. These results are consistent with either or both of two explanations. (i) Olfactory receptor cells were invaded directly by inhaled virus. (ii) The olfactory receptor cells were invaded centrifugally after the virus invaded the central nervous system.

Thus, these results on the distribution of virus in ill bats implicate the nasal mucosa as a potential portal of entry in natural infection by airborne rabies virus. In addition, they implicate the nasal mucosa as a possible portal from which rabies virus is expelled into the air in particles of respiratory mucus, probably supplementing airborne particles of virus-bearing saliva and possibly urine. Virus might be dispersed in an aerosol through breathing or sneezing. It might also become airborne through vocalization, since aggregations of resting bats chatter constantly, and flying bats navigate by sonar, some of the sounds being emitted in bursts via the nostrils.

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Paraoxon: Effects on Rat Brain Cholinesterase and on Growth Hormone and Prolactin of Pituitary

Abstract. *Cholinesterase activity of brain and content of growth hormone and prolactin in the pituitary were compared after short-term (3 days) and long-term (14 days) treatment with paraoxon in male and female rats. Within 3 days cholinesterase activity was reduced to between 5 and 15 percent of that in controls. The content of growth hormone in the pituitary was increased in long-term experiments by 50 percent. This increase in paraoxon-treated animals suggests a possible role of a cholinergic mechanism in the regulation of growth hormone secretion.*

The effects of organophosphorus inhibitors of cholinesterase (ChE) are often attributed solely to their action on its activity (*1*). In contrast to short-term changes in the cholinergic system induced by organophosphorus agents, long-term changes have been investigated little, including generalized toxic actions of these inhibitors. Exceptions include inhibition of Na^+,K^+ adenosine triphosphatase (*2*, *3*), demyelination (*4*), and inhibition of enzymes involved in carbohydrate metabolism (*5*). Most of these nonspecific observations have been made in vitro, however. An observation of specific interest to us was the stimulating action of organophosphorus inhibitor of ChE on [^{14}C]lysine incorporation into protein of the rat brain (*6*). Since the pool of the soluble microsomal fraction was unchanged, it was concluded that the effect was not due to an increase in permeability but to a general stimulation of the synthesis of protein. More recently (*7*), it has been shown that prior treatment of isolated nerve fibers with paraoxon, an irreversible ChE inhibitor, increases the protein synthesis. Paraoxon is the active metabolite of the commonly used insecticide, parathion. Our observations are concerned with the effect of long- and short-term paraoxon treatment on the

Table 1. Cholinesterase (ChE) activity (acetylcholine hydrolyzed per gram of brain per hour) in rat brain homogenates after rats were treated with paraoxon for 3 days and for 14 days. Results are expressed as averages (± standard error).

Treatment	Animals (No.)	ChE activity (μmole g^{-1} hr^{-1})	Remaining activity (% of control)
None*	19	548.74 ± 12.68	100.00
3 days	28	40.75 ± 7.38	7.30
None*	12	584.00 ± 11.16	100.00
14 days	34	44.85 ± 6.70	7.70

* Control.

major proteins of the rat anterior pituitary of growth hormone (GH) and prolactin (Pr) (*8*).

Ninety-three adult albino Sprague-Dawley rats (male and female), weighing 140 to 200 g, were used. Paraoxon (diethyl-*p*-nitrophenyl phosphate) was obtained from Merck & Co., Inc.; GH and Pr used for standard curves were obtained from the National Institutes of Health (NIH-BGH-B14; NIH-PB1). For GH and Pr determination, polyacrylamide gel electrophoresis, as modified by Lewis (*8, 9*), was used. In long-term experiments, 34 rats received paraoxon (124 μg/kg) in 0.9 percent NaCl (0.2 ml per 100 g of body weight) for 14 days. In short-term experiments, 28 rats received one daily intraperitoneal injection of 600 μg/kg for 3 days. Control animals (31 rats) received an equal volume of 0.9 percent NaCl. These animals were killed 30 to 60 minutes after the third injection. Daily administration of a dose less than the lethal dose (LD_{50}) of paraoxon (0.8 mg/kg) produces within a few day the cholinergic symptoms characteristic for this group of compounds, as seen in piloerection, salivation, generalized tremor, acrocyanosis, muscular fasciculations, contractions of the diaphragm, periodic muscular paralysis, and diarrhea.

After the animals were decapitated (between 10 a.m. and 11 a.m., to eliminate possible circadian variations), the anterior pituitary was dissected, weighed, and homogenized with 0.3 to 0.6 ml of distilled water. Portions (25 to 50 μl) of the homogenate were put immediately on polyacrylamide gels (Canalco disc electrophoresis, cold chamber model 1200). The GH and Pr bands, after being destained, were excised and dissolved in 0.5*M* KOH, and optical density was determined with a spectrometer (Gilford model 2400). Amounts of GH and Pr were expressed as micrograms of hormone per gram of pituitary tissue.

