increase is to be attributed to cholinergic vasodilator fibers (4, 5). Sympathetic nerve
terminals are demonstrated in the vicinity of vagal ganglion cell bodies within the myo-
cardium (6), and the terminals functionally liberate catecholamines which have an inhibitory
effect on transmission through parasympathetic ganglia (7). It is considered that during
sympathetic stimulation the transmission is inhibited via alpha adrenoceptors and on release
a facilitation occurs which, in turn, produces a cholinergic dilatation of the coronary vessels.

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THE CONVERSION OF EXOGENOUSLY ADMINISTERED
L-DOPA TO DOPAMINE IN THE STOMACH AND
LIVER OF RATS

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Dihydroxyphenylalanine (L-DOPA), a neurotransmitter precursor, is synthetized from
tyrosine within chromaffin cells and adrenergic neurons (1). This amino acid has not, how-
ever, been detected in tissues and blood, probably due to the rapid conversion to dopamine.
In 1966, Hakanson and Owman (2) discovered a large number of green fluorescent epithelial
cells in the oxyntic gland area of rat stomach after treatment with L-DOPA and suggested
that this green fluorescent material was dopamine converted from L-DOPA in the enter-
ochromaffin-like cells.

The conversion of exogenously administered L-DOPA to dopamine in the stomach
of rat has been chemically confirmed in the present studies. It was also shown that most
of this amino acid was destroyed by the liver catechol-O-methyltransferase (COMT).

Methods and Materials: Male rats of Wistar strain weighing 180 to 250 g were used.
Animals were fasted for approx. 16 hr prior to oral, intraperitoneal and intravenous
administration of L-DOPA. Intravenous injection of L-DOPA to the femoral vein was
done under pentobarbital sodium anesthesia. The intravenously and intraperitoneally

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injected animals were sacrificed by decapitation. Arterial blood, stomach and liver were removed. In oral administration, the median lobe of the liver was ligated with thread as close as possible to the root under pentobarbital sodium anesthesia and a small section of this lobe removed. Blood from portal vein and abdominal aorta was collected using syringes, all procedures being performed within 2 to 3 min. To separate dopamine from L-DOPA, column chromatography was performed using Dowex 50 (Na⁺ form, 200 mm² · 170 mm). Up to 2 g of tissues, 200 mg of EDTA and 10 mg of sodium metabisulfite were added and homogenized with 20 ml of 0.4 N-HClO₄ in a glass homogenizer. Homogenates were centrifuged at 30,000 g, 0°C for 20 min. Supernatant solutions were adjusted to pH 4.0 with 5 N-NaOH and placed on to a Dowex column. L-DOPA passed through the column. Dopamine adsorbed to Dowex was eluted with 15 ml of 2N-HCl. To perform aluminium oxide adsorption procedure, acid solutions of L-DOPA and dopamine thus separated were adjusted to pH 8.0 with 5 N-NaOH and transferred into polycarbonate centrifuge tubes containing 400 mg of activated aluminium oxide and 2 ml of 2 M Tris-buffer (pH 8.2), and immediately shaken for 5 min. L-DOPA and dopamine were eluted with 5 ml of 0.1 N- and 0.05 N-HCl, respectively and assayed spectrofluorometrically. The extraction and assay procedure, unless otherwise mentioned, was the same as that described by Anton and Sayre.¹

Results and Discussion: The time course of L-DOPA content in the stomach after intravenous administration of 20 mg/kg coincided well with that in the plasma, as shown

![Graph A](image1.png)

![Graph B](image2.png)

**Fig. 1.** L-DOPA and dopamine contents in the stomach, liver and plasma of rats after administration of L-DOPA (20 mg kg i.v.). (A) L-DOPA, (B) Dopamine. Liver, • Stomach, ○ Plasma. Each value represents Mean S.E. of 4 samples.
TABLE 1-A. Regional distribution of L-DOPA and dopamine in the stomach of rats 60 min after administration of L-DOPA (20 mg/kg i.v.). Each value represents Mean ± S.E. of 4 samples.

|          | Rumen  | Antrum | Body  |
|----------|--------|--------|-------|
| L-DOPA (μg/g) | 0.22±0.07 | 0.22±0.04 | 0.24±0.06 |
| Dopamine (μg/g) | 0.05±0.03 | 0.87±0.14 | 2.38±0.42 |

