Table 2. Oral transmission of F1− *Yersinia pestis* strain CPS-2a to rats

No. days after consuming infected mouse carcass	No. rats dead	No. rats with *Y. pestis* infection	No. rats with bacteremia	No. rats with infected spleen	No. rats with infected bubo	Phenotype of *Y.pestis* isolated
4	5	5	1	5	0	F1−
5	4	3	1	3	0	F1−
6	1	0	0	0	0	
7	1	1	0	1	0	F1−
137	1	1	0	0	1	F1−
168–369	8	0	0	0	0	
Total	20	10	2	9	1	

9 rats (45%) died of acute plague (table 2). Two rats developed bacteremia. Another rat, that survived 137 days, was found at necropsy to have a pleural bubo situated anterior to the heart that measured 0.5 cm in diameter. The bubo contained viable F1− *Y.pestis*.

Discussion. These experiments confirm earlier evidence[3] that rats are more resistant than mice to disease from F1− plague bacilli. However, resistance to disease can be overcome in many rats by the oral transmission of large infective doses of F1− *Y.pestis*. Wild rats are omnivorous and will consume corpses[9]. Thus, the experimental data indicate that a potential for rat plague from F1− organisms exists, especially via the consumption of infected material. Agonal bacteremia occurs in some rats, as in most mice[3], so flea-borne transmission of F1− *Y.pestis* also should be possible. All F1− strains we have examined exhibit the complex of pesticin-coagulase-fibrinolytic factor that is required for maintaining *Y.pestis* infections in fleas and for establishing vector capacity[10]. However, fleas frequently transmit moderate infective doses of bacilli[11], which many rats would resist. Consequently, disease from F1− *Y.pestis* may occur only infrequently in wild rats, and the probabilities for human infection may be low. Perhaps this explains why just one human case of plague from naturally-acquired F1− *Y.pestis* has been documented to date[2].

1 The views of the authors do not purport to reflect the position of the Department of the Army or the Department of Defense (para 4–3, AR 360-5).
2 Winter, C.C., Cherry, W.B., and Moody, M.D., Bull. Wld Hlth Org. *23* (1960) 408.
3 Williams, J.E., and Cavanaugh, D.C., Experientia *39* (1983) 408.
4 Williams, J.E., Harrison, D.N., and Cavanaugh, D.C., Trans. R. Soc. trop. Med. Hyg. *69* (1975) 171.
5 Surgalla, M.J., and Beesley, E.D., Appl. Microbiol. *18* (1969) 834.
6 Beesley, E.D., and Surgalla, M.J., Appl. Microbiol. *19* (1970) 915.
7 Higuchi, K., and Smith, J.L., J. Bact. *81* (1961) 605.
8 In conducting the research described in this report, the investigators adhered to the 'Guide for the Care and Use of Laboratory Animals'; promulgated by the Committee on Care and Use of Laboratory Animals of the Institute of Laboratory Animal Resources, National Research Council.
9 Landry, S.O., Q. Rev. Biol. *45* (1970) 351.
10 Brubaker, R.R., Beesley, E.D., and Surgalla, M.J., Science *149* (1965) 422.
11 Bibikova, V.A., and Klassovsky, L.N., Flea transmitted plague. Meditsina, Moscow 1974.

0014-4754/84/070739-02$1.50 + 0.20/0

Effect of 5-hydroxytryptamine antibodies on pigment migration in the erythrophores of the fiddler crab, *Uca pugilator:* Further evidence for 5-hydroxytryptamine as a neurotransmitter that stimulates release of red pigment-dispersing hormone

M.M. Hanumante, T.A. Butler and M. Fingerman

Department of Biology, Tulane University, New Orleans (Louisiana 70118, USA), 17 August 1983

Summary. 5-Hydroxytryptamine (5-HT) antibodies inhibit red pigment dispersion in the fiddler crab, *Uca pugilator*. This observation supports the hypothesis that 5-HT stimulates release of red pigment-dispersing hormone.

