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ARGENTAFFIN CARCINOMA OF ILEUM WITH RAISED SERUM 5-HYDROXYTRYPTAMINE

REPORT OF A CASE

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Gastro-intestinal argentaffin carcinoma has lately been recognized as an endocrine growth which may cause flushing attacks and cyanosis, and perhaps stenosis of the pulmonary valve of the heart. The existence of this syndrome was suggested by Biörck *et al.* (1952), who described the post-mortem discovery of argentaffin carcinoma of the ileum in a patient who had complained of cyanosis, flushing attacks, dyspnoea, abdominal pain, and diarrhoea, and who had a pulmonary valvular stenosis. In the same year similar features were recognized by Waldenström and Ljungberg in a patient who had had an argentaffin carcinoma excised in 1944, and by Björkman in another case at necropsy. The observation of changes in skin colour and of flushing attacks led to laparotomy in two further patients, and argentaffin carcinoma was found. To a

detailed description of these five cases Thorson *et al.* (1954) have added one from their hospital records and have considered three described by Isler and Hedinger (1953), two by Rosenbaum *et al.* (1953), and five reports from earlier literature. Other probable cases have been described after death by Ratzenhofer and Lembeck (1954), Branwood and Bain (1954), Jenkins and Butcher (1955), and Fraser (1955), while Page *et al.* (1955) mention a patient with characteristics of the same syndrome.

We have recently encountered a patient in whom, following resection of a stenosing tumour of the ileum, the probable histological diagnosis was postulated on the basis of the reddish-blue colour of the patient's skin. Affinity of the tumour cells for silver was not demonstrated, but we believe that the finding of raised levels of serum 5-hydroxytryptamine (5-H.T.) and urinary 5-hydroxyindoleacetic acid (5-H.I.A.A.) confirms that the tumour originated from argentaffin cells.

Case Report

A man aged 23 was admitted to hospital in January, 1955, with intestinal obstruction and a history of flushing attacks and cyanosis.

Skin Colour.—Since the age of 18 years his face had become red. It felt warm during almost every meal, and this flushed feeling usually subsided in about half an hour. In the past year there had also been some blueness of the face and hands out of doors, and he attributed this to the cold. He had become used to his colour, which caused him no concern. On examination at rest the face was red with a bluish tinge. Dilated capillaries were easily seen with the aid of a loupe. There was no clubbing of the fingers.

Abdominal Condition.—For ten months he had suffered repeated bouts of spasmodic central abdominal pain lasting about 24 hours, and accompanied by vomiting. At these times he could palpate a swelling in the lower abdomen and could feel it move. The attacks would often subside after a series of loose black stools, but there was no diarrhoea at other times. There had been some flatulence and borborygmi, and heartburn had been troublesome. His appetite was fair and he was not aware of any change in weight. For two weeks before admission he had suffered pain every day, and in the last 36 hours he had vomited repeatedly. On examination there was no abdominal distension. During a spasm of pain a tender, mobile, sausage-shaped swelling was palpated in the hypogastric region, and there was transient visible peristalsis in the same area. When the spasm had subsided the abdomen appeared normal.

Laparotomy revealed a stricture of the ileum 60 cm. from the ileo-caecal valve, associated with contracted mesentery containing two enlarged lymph nodes. In the liver were numerous hard nodules up to 2 cm. in diameter. The thickened mesentery and glands were excised with the stricture, and continuity of the ileum was restored by side-to-side anastomosis.

Investigations

Pathology.—The specimen consisted of a segment of small intestine with obstruction caused by a ring stricture. Above this there was distended loop of hypertrophied ileum, and below was a segment of collapsed bowel. In the adjacent mesentery the lymph nodes were enlarged and had obviously been invaded by secondary tumour. Microscopically the muscle wall was invaded by sheets of eosinophilic cells with poorly demarcated outlines and pale-staining nuclei with a well-marked membrane. In some areas a pseudo-adenomatous arrangement was present (see Fig.). The sections resembled Kulitschitzky-cell carcinoma more closely than adenocarcinoma, but affinity of the cells

for silver could not be demonstrated. The lymph nodes were invaded by sheets of cells similar to those of the primary growth in the bowel wall.

Blood.—Haemoglobin, 72% ; red cells, 4,310,000. The blood contained no sulphaemoglobin or methaemoglobin.

Serum 5-Hydroxytryptamine.—The patient's serum 5-H.T. content was estimated from blood collected between flushing attacks nine days after operation, and was found to be 3 μ g. per ml., almost ten times the highest value obtained from six normal human subjects by Cottrell *et al.* (1955), who recorded the following results: 0.05, 0.1, 0.175, 0.182, 0.26, and 0.354 μ g. of 5-H.T. per ml. of serum. The method used throughout was that of Amin *et al.* (1954).

Photomicrograph of section of primary tumour.

Urinary 5-Hydroxyindoleacetic Acid.—The 5-H.I.A.A. content of a 24-hour sample of the patient's urine was estimated at approximately 80 mg., or eight times the highest normal value recorded by Titus and Udenfriend (1954), who found 5–10 mg. of 5-H.I.A.A. in 24 hours.

