

CATECHOLAMINE METABOLISM IN A FUNCTIONAL NEURAL TUMOR*

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In recent years the association of chronic diarrhea with tumors of neural origin has been reported by several authors (1-5). Green, Cooke and Lattanzi (5), in their report of three such cases, focused attention on the possible secretory nature of these tumors by emphasizing the cessation of symptomatology that results following their operative removal. Evidence of increased production of catecholamines in such cases was then presented simultaneously by Dicke and co-workers (6) and by Greenberg and Gardner (7). More recently, two additional cases with similar results have been reported (8, 9).

As noted by Sjoerdsma, the study of catecholamine metabolism in humans can, perhaps, be most easily accomplished by the investigation of the biosynthesis and fate of catecholamines in patients with pheochromocytoma (10). Urine from such patients has proved to be a good source for the identification of the metabolites of pressor amines.

The demonstration of the hormonal nature of other tumors of nervous tissue opens up a new area for the study of catecholamine metabolism by human nervous tissue. This paper is a report of studies carried out in a two and one-half year old girl who manifested chronic, severe diarrhea and a normal postoperative course in association with a posterior mediastinal ganglioneuroma.

CASE HISTORY

C.M., SMH 423041-3, a 2.5 year old white female, was admitted to Syracuse Memorial Hospital on May 24, 1959, with a chief complaint of chronic diarrhea of 1

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year's duration. At the age of 15 months she developed a watery diarrhea, 6 to 8 stools per day, that persisted up to the time of admission, associated with increasing abdominal distention and failure to gain weight for the preceding 8 months. There was no anorexia; an intake of 8 to 10 glasses of water per day was noticed with an apparent polyuria. There was no history of increased perspiration, skin rashes, flushing or episodic abdominal pain. Coldness of the distal parts of the extremities was not observed, and she remained afebrile throughout. Prenatal and birth histories were entirely normal, as were her early development and feeding. There are 4 normal siblings in the family and a negative family history for significant abnormalities or diseases.

Physical examination. Observation revealed a small, alert wiry girl with a protuberant abdomen and a paucity of subcutaneous adipose tissue. Blood pressure was 105/70; weight 21 pounds; height 32.5 inches; head circumference 18.25 inches; head to pubis 18.75 inches; arm span 31.38 inches; upper/lower ratio, 1.35. Significant findings were limited to the abdomen which was distended and tympanitic and quiet to auscultation. No visceromegaly was present; rectal examination was normal; the stool was watery, brown and nonfatty.

Radiological studies. Radiographic and fluoroscopic examination of the chest with barium swallow revealed a large circumscribed area of increased density in the posterior superior mediastinum. An extrapleural shadow was present in both apices (Dr. Edward Carsky). Examination of both hands and wrists showed normal bone architecture and a skeletal maturation corresponding to the chronological age.

Serum chemistries. Chemical measurements on serum are recorded in Table I. Hypokalemia was present. Associated with this was a failure to conserve water; i.e., after a 12 hour period of oral fluid deprivation, the urine specific gravity was 1.009. Urine collections for 6 consecutive preoperative 24-hour periods revealed volumes of 960, 765, 740, 550, 1005 and 960 ml, with specific gravities ranging between 1.005 and 1.010. Hypophosphatemia of marked degree was found, in association with slightly diminished alkaline phosphatase activity.

Hospital course. The patient continued to manifest persistent watery diarrhea until surgery. She showed no hypertension throughout her hospital stay. On June 5, 1959, a right thoracotomy was performed by Dr. Lawrence K. Pickett with the removal of a smooth, encapsulated tumor mass from the right superior mediastinum, measuring $4.5 \times 1.5 \times 2$ cm and weighing 42 g. Part of the