Centrifugal translocation of the pigment in the erythrophores of the fiddler crab, *Uca pugilator*, is mediated by a red pigment-dispersing hormone (RPDH)[1]. 5-Hydroxytryptamine (5-HT) also produces red pigment dispersion when injected into fiddler crabs, but unlike RPDH, has no effect on the erythrophores of isolated legs[2]. 5-HT, which is present in the central nervous system of the fiddler crab[3], thus may act as a neurotransmitter stimulating RPDH release in the fiddler crab. Data obtained using a variety of pharmacological agents known to affect 5-HT neurotransmission, at least in mammals, have strengthened the putative role of 5-HT as a neurotransmitter eliciting release of RPDH in *Uca pugilator*[4–6]. On the other hand, dopamine appears to be the neurotransmitter stimulating release of the RPDH antagonist, the red pigment-concentrating hormone (RPCH)[7].

The availability of antibodies against 5-HT has provided a powerful tool to demonstrate its presence. 5-HT antibodies should react with 5-HT in *Uca pugilator* and thereby decrease the amount available to stimulate RPDH release. In the present study 5-HT antibodies were injected into fiddler crabs either maintained on a white or a black background, shifted from a white to a black background or shifted from a black to a white background. The effects of the antibodies on the degree of red pigment dispersion were then observed. A white background fosters red pigment concentration whereas a black background fosters red pigment dispersion[1].

Materials and methods. Intact adult male fiddler crabs, *Uca pugilator*, from the vicinity of Panacea, FL (Gulf Specimen Co.) were used. The crabs were exposed to an illumination of 1190 lx during the experiments, which were performed at 24 °C. The erythrophores seen through the cuticle on the anteroventral surface of the second walking leg on the right side

were the ones observed. They were staged by the system of Hogben and Slome[8], where stage 1.0 represents maximal pigment aggregation, stage 5.0 maximal dispersion, and stages 2.0, 3.0 and 4.0, the intermediate conditions. These erythrophores were staged at the time of injection and 15, 30, 60, 90 and 120 min thereafter.

The data presented are the mean erythrophore stages for 10 crabs and were analyzed by means of the Wilcoxon Rank Sum Test (1-tailed) with significance set at the 95% confidence interval. The 5-HT antibodies[9] were dissolved in Pantin's crustacean saline[10], 20 µg/dose. 5-HT creatinine sulfate, tryptamine hydrochloride and 5-methoxytryptamine hydrochloride were purchased from Sigma, and dissolved in crustacean saline to provide 2×10^{-3} M solutions. In all experiments, the dose injected into each crab was 0.05 ml. Control crabs received an injection of saline alone.

Results and discussion. The 5-HT antibodies had no significant effect on the stage of the red pigment of the crabs initially having maximally concentrated red pigment (stage 1.0) and that were retained on a white background throughout the experiment (table 1). This observation is consistent with the ability of a white background to foster red pigment concentration through the action of RPCH. The significant decrement, after 30 min, of red pigment dispersion in the experimental crabs that initially had maximally dispersed red pigment (stage 5.0), and which were maintained throughout the experiment on a black background, was presumably caused by binding of the antibodies to 5-HT. In accordance with the hypothesis that 5-HT neurotransmission stimulates the release of RPDH, 5-HT would be released in crabs kept on a black background to stimulate RPDH secretion from the neuroendocrine cells that synthesize and release it to keep the red pigment dispersed. However, the injected 5-HT antibodies presumably combined with the 5-HT, thereby resulting in less 5-HT being available to stimulate release of RPDH into the hemolymph of the experimental crabs than in the controls. A similar explanation appears to apply to the significantly reduced degree of dispersion of the red pigment in the erythrophores of the crabs that received the antibodies at the time they were transferred from a white to a black background. In the crabs administered 5-HT antibodies and which underwent a background change from black to white, significant augmentation of red pigment dispersion occurred. This result was presumably due to inactivation of 5-HT that was being released presynaptically just before the onset of the red pigment-concentrating events that were induced when the crabs were transferred to a white background. Consequently, these experimental animals must have had less RPDH in their hemolymph than did the controls. Beltz and Kravitz[11] used 5-HT antibodies to map the 5-HT immunoreactivity in the nervous system of the lobster, *Homarus americanus*, and stated that the central ganglia were 'readily penetrated' by the antibody preparations. 5-HT antibodies have been used also in vivo on mice to inhibit cutaneous reactions to intradermally injected 5-HT[12]. Even though the 5-HT antibody preparation we used was polyclonal, it does not cross-react with dopamine, norepinephrine or epinephrine[13]. It does, however, react not only with 5-HT, but also with 5-methoxytryptamine and to some extent tryptamine[14]. 5-Methoxytryptamine is almost as effective as 5-HT in being bound by the antibody while tryptamine is about 26 times weaker. These were the only 3 compounds tested that showed nanogram sensitivity to binding by the antibody. When 5-HT, tryptamine and 5-methoxytryptamine were injected into fiddler crabs with maximally concentrated red pigment and which were kept on a white background 5-HT produced significant red pigment dispersion, as expected (table 2). But there was no significant response to the tryptamine or 5-methoxytryptamine. The slight pigment dispersion that occurred in the control crabs kept on the white background (tables 1 and 2) was probably due to factors such as excitement from handling, the saline injection they received and their circadian rhythm of red pigment dispersion. These results with 5-HT, tryptamine and 5-methoxytryptamine are consistent with those of Burgers[15] who injected these same compounds into the prawn, *Palaemon serratus*, and also found, as herein, that only 5-HT produced red pigment dispersion. He concluded that the 5-hydroxy group on the indole nucleus is essential for the red pigment-dispersing action of 5-HT.

