Table 1. Stepwise free energies of interaction on liganding. See the text for definitions of the models and the energy parameter U.

ΔG_i^I	Model		
	Pauling (Eq. 11)	Coryell (Eq. 12)	Mixed interactions (Eq. 13)
ΔG_1^I	0	$3U$	$6U$
ΔG_2^I	$-U$	$2U$	$2U$
ΔG_3^I	$-2U$	U	$-2U$
ΔG_4^I	$-3U$	0	$-6U$
$\overline{\Delta G^I}$*	$-3/2U$	$3/2U$	0

*$\overline{\Delta G^I} = \frac{1}{4}\Sigma\Delta G_i^I$

observed limiting behavior of hemoglobin and the β chains. We note that in the Coryell model three of the six pairwise interaction energies are broken on binding of the first ligand molecule. Because of symmetry, the sum of these three energies, which is equal to ΔG_1^I, must be equal to one-half the total interaction energy. Thus, $\overline{\Delta G^I} = \frac{1}{2}\Delta G_1^I$, or about 1150 cal/mole. It may be verified that this result is valid even if the subunits do not interact isotropically and the molecule possesses only dihedral symmetry rather than the full tetrahedral symmetry specified earlier. However, if the assumption of pairwise-additive interactions is invalid, then we cannot use the symmetry argument given above to relate ΔG_1^I to the total energy of interaction and hence $\overline{\Delta G^I}$.

In conclusion we note that, following Wyman (*1*), many workers (*2, 9, 10*) have identified the quantity $2.303\sqrt{2}$ *RTN* obtained from a Hill plot as the average interaction energy per site, or $\overline{\Delta G^I}$ in our terminology. It is shown here that this quantity is not $\overline{\Delta G^I}$, but rather $(-\Delta G_4^I + \Delta G_1^I)$, and that it cannot be related to the average free energy of interaction per site without reference to a specific model for cooperative ligand binding.

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References and Notes

1. J. Wyman, *Advan. Protein Chem.* **19**, 223 (1964).
2. See, for example, E. Antonini, J. Wyman, M. Brunori, J. F. Taylor, A. Rossi-Fanelli, A. Caputo, *J. Biol. Chem.* **239**, 907 (1964); R. Banerjee and R. Cassoly, *J. Mol. Biol.* **42**, 351 (1969).
3. D. E. Koshland, G. Nemethy, P. Filmer, *Biochemistry* **5**, 365 (1966).
4. J. Monod, J. Wyman, J. P. Changeux, *J. Mol. Biol.* **12**, 88 (1965).
5. L. Pauling, *Proc. Nat. Acad. Sci. U.S.* **21**, 186 (1935).
6. C. D. Coryell, *J. Phys. Chem.* **43**, 841 (1939).
7. H. A. Saroff and W. T. Yap, *Biopolymers*, in press.
8. The apparent Hill n is a parameter appearing in the empirical Hill equation which approximately describes the saturation y as a function of oxygen partial pressure c_o. The equation is $y = Kc_o^n/(1 + Kc_o^n)$.
9. I. Tyuma, S. Shimizu, K. Iwai, *Biochem. Biophys. Res. Commun.* **43**, 423 (1971).
10. I. Tyuma, K. Imai, S. Shimizu, *ibid.* **44**, 682 (1971).

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Rabies Virus in Nasal Mucosa of Naturally Infected Bats

Abstract. *Rabies virus was demonstrated in the olfactory mucosa of naturally infected bats by staining with fluorescent antibody and by isolation of the virus from the nasal tissues. The olfactory mucosa is a potential portal of entry and exit for airborne rabies virus in bat caves.*

During a study of the pathogenesis of airborne infection with rabies virus, we detected the virus in the nasal mucosa of naturally infected bats, both by virus isolation and by fluorescent antibody staining techniques. This finding suggests that nasal tissues may play a role in airborne transmission of rabies —a mode of transmission previously shown to be associated with certain bat habitats.