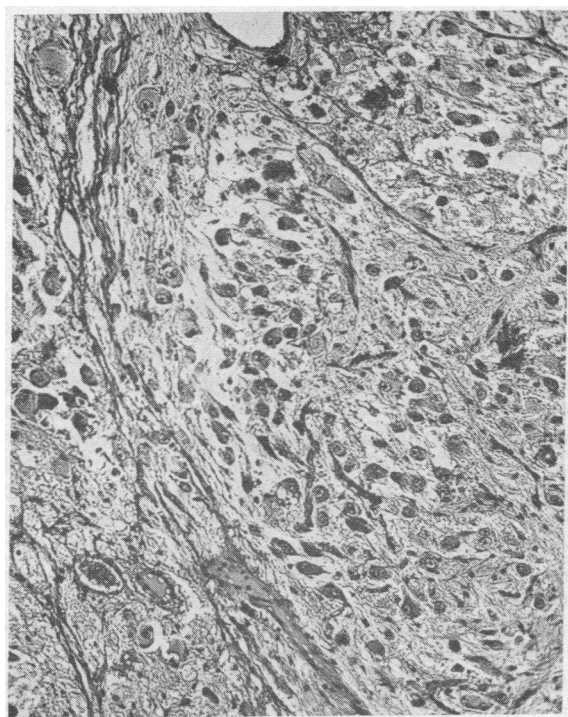


FIG. 1. PHOTOMICROGRAPH OF GANGLIONEUROMA FROM PATIENT C.M. ($\times 120$). Note numerous well differentiated ganglion cells.

tumor was frozen in dry ice-alcohol within 15 minutes after removal. The histologic appearance of the tumor was characteristic of a ganglioneuroma with focal calcification (Dr. John T. Prior); as shown in Figure 1. The postoperative course was completely uneventful with immediate cessation of diarrhea and resumption of a normal stool pattern.

METHODS

1. *Assay of urinary hormones and metabolites.* The major metabolite of 5-hydroxytryptamine (serotonin), 5-hy-

droxyindoleacetic acid (5-HIAA), was determined by the method of Udenfriend, Titus and Weissbach (11). Free norepinephrine and epinephrine were determined on the same specimens (collected in 5 ml 6 N HCl and maintained in a frozen state) by two means: *a*) the method of von Euler and Floding, using iodine as the oxidant (12) and *b*) the method of von Euler and Lishajko (13).

Dopamine was determined according to Carlsson and Waldeck (14), after elution from an alumina column with 0.25 N acetic acid. Norepinephrine, epinephrine, dihydroxyphenylacetic acid (dopac) and metanephrine produced less than 2 per cent fluorescence in equimolar amounts when compared with dopamine under the conditions of this method.

3-Methoxy-4-hydroxymandelic acid (VMA), a major metabolite of norepinephrine (and epinephrine) was determined by the semiquantitative paper chromatographic method of Armstrong, Shaw and Wall (15, 16). Confirmatory determinations were done by the method of von Studnitz and Hanson (17).¹ Homovanillic acid, a major metabolite of both dopa and dopamine, was determined by high voltage paper electrophoresis (18).

3-Methoxy-4-hydroxyphenylalanine (3-methoxydopa), a newly identified amino acid, was isolated in the pre-operative urine,¹ confirming a previous finding in a similar case (18).

2. *Biochemical studies of the tumor.* Five per cent trichloroacetic acid extracts of the tumor were passed through an alumina column at pH 8.3 and eluted with 0.25 N acetic acid. Norepinephrine, epinephrine and dopamine were then determined by previously described methods (13, 14).

Approximately 3 g of the tumor was extracted and passed through an alumina column as above; the eluate was evaporated *in vacuo* at less than 35° C to 2 ml, extracted twice with 5 vol of butanol. After evaporation, the butanol extract was applied to Whatman no. 1 chromatographic paper. After a 24 hour run, descending,

¹ These determinations were kindly done by Dr. Wilfried von Studnitz, Department of Clinical Chemistry, University of Lund, General Hospital, Malmö, Sweden.

TABLE I
Serum chemistries demonstrating hypokalemia and hypophosphatemia

Date	Na ⁺	K ⁺	CO ₂	Cl ⁻	BUN	Total proteins	Serum albumin	Ca	P	Alk. phosphatase
	<i>mEq/L</i>	<i>mEq/L</i>	<i>mEq/L</i>	<i>mEq/L</i>	<i>mg %</i>	<i>mg %</i>	<i>mg %</i>	<i>mg %</i>	<i>mg %</i>	<i>B-L units*</i>
5/26/59	140	2.9	16	106	30					
5/27/59					23					
5/29/59	135	3.0	22	99				10.6	1.6	
6/1/59†	128	3.9	18	90						2.4
6/3/59†	139	3.3	18.5	98		7.2	5.8		2.8	2.6
6/5/59	Surgery									
6/10/59	133	5.6	26	97	14	6.8	4.5	10.6	4.2	

* The method of Bessey, Lowry and Brock was used for serum alkaline phosphatase (J. biol. Chem. 1946, **164**, 321). The normal range for children in this laboratory is 2.8 to 6.7 B-L units.

