of the infectious potential of the animal and therefore did not touch it. In a moment the bat flew away and the men entered their cabin. When they emerged about 3 or 4 minutes later, the bat again lit on the shirt and again bit it. It was captured in a jar and brought to the laboratory the next day.

The bat was induced to bite one thigh of each of three 2-day-old mice. Its attack upon the mice was not exceptionally vicious. Three other suckling mice of the same litter were not submitted to bites but were kept in the litter as controls. This measure was found necessary because of occasional nonspecific mortality in litters. Neither the injected nor the normal mice were marked to distinguish them, since this might induce cannibalism in, or abandonment by, the mother.

The bat was kept in a glass jar with water and canned dog food available. Under these conditions, normal bats usually survive for a month or more but this one died 7 days after capture. The carcass was stored in Dry Ice until it could be examined.

On the 13th day after being bitten, two mice, and on the 14th day a third mouse, exhibited partial paralysis of the hindquarters. Their brains were removed for transmission. The remaining mice were observed for 28 days and remained normal throughout that period.

When it appeared that an infectious agent was present in the bitten mice, brain tissue and salivary glands of the bat were triturated separately in albumen-saline diluent and injected intracerebrally into 21-day-old white Swiss mice. Salivary-gland suspension was also injected intramuscularly. All injections were 0.02-ml volumes of 5- to 10-percent suspensions. On the 10th day afterward, four of six mice injected intracerebrally with brain suspension were obviously ill and were killed. Another mouse was sick on the 11th day, the sixth mouse on the 12th day; both were dead on the 14th day. None of the mice injected with salivary-gland suspension showed signs of illness during a 28-day period of observation.

An aliquot of the salivary-gland suspension not used in the first injections and which had been stored in a Dry Ice chest was then injected intracerebrally into six 21-day-old mice and into a litter of five 2-day-old mice. One of the litter became ill on the 14th day and was killed. Another, sick on the 21st day and held for observation, was dead the next day and was discarded. Three of the litter remained normal. None of the 21-day-old mice showed evidence of infection.

A transmissible agent was demonstrated in tissues of mice injected with tissues from, or bitten by, the bat. These tissues included brain of mice infected by biting or by injection of salivary glands and brain, and salivary glands of mice infected by biting. The isolates were neutralized by immune serum prepared in rabbits by hyperimmunization with PV-1 strain of rabies virus. Mice immunized with H.E.P. Flury strain virus (*7*) were immune to intracerebral injection of the bat isolates.

A hemisphere of brain from a mouse bitten by the bat was fixed and stained with William's stain. It contained numerous Negri bodies (*8*).

The occurrence of human rabies following bat bite and this demonstration of infection in bitten laboratory animals establish the infectiousness of bites of insectivorous species. While little doubt existed that such would be found eventually, the potential is now clearly demonstrated. The fact that three mice were bitten and three, presumably the same ones, succumbed at about the same time probably indicates that all were infected. Greater susceptibility of young mice (*9*) infected by any route may have, in part, contributed to the efficacy of transmission in this case. This certainly seemed to be true when a salivary gland suspension was injected into suckling and into weanling mice. Perhaps the thin skin of 2-day-old mice may also have facilitated transmission.

Because the bat died before the bitten mice became ill, a second test of the bite to determine persistence of infectiousness was not made. Unusual behavior in captivity was not noted, but opportunities for activity were limited in the close confines of the jar. It has yet to be demonstrated that insectivorous bats can become true carriers of infection—that is, infective but symptom-free. If such were the case, and if they lacked the aggressive urge of furious rabies, they, unlike the vampire bats, would not constitute a direct and serious menace to animals other than bats. However, the potential for spread within a colony as a result of intraspecific strife might be increased.

J. Frederick Bell
National Institute of Allergy and Infectious Diseases, Hamilton, Montana

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Identification and Assay of 5-Hydroxytryptamine in Molluscan Tissues by Fluorescence Method

Abstract. The fluorescence assay method has been used in the identification and quantitative estimation of 5-hydroxytryptamine (5-HT) in mollusks. Ganglia of a representative pelecypod contain more 5-HT than those of a gastropod. Most non-nerve tissues have low levels of 5-HT, which, except in "kidneys," may derive from nerve endings.

