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5-Hydroxytryptamine in the Gastrointestinal Tract After Intestinal Obstruction or Hepatectomy. (29118)

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5-Hydroxytryptamine occurs in the gastrointestinal mucosa of the dog in concentrations of 7 to 14 μg/g with the pyloric region containing the highest concentration(1). In the rabbit, 5-hydroxytryptamine has a half-life of 10 and 17 hours in the mucosa of the stomach and intestine, respectively(2). Such a rapid turnover of considerable quantities of 5-hydroxytryptamine has suggested that it may have an important function in the gastrointestinal tract, possibly in regulation of motility(3,4). 5-Hydroxytryptamine has been found to have a very potent action on the motility of the gastrointestinal tract(5) and methods for assay of this substance are dependent on the extreme sensitivity of the ileum of guinea pig and colon of rat to nanogram quantities(1,6). Hypermotility of the gastrointestinal tract accompanied by reversed peristalsis occurs proximal to an intestinal obstruction and also is present after hepatectomy and in intoxicated Eck's-fistula dogs.

Rosenberg and his colleagues(7) reported that no changes occurred in the amounts of 5-hydroxyindoleacetic acid in urine or of 5-hydroxytryptamine in plasma in 7 dogs after simple intestinal obstruction; measurements were not made of the concentration of 5-hydroxytryptamine in the gastrointestinal mucosa. Sigel and co-workers(8) found that urinary excretion of 5-hydroxyindoleacetic acid did not change from normal in 6 dogs after ligation of the arterial supply to the mesenteric small bowel and strangulation of the middle 2 feet of the small intestine with cord. The purpose of the present investigation was to compare the concentration of 5-hydroxytryptamine in the gastrointestinal mucosa of dogs after hepatectomy or intestinal obstruction with that in dogs after laparotomy. Measurements also have been made of concentrations of 5-hydroxytryptamine in blood and in brain and of 5-hydroxyindoleacetic acid in urine.

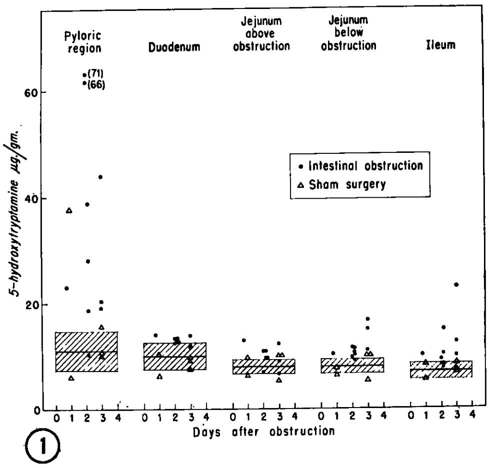
Methods and materials. Dogs weighed from 7 to 15 kg. All bowel obstructions were made in the jejunum about 12 inches from the ligament of Treitz; some were tied acutely and others were created by pulling up a previously placed wire snare. A number of control procedures were used; in 7 dogs wire loops were placed around the jejunum but were not pulled up to create intestinal obstruction. In 3 other dogs, the abdomen was opened and the stomach and duodenum were retracted for 20 minutes. Another group of dogs was studied 2 days after cannulation of the bile duct and cholecystectomy to produce an external biliary fistula.

Total hepatectomy was performed by the 2-stage method of Grindlay and Mann(9). Postoperatively the dogs received glucose solution by continuous infusion. The Eck's fistulas were created by end-to-side portal-caval anastomosis.

The dogs were anesthesized with sodium pentothal and segments of the pyloric region of the stomach, the duodenum, the jejunum, and the ileum were removed. The mucosa was scraped and dissected from the underlying muscle and frozen in powdered solid CO_2. The concentration of 5-hydroxytryptamine in homogenates of mucosa was determined fluorometrically by the method of Weissbach and Waalkes(10). The concentration of 5-hydroxytryptamine in whole blood was measured by the method of Udenfriend and associates(11). Urinary 5-hydroxyindoleacetic acid was determined by the procedure of Macfarlane and associates (12).

Because 5-hydroxytryptamine has been found to have a well defined distribution in the brain(13), brains were dissected into 4 parts which were analyzed for 5-hydroxytryptamine separately(14). A transverse cut 5 mm below the obex marked the posterior limit of the brain stem region. Laterally, the region was separated from the cerebellum by transecting the cerebellar peduncles; the anterior limit was a line through the posterior rim of the mammillary body to the anterior limit of the superior colliculus. The hypothalamic region was defined as follows: the anterior limit was a line through the midpoint of the optic chiasm; the posterior limit was the posterior rim of the mammillary body; laterally, the region was limited by the lateral edge of the mammillary body and the midpoint of the perforated space. The depth of the hypothalamic region was cut to 8 mm. Sagittal cuts were made in both of the cerebral hemispheres 1 cm from the midline and the caudate nuclei were exposed by reflecting the cerebral tissue laterally.

