Hallinan *et al.* (*8*) submitted electron-micrographic evidence that rat reticulocytes contain mitochondria. These intracellular structures are absent from the fully mature erythrocyte. The cationic component of rat liver GOT is confined predominantly to the mitochondrial fraction, whereas the anionic component is almost exclusively in the supernatant fraction (*2*). Our results with the rabbit are in agreement with the earlier findings. The mature erythrocyte contains only the anionic GOT, whereas the reticulocytes contain the cationic type. Ellis *et al.* (*9*) have reported that during the transition from reticulocyte to erythrocyte much of the cellular protein, other than hemoglobin, disappears and that the reticulocytes show substantial increases in acid cathepsin acting at *p*H 3.2, in several peptidases, and in acid and alkaline phosphatases. Hallinan *et al.* (*8*) have pointed out that in sections prepared for electron microscopy the mitochondria of rat reticulocytes frequently show signs of alterations, with disoriented cristae and even ruptured outer membranes, whereas the mitochondria of monocytes in the same section are relatively unaltered. As noted above, there was some indication that the intensity of the anionic band of GOT was inversely related to the intensity of the cationic band as reticulocytosis was induced and allowed to recede.

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25 May 1965

Serotonin: Synthesis and Release from the Myenteric Plexus of the Mouse Intestine

Abstract. *After injection of its radioactive precursor, 5-hydroxytryptophan, radioactive serotonin was biosynthesized and bound in the myenteric plexus of the mouse intestine. Addition of nonradioactive serotonin to preparations in vitro caused a net release of radioactive serotonin from the plexus. This release appeared to result from activity in the intramural nervous system of the intestine. A neurotransmitter role between sensory and motor neurons in the peristaltic reflex pathway is suggested as a working hypothesis to explain the action of serotonin.*

A radioautographic method, utilizing the tritiated precursor of serotonin, H^3-labeled 5-hydroxytryptophan (5HTP-H^3), has recently been devised for the purpose of localizing tissue sites that synthesize and bind serotonin (*1, 2*). The method has shown the myenteric plexus of the mouse intestine to be such a site. Surprisingly, and in marked contrast with the stomach where the argentaffin cells of the mucosa also become labeled, the myenteric plexus is the only site shown to synthesize and bind serotonin in the small intestine after a single intravenous injection of 0.4 to 4.0 mc of 5HTP-H^3 (*2, 3*). We now report additional experiments intended to define the localization and fate of intestinal serotonin; certain results may be interpreted to suggest that serotonin is a neurotransmitter between sensory and motor nerve cells in the intramural nervous system of the intestine.

One hour after intraperitoneal injection of 60 mg/kg of β-phenylisopropylhydrazine (a monoamine oxidase inhibitor), mice were injected intravenously with 0.4 to 4.0 mc of 5HTP-H^3 or 5.0 μc of C^{14}-labeled 5HTP. Stomach and intestines were removed 4 hours later, and strips of gut were opened longitudinally and bathed in oxygenated Krebs solution at 37°C.

The resultant outflow of radioactive material can be described by a multicompartmental washout curve (*4*). Three components were resolved: the first was extremely rapid, with a half time ($t_{1/2}$) of about 40 seconds; the $t_{1/2}$ of the second averaged 9 minutes; the third component was very slow, having a $t_{1/2}$ averaging 560 minutes. The radioactive compounds present in the effluent were identified by paper chromatography. In addition, the radioactive compounds initially present, and those remaining after washout, were extracted from the intestinal strips with 70-percent ethanol and similarly assayed. Most of the material accounting for the radioactivity of the two faster components was either 5HTP-H^3 or tritiated serotonin-*O*-glucuronide. Radioactive serotonin was detected in the bath only in small amounts, briefly, during the first minute of washout, and not again until 60 minutes later.

Since very little radioactive serotonin leaves the intestinal strips, the proportion of tissue radioactivity due to tritiated serotonin increases with washout time (Fig. 1). It was concluded therefore that the rapid components represent washout of the serotonin precursor and metabolite which are unbound, whereas the slow component reflects the existence of a barrier to the free diffusion of serotonin from the tissues (*4*). The extremely rapid phase may depict washout from extracellular space and perhaps from blood platelets.