By means of paper chromatography and bioassay, 5-hydroxytryptamine (serotonin, enteramine) was found in nerve tissue of the mollusks *Venus mercenaria* and *Busycon canaliculatum* (*1*). This earlier identification has now been confirmed, non-nerve tissues have been examined, and the quantities of 5-HT have been more precisely determined, by the spectrofluorometric method developed at the National Institutes of Health (*2*). We have followed the analytical procedure outlined by Bogdanski *et al.* (*3*), with minor modifications, such as a lower ratio of tissue weight to solvent

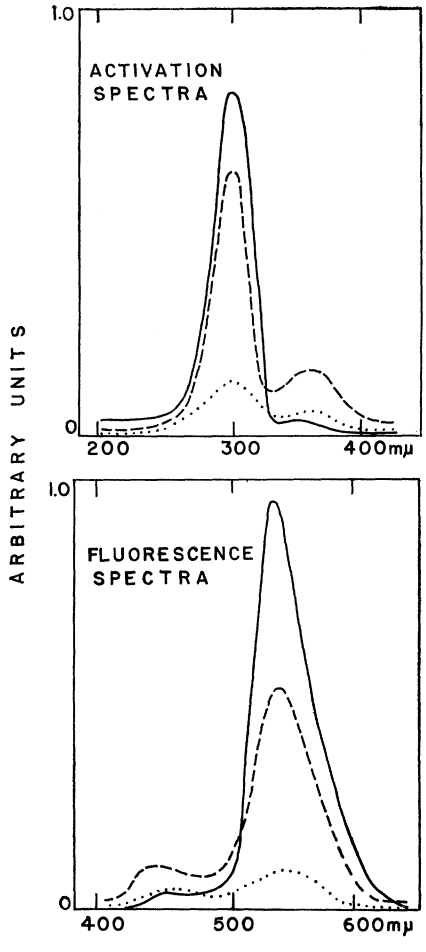

Fig. 1. Activation and fluorescence spectra of authentic 5-HT (solid line), extract of *Venus* ganglia (dashed line), and extract of *Busycon* ganglia (dotted line), all in 3*N* HCl.

volumes, made necessary by the small amounts of tissue available. With this method, we obtained activation and fluorescence spectra of extracts of nerve tissues and of authentic 5-HT in 3*N* HCl, such as are shown in Fig. 1. These are the observed curves; activation maxima appear at 305 mμ and fluorescence maxima at 540 mμ (*4*). In publications from the National Institutes of Health (*2, 3*), the activation maximum of 5-HT in 3*N* HCl is given as 295 mμ, that for fluorescence as 550 mμ. However, the maxima for the extracts and the authentic 5-HT, as is shown in Fig. 1, are in good agreement, and it is now reasonably certain that 5-HT has been correctly identified in tissues of mollusks. Dimethyl-5-hydroxytryptamine (bufotenin) has the same activation and fluorescence maxima as 5-HT, but it has not been seen on chromatograms of *Venus* or *Busycon* ganglia.

Table 1. 5-Hydroxytryptamine content of tissues of *Venus mercenaria* and *Busycon canaliculatum*.

Tissue	5-HT (μg/g)	Range
Venus mercenaria		
Ganglia (pooled)	30.0	12–52*
Blood†	< 0.004	
Digestive		
Intestine (visceral portion)	0.66	
Intestine (rectal portion)	0.60	
Digestive gland ("liver")	0.10	
Gill	0.53	
Heart		
Auricles	0.20	
Ventricle	0.50	
Bulbus arteriosus	0.04	
Kidney	0.26	
Mantle (central portion)	0.37	
Mantle (edge)	0.75	
Busycon canaliculatum		
Ganglia (pooled)	9.2	8.4–9.7‡
Nerve (connectives)	2.0, 2.5	
Ganglia and attached nerves	4.3, 5.5	
Blood	0.02	
Digestive		
Esophagus	0.06	
Intestine (rectal portion)	0.11	
Gill	0.23	
Heart (auricle and ventricle)	0.36	
Hypobranchial gland	0.08	
Kidney	2.0	1.4–2.3‡
Mantle	0.08	
Radula and odontophore muscles	0.07, 0.09	
Salivary glands	< 0.01	

* Eleven determinations. † With some mantle fluid. ‡ Four determinations.