† During the period 6/1 to 6/3, oral K⁺ supplement was 45 mEq/day.

TABLE II
Summary of determinations of catecholamines and metabolites in pre- and postoperative urine (see text)

	Norepinephrine		Epinephrine		3-Methoxy-4-hydroxy-mandelic acid		Dopamine	Homovanillic acid	
	A	B	A	B	C	D			
	$\mu\text{g}/24 \text{ hrs}$		$\mu\text{g}/24 \text{ hrs}$		$\mu\text{g}/\text{mg urinary creatinine}$	$\text{mg}/24 \text{ hrs}$	$\mu\text{g}/24 \text{ hrs}$	$\mu\text{g}/\text{mg urinary creatinine}$	$\text{mg}/24 \text{ hrs}$
Preoperative	148	232	3.6	0.0	25	90	13.0	2,833	402
Postoperative	17.1	22	0.65	2.9	1.25	14.5	1.4	100	120
Normal values	8.0 ± 4.0		1.2 ± 0.5		1.5–3.0*	2.8–7.5*		5–10X norepinephrine values†	58–12.5
									Not available

* These values refer to adults and children above 3 years of age. Below that age the values are slightly higher; creatinine is used as a basis for comparison, with the relatively lower muscle mass of young children resulting in an apparently increased excretion of 3-methoxy-4-hydroxymandelic acid.

† Normal values obtained by use of same method in newborns (25) and adults (26).

with butanol saturated with 1 N HCl as the mobile phase, the chromatogram was sprayed with ethylenediamine 10 per cent (vol/vol); the reference spots, representing approximately 10 μg pure substance, were located by exposure to ultraviolet light and the paper was cut into 2 \times 1 cm strips, eluted overnight in dilute NH_4OH (pH 10), and the fluorescence was read in a Coleman spectrofluorometer.

The histamine content of the tumor was determined² by the fluorescent method of Shore, Burkhalter and Cohn (19).

3. *In vitro* biologic activity of the tumor. A trichloroacetic acid extract of 0.192 g of the tumor was washed 3 times with peroxide-free ether and taken to pH 4.5 with 1 N NaOH, then bioassayed on a hen's isolated rectal cecum preparation, according to the method of von Euler (20).

4. Assay of catechol-O-methyl transferase activity. When the soluble supernatant fraction from a wide variety of tissues is incubated with S-adenosylmethionine and epinephrine, in the presence of a cation (especially Mg^{2+}), metanephrine (3-methoxy derivative of epinephrine) is formed, as described by Axelrod (21–23). Tissue, representing 0.5778 g wet weight of tumor, was assayed for its ability to form metanephrine according to the method of Axelrod and Tomchick (22). Attempts were made to isolate normetanephrine from the tumor by paper chromatography of organic solvent extracts of the filtrate of trichloroacetic extracts of the tumor, which had been passed through alumina columns. Extractions of the filtrates were done with butanol, followed by heptane, and also with isoamylalcohol:toluene, 3:2, both at pH 10, with re-extraction in both cases with a small volume of 0.05 N HCl.

² These determinations were kindly performed by Dr. Helen Treadway Graham, Department of Pharmacology, Washington University School of Medicine, St. Louis, Mo.

RESULTS

In three consecutive preoperative urine collections, 1.61, 1.69 and 2.06 mg of 5-HIAA per 24 hours were present. Normal levels for children of comparable age are not available; however, normal adult levels, using the same method, range from 1.7 to 5.5 mg per 24 hours (24).

The preoperative excretion of norepinephrine, VMA, dopamine and homovanillic acid was elevated when compared with normal values and/or postoperative levels. The results of these determinations are summarized in Table II. Columns designated A and B refer, respectively, to figures derived from the methods of von Euler and Floding (12) and von Euler and Lishajko (13). The normal levels, obtained by the use of the former method, are abstracted from Zeisel and Kuschke (27) and are in agreement with our unpublished observations. The slightly elevated postoperative levels are believed to be of no significance in view of the normal course of the patient during the six months since surgery.

The identity of the activation and fluorescent spectra (as determined in an Aminco-Bowman spectrophotofluorometer) of dopamine in the preoperative urine with standard dopamine is demonstrated in Figure 2.