Since tritiated serotonin accounts for most of the radioactivity remaining in the gut after the slow component is reached, any label detected by radioautography in tissue fixed at this time probably indicates the presence of tri-

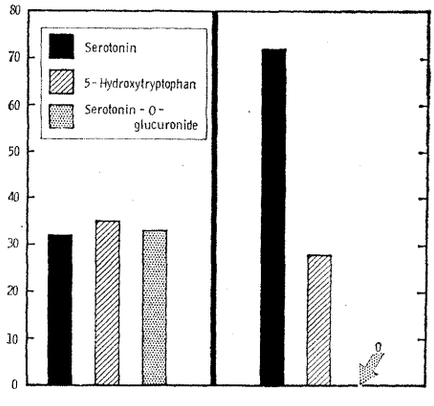

Fig. 1. Radioactive compounds extracted from intestinal strips with 70-percent ethanol 4 hours after injection of C^{14}-labeled 5-hydroxytryptophan and isolated and identified by paper chromatography; their amounts were determined by counting in a liquid-scintillation spectrometer. The compounds initially present, expressed as percentages of total extractable radioactivity, are shown at left; those present after 1 hour of washout, on the right.

tiated serotonin. The accuracy of this identification is substantiated by comparing radioautographs of tissue fixed before and after washout and attributing to tritiated serotonin only the labeling that was undiminished by washout. Similarly, increases in the rate of washout noted after the slow component was reached probably indicate release of tritiated serotonin from the intestinal strips. Labeling around myenteric ganglion cells is illustrated in Fig. 2; the pattern of labeling around the cells suggests possible presence of tritiated serotonin in synapses on the surface of the

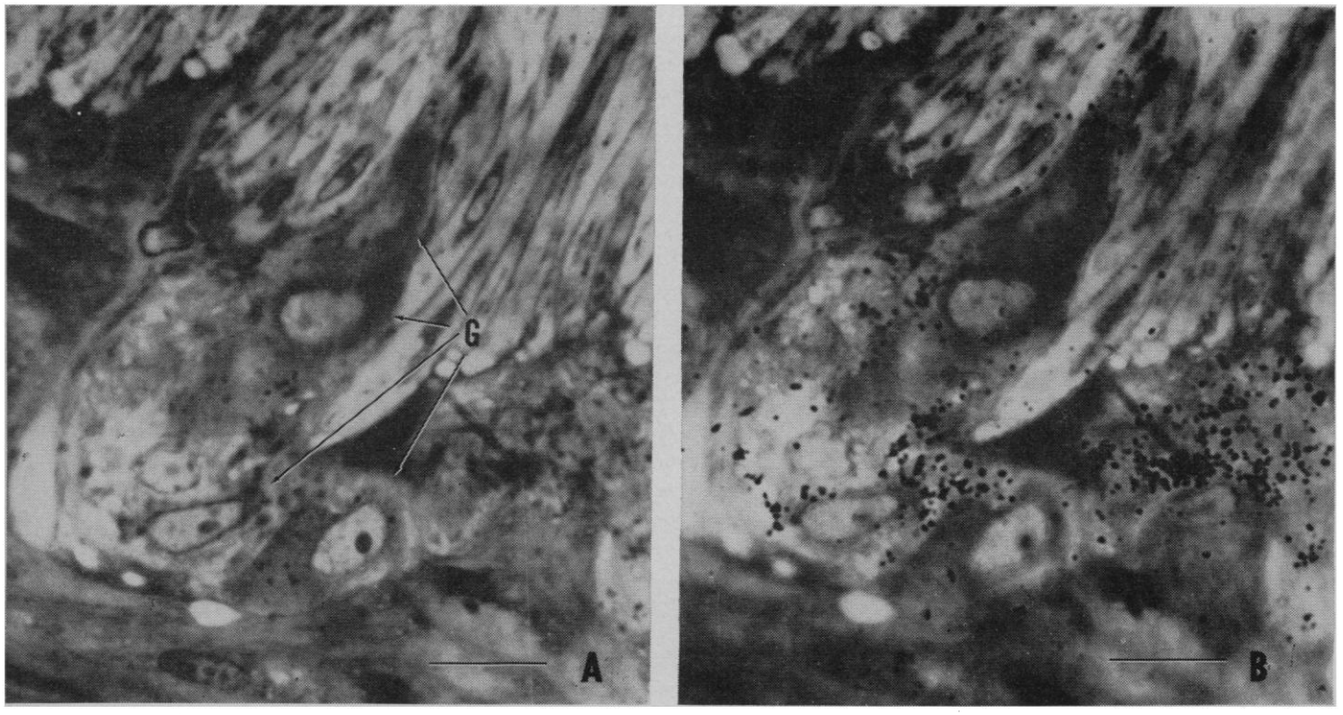

Fig. 2. Radioautographs of the myenteric plexus of the mouse intestine. *A*, Focus on the plane of section, showing ganglion cells (*G*) and surrounding satellite cells and synapses; the inner, circular, layer of muscle lies below, and the outer, longitudinal, layer above, the ganglion. *B*, The plane of the overlying emulsion, showing accumulation of silver grains, indicating the presence of tritiated serotonin around the ganglion cells. The scale line represents 10 μ.

