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*).

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References and Notes

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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*)

$$x^nK = y/(1-y)$$

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.

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