The marked elevation in the urinary excretion of VMA was found when determined by both methods used; in Table II, C refers to the method of Armstrong (16) and D to the method of von Studnitz and Hanson (17). The discrepancy in

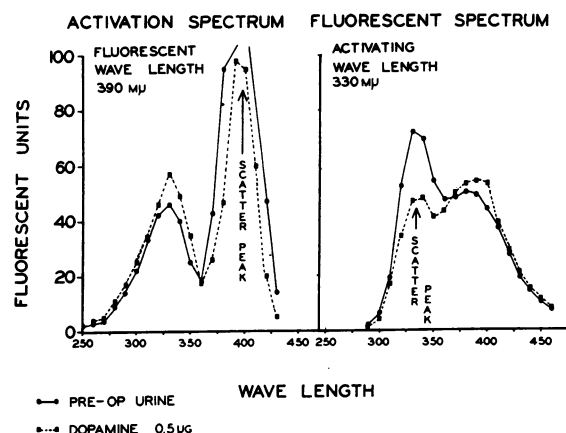


FIG. 2. ACTIVATION AND FLUORESCENT SPECTRA OF AN ALIQUOT OF PREOPERATIVE URINE, DEMONSTRATING THE SIMILARITY TO STANDARD DOPAMINE. See text for methods.

amounts found by the two methods is probably based on a difference in methodology. Homovanillic acid was elevated when compared with the postoperative value; no normal values have been established.

The tumor itself contained 8.6 μg norepinephrine, 0.074 μg epinephrine and 2.09 dopamine per g wet weight. Figure 3 depicts the identity of fluorescent peaks of an extract of the tumor with standard norepinephrine and dopamine, when chromatographed in butanol:HCl (see Discussion). Two separate homogenates of the tumor were assayed for histamine content, the second of which contained more connective tissue than the

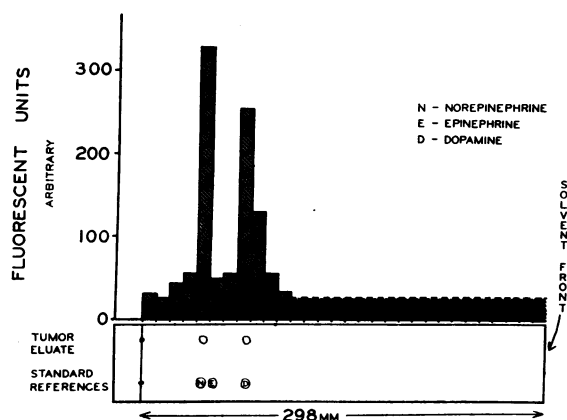
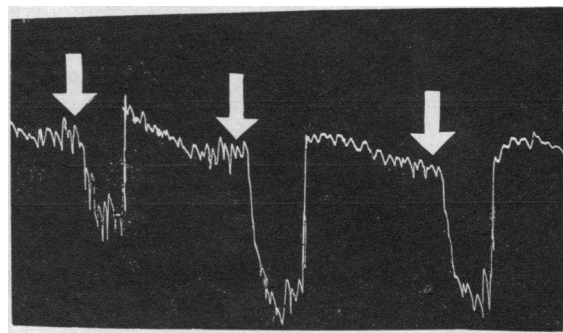


FIG. 3. QUANTITATIVE PAPER CHROMATOGRAPHY OF AN ELUATE FROM THE GANGLIONEUROMA. Ethylenediamine condensation method; N = norepinephrine, E = epinephrine, D = dopamine.



0.1 cc 0.2 cc 200
Tumor Tumor mμg
Extract Extract NE
(0.1 cc extract = 6.3 mg tumor wet wt.)

FIG. 4. BIOASSAY OF TUMOR EXTRACT ON HEN'S ISOLATED RECTAL CECUM, METHOD OF VON EULER (20).

first. Respectively, the values found were 0.37 and 0.261 μg per g wet weight of tissue. These values correspond to the content found in normal brain tissue by the same method.

When a crude extract of the tumor was bioassayed on a hen's isolated rectal cecum, relaxation of the intestinal smooth muscle was produced, as shown in Figure 4. By this method, 15.2 μg of norepinephrine-equivalents per g wet weight of tumor was calculated to be present.