Cholinesterase in the brain was determined by a titrimetric method (*10*). The titrating agent was 0.005*M* NaOH, which was prepared fresh on the day of use from an NaOH stock solution and was adjusted by titration with 0.002*M* potassium hydrogen phthalate to *p*H 7.90 to 7.95. The *p*H end point was set to *p*H 7.5, and the reaction was run at room temperature (20° to 23°C). Acetylcholine iodide ($5 \times 10^{-3}M$) was used to determine enzyme activity. The substrate was dissolved in unbuffered Locke's solution.

The effects of paraoxon on the ChE activity of whole brain are summarized in Table 1. The brains from rats treated for the longer period (14 days) showed an enzyme activity remaining that was only about 8 percent of that found in control animals. By the time of the sixth injection, the more general symptoms of cholinergic poisoning could be observed (diarrhea, miosis, hypersalivation). In addition, a generalized tremor was observed, beginning 20 minutes after the injection and lasting 3 hours. Animals that died during the treatment period (10 percent of the animals) were discarded. In general, animals with the more severe symptoms showed more drastically reduced ChE levels and a significant drop in body weight (about 20 percent from their initial weight). In the short-term (3 days) experiments, the animals were killed 30 to 60 minutes after the last injection. Symptoms such as generalized tremor were seen as early as 20 minutes after the first injection. When the animals were killed, the brain ChE in the animals treated for 3 days was reduced to less than 7 percent of those of controls. To be certain that ChE activity determined at the time of killing corresponded to the actual level of enzyme activity in the living animal (*11*), we added a known amount of acetylcholinesterase (AChE) (commercially available red blood cell AChE) to brain tissue from paraoxon-treated rats before the brain tissue was homogenized. No reduction in the activity of the added known amount of AChE was observed. The same observation was made when a control half-brain and a brain from a paraoxon-treated animal were homogenized together. Brains from animals that received their last paraoxon injection 24 hours before the enzyme determination had a threefold (15 to 27 percent of controls) higher ChE activity than those brains from animals killed 60 minutes after the last injection of paraoxon.

Table 2. Growth hormone (GH) and prolactin (Pr) content in rat pituitaries after long-term treatment with paraoxon. Results are averages (± standard error) of two experiments with six rats in each group, with a total of 93 rats.

Treatment	GH (μg/mg)	Pr (μg/mg)
	Male	
Control (NaCl)	54.39 ± 5.28	8.27 ± 1.34
Paraoxon	79.99 ± 11.8*	9.87 ± 4.18†
	Female	
Control (NaCl)	38.88 ± 3.12	14.95 ± 2.46
Paraoxon	60.36 ± 12.4*	17.84 ± 5.16†

* By Student's *t*-test, $P < .001$. † $P > .1$ (not significant).

The effects of 14-day treatment with paraoxon on GH and Pr are presented in Table 2. In both sexes the GH content in the pituitary was significantly increased. No significant increase was observed in Pr content.

Table 3 shows that short-term paraoxon treatment produces a significant increase of GH in both male and female. Some increase of Pr was observed, especially in females. The observed increase of GH may be due to a block of release, or to increased synthesis, of GH. The relatively small differences between GH in short- and long-term experiments favors the second integration. These results are consistent with the observation of Welsch and Dettbarn (*7*) that paraoxon stimulates protein synthesis in peripheral nerve. Little information is available on the function of ChE in the anterior pituitary. In contrast to the ChE activity in rat brains, found mainly in the microsomal and supernatant fractions, ChE is uniformly distributed throughout all subcellular fractions of the anterior and posterior pituitary. Comparisons of the relative rates of hydrolysis of specific substrates indicated that the brain contains a much higher amount of AChE than either the anterior or the posterior lobe of the pituitary (*12*).

Table 3. Growth hormone (GH) and prolactin (Pr) content in rat pituitary after short-term treatment with paraoxon. Results are averages (± standard error) of two experiments with six rats in each group, with a total of 93 rats.

Treatment	GH (μg/mg)	Pr (μg/mg)
	Male	
Control (NaCl)	50.57 ± 4.53	6.87 ± 1.16
Paraoxon	70.04 ± 9.88*	7.15 ± 1.32†
	Female	
Control (NaCl)	36.15 ± 4.98	14.00 ± 1.13
Paraoxon‡	48.64 ± 4.58§	18.97 ± 1.11‖

* $P < .001$. † $P > .1$ (not significant). ‡ In some animals in this group, there were no symptoms with normal GH and Pr values and relatively lower values of cholinesterase in brain. § $P < .01$. ‖ $P > .05$.