Levels of 5-HT in various representative tissues of *Venus* and *Busycon* are given in Table 1. No correction has been made for the failure of the method to extract all the 5-HT, and with our modified procedure we recover about 70 percent of added 5-HT.

The *Venus* ganglia examined included the cerebropleural, visceral, and pedal. The *Busycon* ganglia examined included the entire esophageal complex but not the visceral ganglia. In two experiments, for which results are not shown, groups of similar ganglia of *Venus* were pooled and extracted separately. No consistent differences between the three groups were found. In each of the 11 separate determinations on *Venus* ganglia of Table 1, pooled tissues from two to ten animals were used. The rather large spread of values is due in part to seasonal and individual variation in 5-HT content and in part to the difficulty of freeing the small, fragile *Venus* ganglia of surrounding tissue. The mean value of 30 μg of 5-HT per gram of fresh tissue is much higher than has been found in nerve tissue of any vertebrate, and we have found equally high values only in ganglia of other species of pelecypod (bivalve) mollusks. Only in organs where 5-HT is a component of a venom are levels of 5-HT very much in excess of 30 μg/g (*5*). The 5-HT content of *Venus* tissues other than ganglia is low. The mantle edge, a well-innervated, muscular structure, has only 0.75 μg of 5-HT per gram, while a presumably poorly innervated organ such as the digestive gland has only 0.1 μg of 5-HT per gram. One determination on *Venus* blood failed to give a detectable amount of 5-HT.

Ganglia of *Busycon* were found to contain about one-third as much 5-HT as those of *Venus*. Ganglia of several other species of gastropod mollusks have less 5-HT than do those of most bivalves. From *Busycon* it is possible to obtain nerve connectives. These were found to contain considerably less 5-HT than equal weights of ganglion tissue. Most non-nerve tissues of *Busycon*, like those of *Venus*, yield relatively small amounts of 5-HT. Of the tissues examined, only salivary glands failed to yield a detectable amount. The *Busycon* kidney was found to contain more 5-HT than any other non-nerve tissue.

The relatively large amount of 5-HT found in *Busycon* kidneys made it of interest to determine whether kidney homogenates would decarboxylate added 5-hydroxytryptophan. The homogenates were not able to do so; this finding suggests that the *Busycon* kidney may be concentrating and excreting intact 5-HT, rather than synthesizing it for a local function. Excretory organs of several other invertebrate species have been found to have a high content of 5-HT, and in *Limulus*, the horseshoe crab, the coxal glands yield considerably more 5-HT than an equal weight of tissue of the central nervous system (*6*).

JOHN H. WELSH
MERILYN MOORHEAD
Biological Laboratories,
Harvard University,
Cambridge, Massachusetts

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6. The work reported here is a portion of a more extensive survey of the occurrence of 5-HT in invertebrates, especially in their nervous systems. The survey is supported by research grant B-623 from the National Institute of Neurological Diseases and Blindness, National Institutes of Health.

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Linear Titration Curves

In an interesting note in this journal (*1*), N. R. Joseph pointed to the advantage of bringing the sigmoid form of titration curves based on the mass action law into a linear form by logarithmic transformation. This transformation was applied by Joseph especially to the Henderson-Hasselbalch equation; he constructed a semilogarithmic paper as well as a slide rule for estimation of the *pK* values.

For such reasons some years ago I proposed a similar logarithmic transformation of the equation of the mass action law in its general form (*2*)

$$x^n K = y/(1-y)$$

yielding

$$n \log x - pK = \log [y/(1-y)]$$

At the same time the production of a corresponding (double) logarithmic paper for a linear representation of such titration curves was recommended, in which $\log [y/(1-y)]$ is plotted on the ordinate as a percentage of $y/(1-y)$ and $n \log x - pK$ is plotted on the abscissa. This paper is now available (*3*) and may be useful for special purposes.

H. DRUCKREY
Laboratorium der
chirurgischen Universitätsklinik,
Freiburg, Germany

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