As a means of studying possible mechanisms governing catecholamine release as a result of this tumor, saline extracts were prepared of two 100 mg wet weight portions of the tumor and injected into male rats. No increase in catecholamine excretion was found, suggesting, at least according to this procedure, the absence of any substance stimulating catecholamine excretion from sites other than the tumor itself.

TABLE III
In vitro assay for catechol-O-methyl transferase activity *

	Metanephrine formed
	μg/50 mg tissue/hr
Tumor	0.0
Control (rat liver)	3.29
S-adenosylmethionine omitted	0.0

* Supernatant from tumor and control (rat liver) incubated with L-epinephrine (0.3 μmole), S-adenosylmethionine (0.2 μmole) and MgCl₂ (10 μmoles), with phosphate buffer at pH 7.8 (50 μmoles) at 38° C for 1 hour. Total volume, 1 ml.

Table III indicates the absence of catechol-O-methyl transferase activity in the tumor. It should be noted that the activity of the control rat liver was somewhat lower than that reported in the studies of Axelrod and Tomchick (21, 22). Thus, some enzyme activity of the tumor might not have been detected. However, no normetanephrine could be isolated from the tumor by paper chromatographic techniques. It appears, then, that the tumor was unable to metabolize the norepinephrine formed within it, at least by the important and perhaps primary mechanism of O-methylation (23).

DISCUSSION

Increased amounts of norepinephrine and epinephrine excretion have been found in cases of neuroblastoma by Isaacs, Medalie and Politzer (28) and Mason and associates (29). The presence of pressor amines in the tissue of a neuroblastoma was reported recently by Graham (30). Increased excretion of VMA in cases of ganglioneuroma associated with chronic diarrhea has now been found on four occasions (6-9). These data have been amplified, in this report, by the finding of increased urinary excretion of dopamine and homovanillic acid and the presence of norepinephrine and dopamine in the tumor. Cells comprising

the sympathetic nervous system and chromaffin tissue, both in the adrenal medulla and extra-medullary in location, are derived from a similar embryologic origin (31). It is now apparent that tumors arising from such cells, whether benign or malignant, can form catecholamines and act as true functional or hormone-producing neoplasms.

The original hypothesis of Blaschko (32) on the metabolic route for the biosynthesis of catecholamines is shown with heavy arrows in Figure 5. This has been supported by most studies during the past decade [see reviews by Blaschko (33) and Kirshner (34)]. Of particular interest with respect to this report are the studies of Goodall and Kirshner (35) who demonstrated the formation of norepinephrine, but only very little epinephrine, from tyrosine in sympathetic nerves and ganglia. That norepinephrine can be formed from dopa and dopamine in pheochromocytoma tissue has also been shown by Sjoerdsma, Leeper, Terry and Udenfriend (36) and Gélina, Pellerin and D'Orto (37). The findings, in the present report, of dopamine and norepinephrine in the tumor tissue, increased urinary excretion of homovanillic acid [a major metabolite of both dopa and dopamine (23)], and the presence of the O-methylated derivative of dopa in the preopera-

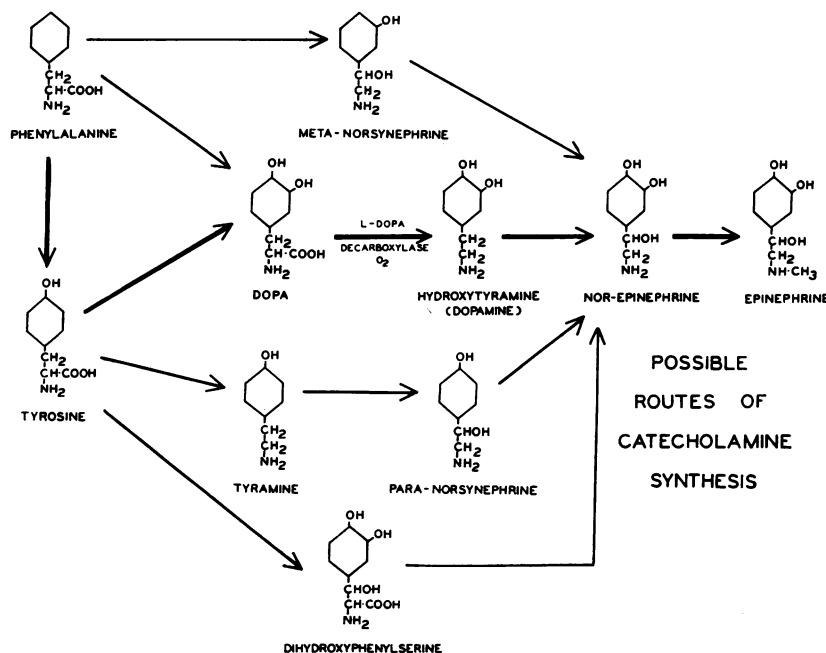


FIG. 5. POSSIBLE ROUTES OF CATECHOLAMINE BIOSYNTHESIS.