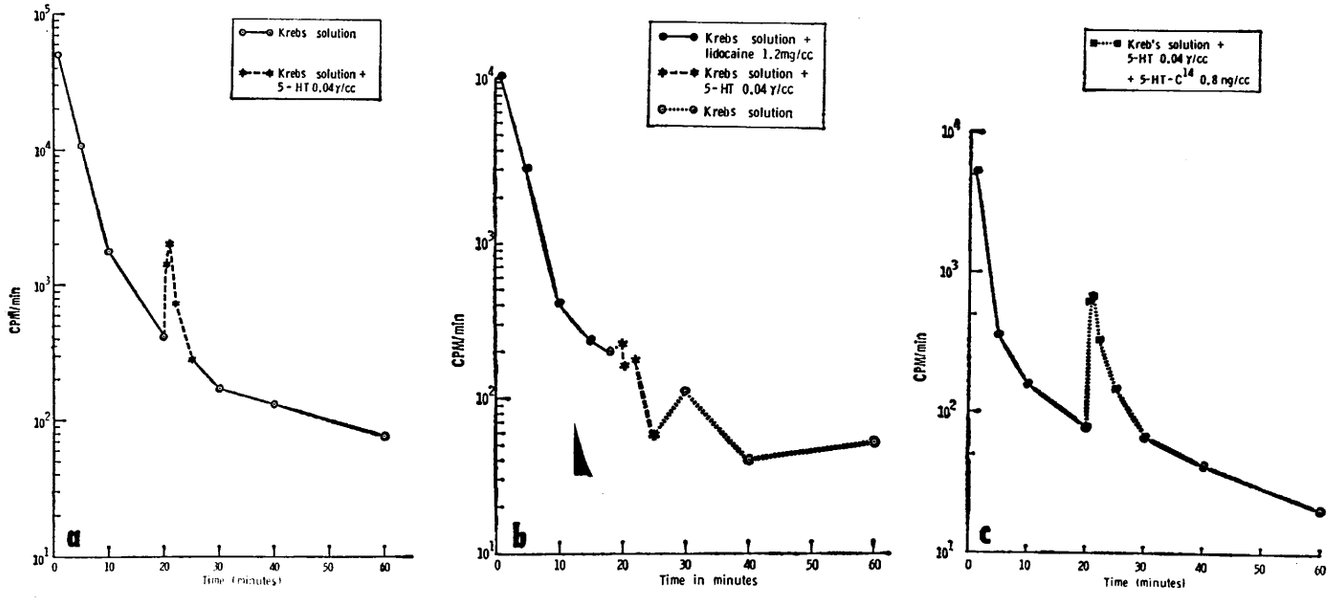

Fig. 3. Representative curves of washout of radioactive material from mouse intestinal strips. *a*, Serotonin autorelease elicited by addition of serotonin (5-HT) to the bath at 0.04 μg/cm^3. In *b*, the solution contained lidocaine for the first 20 minutes of washout; after a 5-minute wash in Krebs solution, serotonin (5-HT) was added in the same concentration as in *a*. In *c*, radioactive serotonin, final specific activity 2.3 $\times$ 10^6 count min^{-1} μmole^{-1}, was added to the bath at the same concentration as in *a*; points on the graph were obtained by subtracting the added radioactivity from the total radioactivity found in the bath; additional radioactive serotonin is released from the intestinal strips.

cells. Since the myenteric plexus is normally the only site labeled in the intestine, any observed release of serotonin would probably be from this site.

Release of radioactive serotonin can be provoked by addition of nonradioactive serotonin to the bathing fluid during the slow component of washout (Fig. 3), a phenomenon we call "autorelease." This release is reflected in radioautographs, which also indicate that the myenteric plexus is the source of the released material. Washout itself does not diminish labeling in the myenteric plexus, yet the addition of serotonin to the bath during washout does deplete the plexus of labeled material.

In order to determine whether this effect represents a net release or self-exchange, radioactive serotonin with a specific activity greater than that measured for intestinal serotonin was added to the bath, and the release of serotonin was again measured. Tissue serotonin was measured spectrophotofluorometrically (*5*). Carbon-14–labeled 5HTP was injected into mice, and the specific activity of intestinal serotonin was $2.3 \pm 2.0 \times 10^5$ count min^{-1} $\mu mole^{-1}$. When serotonin of higher specific activity, 2.3×10^6 count min^{-1} $\mu mole^{-1}$, was added to the bath, and the added radioactivity was subtracted from the total radioactivity found in the bath, the release of radioactive serotonin from the intestine was still evident and was undiminished (Fig. 3*C*). Since additional radioactivity did appear in the bath, in a situation in which exchange would be expected to be between radioactive molecules, the phenomenon was a net release and not self-exchange.