Results. Obstruction. In 9 of 10 dogs, after intestinal obstruction there was a definite increase in concentration of 5-hydroxytryptamine in the mucosa of the pyloric region, ranging up to 6 times the normal values (Fig. 1). Only slight increases were found



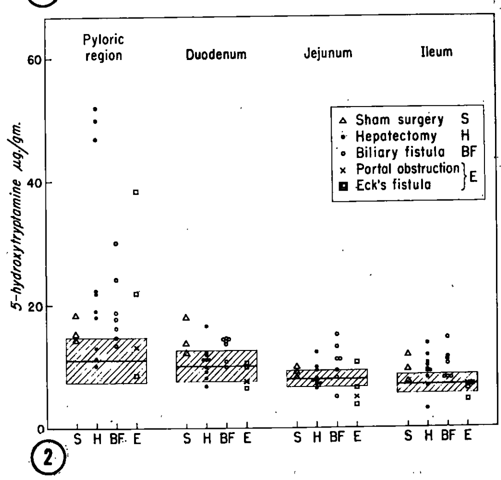


FIG. 1. 5-Hydroxytryptamine in different parts of gastrointestinal mucosa of dogs after intestinal obstruction. Shaded rectangles show corresponding mean concentrations $\pm$ one standard deviation of 5-hydroxytryptamine of 12 normal dogs.

FIG. 2. 5-Hydroxytryptamine in gastrointestinal mucosa of dogs after various surgical procedures. Shaded rectangles show corresponding mean concentrations $\pm$ one standard deviation of 5-hydroxytryptamine of 12 normal dogs.

TABLE I. 5-Hydroxytryptamine in Pyloric Mucosa and Blood and 5-Hydroxyindoleacetic Acid in Urine in Dogs with Obstruction of Jejunum.

Component	Postobstruction (hr)	Experimental dog 1	2	3	4	Normal*
5-Hydroxytryptamine in:						
Pyloric mucosa (μg/g)	52	10	39	19	28	11.0 ± 3.6
Whole blood (μg/ml)	0	.63	.41	.68	.70	.63 ± .21
	6	.58	.46	.72	—	
	24	.59	.45	.95	.90	
†	52	.60	.40	.81	.62	
‡	52	.67	.44	.88	.77	
5-Hydroxyindoleacetic acid in:						
Urine (μg/hr)	1 to 52	32	56	74	37	63 ± 17

* Means and standard deviations. Data were from 12 normal dogs for pyloric mucosa, from 25 normal dogs for blood, and from 64 daily samples from 7 normal dogs for urine.
† Systemic blood. ‡ Portal blood.

in the mucosa of other regions of the gastrointestinal tract. In the 5 dogs which had undergone sham operation (wire loops were not pulled up to create obstruction), the concentration of 5-hydroxytryptamine was in the normal range in all regions except in one dog which had a high concentration in the pyloric region.

Table I shows the results of simultaneous determinations of 5-hydroxytryptamine in the pyloric mucosa, 5-hydroxytryptamine in blood, and 5-hydroxyindoleacetic acid in urine in 4 dogs with obstruction of the duodenum. Concentrations of 5-hydroxytryptamine were slightly higher in the portal blood than in systemic blood, but the differences were not great. Urinary output of 5-hydroxyindoleacetic acid was within the normal range in these 4 obstructed dogs and no correlation was apparent between the concentration of 5-hydroxytryptamine in the pyloric mucosa and that of 5-hydroxytryptamine in whole blood or of 5-hydroxyindoleacetic acid in urine.

The concentration of 5-hydroxytryptamine in the brains of 4 obstructed dogs was found to be within the normal range in the 4 regions of the brain studied (Table II) although 3 of these dogs had increased concentrations of 5-hydroxytryptamine in the pyloric region of the gastrointestinal tract.

Other surgery. Normal concentrations of 5-hydroxytryptamine were found in pyloric mucosa (10 and 11 μg/g) 11 and 14 hours after total hepatectomy (Fig. 2). In 7 of 8 other dogs which were studied 20 hours or longer after hepatectomy, high concentrations of 5-hydroxytryptamine were found in the pyloric area; concentrations of 5-hydroxytryptamine in other regions were similar to those in control dogs.

Of the 3 dogs with Eck's fistula, 2 had high concentrations of 5-hydroxytryptamine in the pyloric region (Fig. 2). One of these dogs was displaying symptoms of intoxication (gross incoordination and apparent blindness) when studied and its pyloric mucosa contained 38 μg of 5-hydroxytryptamine per gram.

Slight increases in concentration of 5-hydroxytryptamine were found in all parts of the gastrointestinal tract of dogs with biliary fistula (Fig. 2). In 4 other dogs, 1 day after sham operation there were slightly increased concentrations of 5-hydroxytryptamine in the pyloric region; concentrations in the other regions of the gastrointestinal tract were normal.

TABLE II. 5-Hydroxytryptamine in Brain* in Normal Dogs and in Dogs with Intestinal Obstruction.