tive urine provide indirect evidence of the existence of the dopa \rightarrow dopamine \rightarrow norepinephrine pathway in this ganglioneuroma. Attention should be directed to the report of Senoh, Creveling, Udenfriend and Witkop (38) which describes the (auto)oxidation of dopamine to form 2,4,5-trihydroxyphenethylamine, a substance that cannot be separated from norepinephrine by chromatography or recrystallization except after treatment with methanolic hydrogen chloride. The relative specificity of the oxidation with ferricyanide substantiates the existence of norepinephrine in the tumor studied in this report.

Recent studies have suggested that dopamine may have a function other than as an intermediate in the synthesis of norepinephrine and epinephrine. Indeed, dopamine has been recognized as a normal constituent in human urine for many years (39, 40). In the brain of all mammalian species studied, norepinephrine and dopamine are present in approximately equal quantities (41). There is, however, a disproportionate distribution of catecholamines in the central nervous system; dopamine is present in high concentration in the caudate nucleus, whereas norepinephrine is most abundant in the hypothalamus. Dopamine, similarly, has been demonstrated in adrenergic nerves (42) and has been isolated in tumor tissue (pheochromocytomas) previously (43-45). The occurrence in ruminants of large amounts of dopamine in lung, spleen (46), duodenum and liver is of interest in view of the close correlation between the distribution of dopamine and a special type of chromaffin cell (47). Such cytological correlations have not, however, been found in either brain or sympathetic nervous tissue. While it seems likely that dopamine has its own physiologic role in those tissues in which its concentration is much higher than its "successor" in the metabolic pathway, norepinephrine, there is no evidence as yet that dopamine serves any physiologic role in the peripheral sympathetic nervous system other than precursor to norepinephrine. Whether this may be altered in the case of dopamine-producing tumors remains to be determined.

Is it possible to relate, in a cause and effect manner, the biochemical findings and symptomatology in the case? The abolition of both symptoms and elevated excretion of catecholamines and metabo-

lites after removal of the tumor is suggestive of a relationship between the two phenomena. Indeed, the precise mechanism of diarrhea in such cases awaits clarification. Increased excretion of catecholamines, as in cases of pheochromocytoma, has often been reported in the absence of disturbed bowel function. The hypokalemia observed in this patient may be secondary to chronic diarrhea or may be associated with the prolonged elevation of blood organic acids. The relationships between catecholamines and potassium balance as reviewed by Ellis (48), are too unclear to support generalizations.

The evidence obtained in this study suggests that the tumor was unable to form the O-methylated metabolites of catecholamines. If so, the O-methylated metabolites found in excess in the preoperative urine must have been formed elsewhere in the patient. The enzyme, catechol-O-methyl transferase, is widely distributed throughout the body, having its highest activity in the liver (22).

SUMMARY

The syndrome of chronic diarrhea in the presence of tumors of neural origin has been found to be associated with increased production of catecholamines and their metabolites. In this report, increased urinary excretions of norepinephrine, 3-methoxy-4-hydroxymandelic acid, dopamine and homovanillic acid were found preoperatively in a 2.5 year old girl who exhibited chronic diarrhea in association with a posterior mediastinal ganglioneuroma. All values returned to near normal following operative removal of the tumor in conjunction with a cessation of symptomatology. The tumor contained norepinephrine and dopamine, as shown by fluorescent and chromatographic techniques. Evidence was obtained that the tumor was incapable of forming the O-methylated metabolites of catecholamines.

Although it appears likely that the production of catecholamines by the tumor is related to the symptom of chronic diarrhea, the exact mechanism of this relationship awaits clarification.

In cases of chronic diarrhea and/or suspected tumors of neural origin, the determination of norepinephrine, dopamine and 3-methoxy-4-hydroxymandelic acid in the urine should prove to be of considerable aid in diagnosis and management.

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