In further experiments we studied the extent to which exogenous serotonin can enter the endogenous serotonin pool in vitro. The experiments of Axelrod and Inscoe (*6*) indicate that there is little or no exchange in vivo. Intestinal strips were bathed for 130 minutes in 5.0 ml of oxygenated Krebs solution containing 2.5×10^5 count min^{-1} ml^{-1} of C^{14}-labeled serotonin at 37°C or at room temperature. The strips were then subjected to washout in Krebs solution until less than 1 percent of the initial radioactivity remained. A multicompartmental desaturation curve was obtained and at least two exponential components could be resolved. The $t_{1/2}$ of the slowest component was 30 ± 5 (standard error) minutes. Since the $t_{1/2}$ of the slowest component of washout of radioactivity, from strips in which endogenous serotonin had been labeled in vivo, was more than 15 times longer than this, the exogenous serotonin probably had not mixed with the endogenous material. Moreover, the addition of nonradioactive serotonin to the bath did not affect the washout of C^{14}-labeled serotonin from intestinal strips loaded with labeled material in vitro. It therefore appears that exchange between exogenous and endogenous serotonin does not occur in the mouse intestine.

Autorelease occurs almost immediately after addition of serotonin to the bath, falls off within less than 1 minute, but can be reelicited after a 15-minute wash in Krebs solution; it follows a dose-response curve, the minimal effective concentration being $4.9 \times 10^{-11} M$ and the maximal response occurring at $9.8 \times 10^{-8} M$. Response is diminished when concentrations greater than $10^{-4} M$ are added to the bath. The phenomenon of autorelease is completely inhibited by a local anaesthetic lidocaine (5 m*M*), and by lowering the temperature to 4°C. Contraction of the longitudinal muscle was measured with a transducer sensitive to 10 μ of linear motion. When, as in these experiments, serotonin is added to both mucosal and serosal surfaces no muscle contraction is elicited by concentrations of serotonin below $10^{-5} M$; autorelease is therefore not secondary to muscle contraction.

The peristaltic reflex, normally initiated by pressure-sensitive mucosal receptors (*7, 8*), is mediated by way of a pathway shown in Fig. 4. Sensory nerve fibers, the pseudounipolar cell bodies of which are situated in the submucosal plexus, interconnect the receptors of the mucosa with the motor ganglion cells of the myenteric plexus (*8, 9*). Serotonin added to the mucosal surface of the intestine stimulates mucosal receptors and, through the intramural nervous system, therefore stimulates peristalsis (*9*). In our investigation the lumen of the intestinal strips was kept open, and consequently the nonradioactive serotonin added to the bath must have reached and thus stimulated mucosal receptors. Addition of this serotonin, however, was also followed by an almost immediate release of radioactive serotonin from the region of the myenteric plexus. These findings suggest that the observed release of endogenous serotonin is the result of activation of the intramural nervous plexus, a suggestion supported by the inhibition of serotonin autorelease by lidocaine and by cold. The pattern of localization of the radioactive serotonin surrounding myenteric ganglion cells indicates that the endings of the sensory neurons are the sites from which the material is released. Further, since pharmacologic evidence suggests that serotonin also depolarizes myenteric ganglion cells (*9, 10*), a working hypothesis may be framed from these data: that serotonin is a neurotransmitter between sensory and motor neurons in the peristaltic reflex pathway.

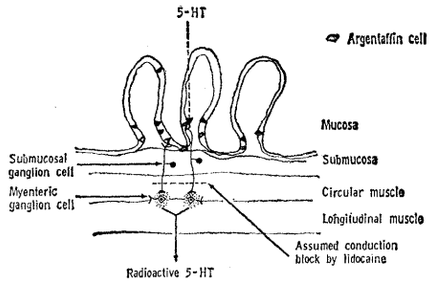

Fig. 4. Proposed mechanism of autorelease of serotonin. In our experiments we considered it likely that serotonin added to intestinal strips in vitro reached and stimulated mucosal sensory nerve fibers; this resulted in release of tritiated serotonin (indicated by stippling) from the myenteric plexus. Such release is blocked by lidocaine. Presence of argentaffin cells, containing a large amount of nonlabeled serotonin in the mucosa, is indicated.

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