B. L-DOPA and dopamine contents in the liver and plasma of rats 30 min after administration of L-DOPA (200 mg/kg per os.). Each value represents Mean ± S.E. of 3 samples.

|          | Liver (μg/g) | Portal vein | Abdominal artery |
|----------|--------------|-------------|------------------|
| L-DOPA   | 0.77±0.41    | 12.88±4.22  | 3.45±1.33        |
| Dopamine | 0.64±0.07    | 0.54±0.07   | 0.05±0.02        |

C. Effects of MAO and COMT inhibitors on dopamine contents in livers of rats 15 min after administration of L-DOPA (100 mg/kg i.p.). Rats were pretreated with carotin 90 min or pyrogallol 15 min before administration of L-DOPA. Each value represents Mean ± S.E. of 3 samples.

| Pretreatment | Dopamine (μg/g) |
|--------------|-----------------|
| None         | 1.86±0.69       |
| Catrin 5 mg/kg i.p. | 1.78±1.34       |
| Pyrogallol 200 mg/kg i.p. | 13.62±1.34     |

in Fig. 1-A. The content of L-DOPA in these organs dropped sharply within 20 min, then gradually declined. Based on theoretical calculation, plasma level of L-DOPA immediately after intravenous administration of this dose should be approx. 200 μg/ml. Disappearance of this amino acid from blood stream was therefore very rapid. This was probably due to a ready degradation of L-DOPA rather than its incorporation into tissues. Wurtman5) reported that approx. 60% of DL-14C-DOPA in an entire mouse was destroyed in the first 5 min after intraperitoneal injection. Dopamine content in the stomach was higher at 20 min than 5 and 60 min after treatment, as shown in Fig. 1-B. Regional distribution studies showed that a large part of dopamine detected in the stomach at 60 min after treatment was derived from the oxyntic gland area, body of stomach (Table 1-A). At this time, the amount of L-DOPA remaining in the stomach was very little and did not region ally differ. These results confirmed the histochemical finding of Håkanson and associates (2, 6).

Dopamine contents in the liver and plasma following 20 mg/kg intravenous injection of this amino acid were consistently very low. Of interest is the fact that the content of L-DOPA in the liver was also very low even at the onset of experiment. By oral administration, the portal-hepatic pathway would be the first site of degradation of L-DOPA following intestinal absorption. A large dose (200 mg/kg) of L-DOPA was given orally and L-DOPA and dopamine in the liver and plasma from different sites were analyzed. Although
the plasma level of L-DOPA in the portal vein 30 min after treatment was very high, contents of L-DOPA as well as dopamine in the liver did not increase extensively (Table 1-B). Plasma level of L-DOPA in the portal vein was four times higher than that in the abdominal artery. Since both L-DOPA and dopamine are excellent substrates for COMT (7), the effect of pyrogallol was examined. Dopamine content in the liver markedly increased with pyrogallol 200 mg/kg i.p. treatment 15 min before the administration of L-DOPA (Table 1-C). Pyrogallol remaining in the samples interfered with the chemical assay of L-DOPA and reliable figures regarding the content of this amino acid was unattainable. Pretreatment with pheniprazine (catron), a monoamine oxidase inhibitor, did not affect dopamine content in the liver.

From these results, it can be concluded that most of L-DOPA in the blood is degraded by a single passage through the liver and in oral administration, only the undegraded part of this amino acid from the liver enters the systemic circulation. Whether the degradation of L-DOPA in the liver is carried out immediately by COMT, or following decarboxylation to dopamine remains obscure.

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SECONDARY ANTIFILARIAL SCREENING IN DOGS USING SETARIA CERVI AS TEST ORGANISM

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It has been reported (1, 2) that intraperitoneal implantation with two male and two female adult Setaria cervi produces microfilaraemia in rats within two weeks and lasts from 54 ± 6 days. When the number of worms implanted is increased, microfilaria appear earlier but the mortality of the host is higher. Complete disappearance of microfilaria from peripheral circulation results after oral and intraperitoneal administration of diethyl carbamazine and 3-acetamido-4-hydroxy-phenyl arsonic acid respectively. Other chemotherapeutic agents effective against various nematode infections, but ineffective against filariasis were also ineffective against microfilaria of Setaria cervi.

For a secondary screening, in a more closely allied host to human beings, adult Setaria