Table 2. Biochemical Differentiation of *P. mors-prunorum* and Pear Isolates of *P. syringae*

Species	Æsculin and arbutin hydrolysis	Gelatin liquefaction	Casein hydrolysis	Tyrosinase activity	Growth in nutrient broth + 5 per cent sucrose
P.-mors-prunorum					
(*a*) Cherry strains	−	−	−	+	white
(*b*) Plum strains	−	−	−	+	white
P. syringae	+ *	+	+	−	yellow

* One isolate failed to hydrolyse æsculin and arbutin.

without passage through the host and appear, therefore, to be relatively stable *in vitro*. The difference between the plum and cherry strains may merely reflect a different history of phage-infection in the field. The cherry strains, for example, may have failed to acquire resistance to *A*2 and *A*7 or related phages because these are absent on cherry or their activity is in some way inhibited. Alternatively, the phage types may be a result of selection by the host plants. This would imply some form of association between phage sensitivity and specific virulence factors in *P. mors-prunorum*.

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Serotonin in the Small Intestine of Conventional and Germ-free Chicks

The function of serotonin (5-hydroxytryptamine) in the tissues of the small intestine is not understood even though it is found in relatively large quantities, particularly in the enterochromaffin cells[1]. The most frequently repeated interpretation ascribes control of intestinal motility and vascular permeability to serotonin[2,3]. Thus, if the tonus of the gut is dependent on serotonin-levels, germ-free animals, which possess poor tonus of the tract and distended cæca[4], might be expected to have a lower serotonin content in the gut than conventional animals. On the contrary, however, this communication provides evidence that intestinal serotonin-levels are actually depressed by the presence of micro-organisms in the lumen of the gut.

White Leghorn chicks were raised in a germ-free environment by methods similar to those previously described[5] on a complete starch-casein diet[6]. Fertile eggs were obtained from local hatcheries after 19 days of incubation, immersed in 2 per cent mercuric chloride solution for 10 min. at 39° C. and put into sterile 'Plexiglas' isolators where they hatched. Control chicks were treated in the same manner but were reared in a non-sterile isolator. After four weeks the chicks were decapitated; the small intestine was excised and washed in cold water. All germ-free animals were examined[7] for bacterial contamination when killed. The various segments of the intestine were homogenized in 0·1 *N* hydrochloric acid in a Ten Broek apparatus at 5° C., and serotonin was assayed[8] on fresh specimens in a Turner photofluorometer (G. K. Turner and Associates, Palo Alto, California).

Table 1. Serotonin in the Small Intestine of Germ-free and Conventional (Contaminated) White Leghorn Chicks at Four Weeks of Age

Treatment	No. of chicks	Serotonin (μgm./gm. fresh tissue) Duodenum	Ileum
Germ-free	20	9·0 ± 1·0 *	11·0 ± 0·7
Conventional	20	8·3 ± 1·3	6·1 ± 0·8
Significance of differences by the '*t*' test		*t* *P*	*t* *P*
Germ-free vs. conventional		0·84 < 0·4	8·8 < 0·01

* Mean ± standard deviation.

A significantly higher concentration of serotonin was found in the ileum of germ-free chicks than in control birds (Table 1). This is consistent with the observation[9] that rats and mice given peroral antibiotics produce significant increases of serotonin in the small intestine. However, no such difference was apparent in the duodenum (Table 1). There is insufficient evidence to determine whether these results reflect differences between the microflora of the duodenum and ileum or an influence of the intense microbial activity of the cæca[10,11] due to the proximity of the ileum to the latter. In any event, the presence of intestinal micro-organisms clearly affects serotonin in the ileum of chicks.

Several reports suggest alternative explanations for the microbial inhibition of serotonin. A few isolates of *Aerobacter aerogenes* and *Alcaligenes faecalis* appear to be capable of oxidizing serotonin *in vitro*[12] although it is not known if this event occurs in the lumen of the intestine. In addition, many species of lactobacilli which are present in large numbers in the chick[13] require pyridoxine or its derivatives[14]; pyridoxal phosphate is required as a co-factor in the decarboxylation of 5-hydroxytryptophan to serotonin[15]. Thus bacterial removal of the coenzyme may inhibit serotonin synthesis. A similar effect might be achieved by the interaction of a bacterial product with the coenzyme such as has been reported for norepinephrine and 3,4-dihydroxyphenylalanine[16].

Further work is required to determine the reason for the difference in intestinal serotonin between germ-free and conventional chicks and the physiological significance of this observation.

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