Table 1. Mean erythrophore stages of crabs administered 5-hydroxytryptamine antibodies or saline, and maintained on a white (W) or a black (B) background, or transferred from a white to a black background (W→B) or from a black to a white background (B→W)

	Background	Time (min) 0	15	30	60	90	120
Experimental	W	1.0	1.0	1.0	1.0	1.0	1.0
Control		1.0	1.1	1.2	1.2	1.2	1.3
Experimental	B	5.0	4.8	4.4*	3.8*	3.4*	2.9*
Control		5.0	5.0	5.0	5.0	5.0	4.9
Experimental	W→B	1.0	1.0*	1.1*	1.0*	1.0*	1.0*
Control		1.0	1.5	1.7	1.9	2.1	2.0
Experimental	B→W	5.0	4.4	3.3*	2.9*	2.5*	2.5*
Control		5.0	5.0	4.8	4.5	4.1	4.1

*Statistically significant, experimental versus corresponding control, $p \leqq 0.05$.

Table 2. Mean erythrophore stages of crabs administered 5-hydroxytryptamine, tryptamine, 5-methoxytryptamine or saline, and maintained on a white background

Drug	Time (min) 0	15	30	60	90	120
5-Hydroxytryptamine	1.0	2.4*	2.5*	2.2*	1.8	1.3
Tryptamine	1.0	1.2	1.2	1.2	1.2	1.2
5-Methoxytryptamine	1.0	1.3	1.2	1.0	1.0	1.0
Control (saline-injected)	1.0	1.3	1.2	1.2	1.2	1.2

*Statistically significant, experimental versus control, $p \leqq 0.05$.

1 Brown, F.A., Jr, Biol. Bull. *98* (1950) 218.
2 Rao, K.R., and Fingerman, M., Comp. gen. Pharmac. *1* (1970) 117.
3 Fingerman, M., Julian, W.E., Spirtes, M.A., and Kostrzewa, R.M., Comp. gen. Pharmac. *5* (1974) 299.
4 Fingerman, M., and Rao, K.R., Comp. gen. Pharmac. *1* (1970) 341.
5 Fingerman, M., Hanumante, M.M., and Fingerman, S.W., Comp. Biochem. Physiol. *68C* (1981) 205.
6 Hanumante, M.M., and Fingerman, M., Gen. comp. Endocr. *45* (1981) 395.
7 Fingerman, M., and Fingerman, S.W., Comp. Biochem. Physiol. *58C* (1977) 121.
8 Hogben, L., and Slome, D., Proc. R. Soc. Lon. B *108* (1931) 10.
9 The 5-HT antibody preparation was a generous gift from Dr S. Spector, Roche Institute of Molecular Biology, Nutley, New Jersey.
10 Pantin, C.F.A., J. exp. Biol. *11* (1934) 11.
11 Beltz, B.S., and Kravitz, E.A., J. Neurosci. *3* (1983) 585.
12 Ranadive, N.S., and Sehon, A.H., Can. J. Biochem. *45* (1967) 1701.
13 Spector, S., Personal communication.
14 Peskar, B., and Spector, S., Science *179* (1973) 1340.
15 Burgers, A.C.J., Pubbl. Staz. zool. Napoli *34* (1965) 500.

0014-4754/84/070740-02$1.50 + 0.20/0