We collected 55 Mexican free-tailed bats, *Tadarida brasiliensis mexicana* (Saussure), at Frio Cave, Uvalde County, Texas, in July 1970. Of these, 15 bats, all immature, were either ill or dead; and 40 bats, including 30 immature animals, were alive and apparently normal. Each live bat was killed by exsanguination from the heart, and serum was saved. Using separate, sterile instruments for each animal, we dissected out the two ethmoturbinals and the nasal septum and made impression smears by touching the surfaces of one ethmoturbinal and the septum against sterile glass slides. The tissues, sealed in glass ampules, were frozen at −70°C for storage. Each bat carcass was sealed in a separate container and also stored at −70°C. The impression smears from nasal mucosa were tested for rabies virus antigen with the fluorescent rabies antibody (FRA) technique (*1*).

Certain frozen ethmoturbinals (pair members not used to make impression smears) were sectioned at a thickness of 2 or 4 μm, and the sections were similarly tested by the FRA technique.

Additional tissues (brain, submaxillary salivary glands, lungs, and kidneys) were later dissected from certain bat carcasses, again with separate, sterile instruments; and cut surfaces of these organs were used to prepare impression smears for FRA staining. These tissues and corresponding nasal tissues were also tested for virus by inoculation of tissue suspensions into separate litters of suckling albino Swiss mice (Rockefeller Foundation strain), with the use of standard techniques (*2, 3*).

Smears from nasal mucosa of 5 of the 15 ill bats were strongly positive for rabies virus antigen when examined by the FRA test. Additionally, ethmoturbinals from two of the five bats were completely sectioned, examined by the FRA technique, and found to be strongly positive. Antigen was prominent in the body and rod (dendrite) of some olfactory cells; the fluorescent-staining cells occurred either individually or in groups.

Mouse inoculation tests for virus in nasal tissues (the septum and one or both turbinals) were positive for only two of the five bats. For one bat, the inoculum (prepared from the septum and one turbinal) produced rabies in one of six mice; and for the other bat, the inoculum (prepared from the septum and two turbinals) produced rabies in all of eight mice.

Additional tests were performed on tissues from the five bats that contained rabies viral antigen in cells of the nasal mucosa. Brain, submaxillary salivary gland, lung, and kidney tissues were tested by FRA and mouse inoculation tests. All of these tissues were positive by one or both tests. (Mouse inoculation tests were negative for virus in lungs and kidneys of one of the bats.)

Impressions from nasal mucosa from the remaining 10 ill bats and from the 40 asymptomatic bats were negative to FRA tests for rabies virus antigen. Negative results were also obtained from additional tests performed on nine of these bats (three ill, six asymptomatic). Nasal, brain, salivary gland, lung, and kidney tissues were tested by the FRA and mouse inoculation techniques; ethmoturbinals were completely sectioned and examined by the FRA test.

Several investigators have successfully infected laboratory animals by the intranasal route, either by instilling rabies virus into the nostrils or by

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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References and Notes

1. E. H. Lennette, J. D. Woodie, K. Nakamura, R. L. Magoffin, *Health Lab. Sci.* **2**, 24 (1965).
2. H. N. Johnson, in *Diagnostic Procedures for Viral and Rickettsial Diseases*, E. H. Lennette and N. J. Schmidt, Eds. (American Public Health Association, New York, 1969), pp. 321–353.
3. One portion (0.02 ml) of each inoculum was inoculated intracranially into each mouse, and another portion (0.03 ml) was inoculated intraperitoneally into the same mouse. Mouse inoculation tests were considered positive when mice developed symptoms compatible with rabies several days or more after inoculation and when their brains were positive by the FRA test. Surviving mice were discarded after 28 days.
4. D. G. Constantine, *Rabies Transmission by Air in Bat Caves* (Publ. No. 1617, Public Health Service, Washington, D.C., 1967).
5. W. G. Winkler, *Bull. Wildl. Dis. Ass.* **4**, 37 (1968).
6. V. Hronovský and R. Benda, *Acta Virol. Prague, Engl. Ed.* **13**, 198 (1968).
7. V. Hronovský, *ibid.* **15**, 58 (1971).
8. H. R. Fischman and M. Schaeffer, *Ann. N.Y. Acad. Sci.* **177**, 78 (1971).
9. J. Schaaf and E. Schaal, *Deut. Tieraerztl. Wochenschr.* **75**, 315 (1968).
10. We thank D. Adams for his anatomical and histological study of the bat nasal cavity, which gave us a guide for locating various tissue components; W. Tyler for consultation; and R. Robenault for making some of the histological preparations.

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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.