Dogs	5-Hydroxytryptamine (μg/g) Hypothalamus	Caudate nucleus	Brain stem	Cerebellum
Normal†	1.28	0.51	0.70	0.11
	±0.19	±0.14	±0.25	±0.04
Obstructed	1.37	0.40	0.62	0.10
	1.01	0.52	0.44	0.09
	0.84	0.49	0.70	0.13
	1.06	0.64	0.90	—

* Dissection into regions described in text.
† Means and standard deviations of data from 8 normal dogs.

Discussion. Significant increases were found in the concentration of 5-hydroxytryptamine in the pyloric mucosa of dogs after obstruction of the jejunum, after total hepatectomy, and after establishment of Eck's fistula, but only occasional slight increases were found in the ileum and increases in the duodenum and the jejunum were not apparent. Concentrations of 5-hydroxytryptamine in blood and in brain and of 5-hydroxyindoleacetic acid in urine were normal in dogs with intestinal obstruction, confirming earlier reports by Rosenberg and co-workers(7) and Sigel and co-workers(8). Small increases in concentration of 5-hydroxytryptamine were sometimes found in the pyloric mucosa following simple laparotomy and in all regions of the gastrointestinal tract 2 days after cannulation of the bile duct to produce an external biliary fistula.

There have been few observations of changes in the concentration of 5-hydroxytryptamine in gastrointestinal mucosa of experimental animals. Increases have been found after injection of the precursor, 5-hydroxytryptophan(15) or in animals maintained on a diet rich in the precursor, tryptophan(16-18). Treatment with antibiotics has been found to increase the concentration of 5-hydroxytryptamine in the gastrointestinal mucosa(19-21). This has been explained on the basis of increased amounts of tryptophan available for synthesis of 5-hydroxytryptamine when bacterial metabolism was eliminated(19). In rabbits, continued administration of ethyl alcohol caused increases in concentration of 5-hydroxytryptamine in the gastrointestinal mucosa; unlike the increases found in our experiments, which were chiefly in the pyloric region, these increases were of the same order in the small intestine and in the pyloric and fundic regions of the stomach(22). Decreased concentrations of 5-hydroxytryptamine have been found in the gastrointestinal mucosa of animals after administration of reserpine(23,24), maintenance on a tryptophan-deficient diet(25), or daily injections of a number of synthetic corticosteroids(26).

The significance of the increased concentrations of 5-hydroxytryptamine in the pyloric mucosa of hepatectomized dogs and dogs with intestinal obstruction is not understood, but reversed peristalsis and hypermotility are common in these dogs.

Summary. Increased concentrations of 5-hydroxytryptamine were found in the pyloric mucosa of dogs after hepatectomy, establishment of Eck's fistula, or obstruction of the jejunum. In dogs with intestinal obstruction, no change occurred in the concentration of 5-hydroxytryptamine in blood or in brain or of 5-hydroxyindoleacetic acid in urine.

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Purification of Luteinizing Hormone-Releasing Factor from Beef Hypothalamus.* (29119)

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Experimental work from a number of laboratories has convincingly demonstrated a gonadotrophin-releasing action of extracts from the stalk-median eminence region of several species. Campbell *et al* showed that direct injection of crude acidic extracts of beef stalk-median eminence into the anterior lobe of the pituitary of rabbits would evoke ovulation(1), and similar results were obtained in the rat by Nikitovitch-Winer(2). More recently, Johnson has obtained ovulation in androgen-sterilized rats after intravenous (i.v.) injection of beef hypothalamic extracts(3). We were able to demonstrate an LH-releasing action of rat stalk-median eminence extracts with the use of the ovarian ascorbic acid depletion assay for LH(4,5). Since the LH-releasing activity was diminished by peptic and eliminated by tryptic digestion, we concluded that the LH-releasing factor (LH-RF) was a polypeptide(5). Other studies showed that LH-releasing action of the extract was not accounted for by known pharmacologically active agents in the hypothalamus, such as substance P and the neurohypophysial peptides, vasopressin and oxytocin(4,5). Subsequently, we were able to show that beef stalk-median eminence tissue also had LH-releasing activity(6). This provided us with a more abundant source of the LH-RF for use in the fractionation studies reported here.

Methods. Preparation of crude extract. Stalk-median eminence fragments were collected from freshly slaughtered steers§ and placed in a beaker on dry ice. The average wet weight of the fragments was 0.12 g. They were ground with sand in a pestle and mortar and extracted several times with small volumes of 0.1 N HCl to a total volume of one ml of acid per stalk-median eminence. After centrifugation, the opalescent supernatant was heated for 10 minutes in a boiling water bath, a procedure which destroys about 70% of beef hypophysial LH(6). The supernatant obtained after recentrifugation constituted the crude extract.

Fractionation of the crude extract. Ten ml of this starting material, representing extract derived from 10 hypothalami, were concentrated by evaporation in a boiling water bath under an air current for 20 minutes to a final volume of 2 ml. The precipitate which formed was discarded, and the concentrated crude extract was then neutralized

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§ Kosher killed; this important since it results in fragments largely free of adherent blood clots.