The mechanism of action of paraoxon on the GH content in the pituitary is obscure; it is possibly but not clearly related to cholinergic mechanisms and bloodflow (*13*). The role of adenosine 3′,5′-monophosphate (cyclic AMP) as second messenger in hormone function (*14*) has been extended to the control of pituitary hormone secretion (*15*). Evidence of the involvement of cyclic AMP in the release of GH has been reported (*9, 16*), and more recently it has been shown that

guanosine 3′,5′-monophosphate (cyclic GMP) stimulates GH synthesis in the rat pituitary (*17*). Experiments in vitro have demonstrated stimulation of protein synthesis in the rat pituitary by dibutyryl cyclic AMP (*18*). Studies by George and co-workers (*19*) showed that acetylcholine (ACh) perfusion of isolated rat heart causes an increase in myocardial cyclic GMP. This latter observation and the more recent finding that oxotremorine, which raises the ACh content in the mouse brain, produces a large increase in cyclic GMP in cerebral cortex and cerebellum (*20*) support a possible relation between ACh and the cyclic nucleotide mechanism. The findings that inhibitors of ChE stimulate protein synthesis, that ACh may increase cyclic GMP, and that the latter has a role in the control of synthesis and release of GH suggest a possible cholinergic mechanism in the synthesis and release of GH in the rat pituitary.

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Predation of Schistosomiasis Vector Snails by Ostracoda (Crustacea)

Abstract. *An ostracod species of* Cypretta *is an effective predator in laboratory experiments on 1- to 3-day-old* Biomphalaria glabrata, *a vector snail of the blood fluke that causes the tropical and subtropical disease schistosomiasis.*

Schistosomiasis is a disease in tropical and subtropical areas that is caused by several species of human blood flukes which require certain species of snails as intermediate hosts. Deschiens *et al.* (*1*) noted that the ostracod *Cypridopsis hartwigi* Müller, 1900, attacked and killed the intermediate host snails *Bulinus contortus* (Michaud, 1829) and *Planorbis glabratus* Say, 1818 (= *Biomphalaria glabrata*); although Deschiens *et al.* did not record quantitative data, they speculated whether or not this ostracod could be used for the biological control of the snails. After observing ostracods killing vector snails in breeding aquariums, Lo (*2*) experimented with *Cypridopsis vidua* (O. F. Müller, 1776) collected near Ann Arbor, Michigan, and 2-day-old *B. glabrata* from Puerto Rico. He used ten groups, each with 1 snail and 5, 15, or 45 ostracods; in his experiments, 50 percent of the snails died in little more than 15 days when 5 ostracods were present and in 8 days when 15 and 45 ostracods were present. Kawata (*3*) noted that in his cultures of *B. glabrata* an ostracod species was an efficient predator on young snails and that the ostracods so irritated adult snails that the snails left the water, then weakened, and either died or were killed by the ostracods.

Our experiments were performed with 1- to 3-day-old snails of the red mutant (albino) strain of *Biomphalaria glabrata* and adults of the ostracod species *Cypretta kawatai* Sohn and Kornicker (*4, 5*). We placed 25 to 500 ostracods with 5 snails in dishes 80 mm in diameter containing distilled water maintained at a constant depth of 20 mm. A small amount of $CaCO_3$ slurry and lettuce was added to each experiment and control group as a source of calcium and as food for the snails and the ostracods. All were kept at room temperature (24° to 26°C). In each experimental and control group, the number of days it took for 50 percent of the original snail population to die was determined by observation. The results are shown in Fig. 1.

These data indicate that under laboratory conditions *C. kawatai* is an effective predator on the young of *B. glabrata* and that the rate of predation increases dramatically with an increase in the number of ostracods. Fifty percent of the snails in the control group (no ostracods present) died in an average of approximately 46 days. In the experiments involving 25 ostra-

Table 1. Experiments with 10 to 50 snails; the diameter of the dish and the number of ostracods are varied. The water depth is 20 mm and the temperature 24° to 26°C; *N* is the number of experiments. The limits of error are for 1 standard error of the mean.

N	Diameter (mm)	Snails (No.)	Ostracods (No.)	Ostracods per milliliter of water	Mean days, 50 percent mortality	Range (days)
5	105	10	500	2.89	0.50 ± 0.02	0.5– 0.6
5	105	10	250	1.45	0.64 ± 0.09	0.5– 1.0
5	190	10	500	0.88	2.40 ± 1.16	0.6– 7.0
5	80	10	50	0.50	2.85 ± 0.84	1.0– 6.0
6	80	10	25	0.25	12.15 ± 3.96	1.7–21.0
3	80	20	100	0.99	3.28	1.7– 4.5
2	80	30	250	2.49	1.54	0.6– 2.5
2	80	40	100	0.99	2.0	1.7 –2.3
1	80	50	250	2.49	1.8	