Heart.—The heart rate was 74 and the blood pressure 105/65. No murmur was detected on auscultation. An electrocardiogram showed clockwise rotation of a semi-vertical heart. The P wave in lead II was 3.5 mm. tall. Radiological examination showed that the heart was normal in size and shape. There was no right ventricular enlargement and no abnormality of the pulmonary conus or pulmonary artery to suggest pulmonary stenosis. The vascularity of the lung fields was within normal limits. Examination at another hospital within the previous three months had shown a coarse systolic murmur over the xiphoid process. A phonocardiogram had indicated a systolic murmur at the apex and a short diastolic murmur over the lower left sternal border. A radiologist suspected pulmonary stenosis. Barium-meal and barium-enema examinations had been negative, and a "follow-through" examination had been advised.

Progress

Peristalsis returned within 36 hours after operation, and the bowels began to move four or five times daily.

The red colour of the face persisted and the patient continued to experience flushing. Walking 100 yards caused him no distress, but produced a deep uniform cyanosis of the whole of the face, head, and neck, and, to a less extent, of the limbs and trunk. After a few minutes at rest pink thumb-nail patches appeared in the blue skin of the face and chest and persisted as the cyanosis decreased.

Five months later he was reported to have been at work as an electrician for three months and was feeling well. The changes in skin colour had continued.

Discussion

5-Hydroxytryptamine

This was first detected as "enteramine" (Vialli and Erspamer, 1933), which was assumed to originate in the

Kultschitzky cells of gastro-intestinal mucosa (Erspamer, 1940). It was later isolated from blood as "serotonin" (Rapport *et al.*, 1948), and was synthesized (Hamlin and Fischer, 1951).

Feyrter suggested that the scattered gastro-intestinal argentaffin cells might possess an endocrine functional unity, and pharmacological assay of argentaffin tumours was attempted by Feyrter and Unna (1936), and by Selberg (1941), but with inconclusive results. Lembeck (1953) obtained a post-mortem specimen of argentaffin tumour for assay, and found at least 2.5 mg. of 5-H.T. per g. of fresh tumour tissue. He detected also another smooth-muscle-stimulating substance which was not identified. Similar evidence of the high 5-H.T. content of argentaffin tumour tissue was found by Page *et al.*, who estimated 0.9 mg. of 5-H.T. per g. from a biopsy specimen.

Thorson *et al.* suggested that excessive secretion of 5-H.T. by argentaffin tumours might be the cause of the syndrome which they had observed. Blood collected from two patients during flushing attacks was examined by Pernow and Waldenström (1954), who found 12 μ g. and 15 μ g. of 5-H.T. per ml. of serum, and compared these figures with normal levels of 0.03–0.2 μ g. per ml. recorded by Erspamer (1954). They concluded that their results could be attributed to secretion of 5-H.T. by metastases, since the primary tumour had been removed in each case.

In normal subjects a proportion of 5-H.T. is excreted in the urine as 5-H.I.A.A. and has been estimated at 5–10 mg. of 5-H.I.A.A. daily (Titus and Udenfriend, 1954). Urine from three patients with argentaffin tumours contained 65–350 mg. of 5-H.I.A.A. a day, although only one of these patients is known to have presented the characteristics of the syndrome (Page *et al.*, 1955). Reviewing 157 contributions to the literature, Page (1954) states that consideration of the functional significance of 5-H.T. depends on conjecture alone. Possible roles include control of peristalsis, stimulation of glandular secretion, antidiuretic action, some part in vascular homoeostasis and in nerve metabolism, and regulation of the excitatory nerves of the heart. 5-H.T. is present in blood platelets and may be released from platelet plugs at sites of vascular injury. It may thus be concerned in the effects of coronary thrombosis, pulmonary embolism, and traumatic shock.

The Primary Tumour

Argentaffin-cell tumours may arise in the stomach, intestine, pancreas, or gall-bladder, and are the commonest new growths of the appendix and of Meckel's diverticulum. Willis (1948) finds the majority are histologically distinctive and can be identified without the unreliable silver-staining test. He acquires the term "carcinoid" because many of them are invasive and metastasizing growths, and prefers to call them argentaffin or Kultschitzky-cell carcinomas. In the small intestine they may form multiple nodules or plaques, or single growths may encircle the bowel and cause stenosis. Spread to mesenteric lymph nodes and liver is frequent, and remote metastases may appear in lung, kidney, adrenal, brain, and skin. Isler and Hedinger mention a metastasis in a rib. Some of these tumours show very slow progress: one patient described by Thorson *et al.* had abdominal symptoms for 20 years before the obstruction caused by an argentaffin carcinoma of the ileum was relieved by a lateral anastomosis, leaving the tumour undisturbed. The patient survived 16 years after this operation.

It has been suggested that the argentaffin granules in Kultschitzky cells are produced by the interaction of 5-H.T. and formalin, which produces also the yellow colour of carcinoid tumours (Ratzenhofer and Lembeck, 1954). These authors believe that failure of silver staining may be attributable to autolysis when formalin fixation is delayed after death or after removal of the tumour at operation. An interval of not more than two hours elapsed between excision of our specimen and its immersion in 10% formalin

solution. The cut surfaces of the primary and secondary tumours in the fixed specimen were greyish white and not yellow.

Hepatic Metastases

These have been present in all cases of the syndrome (see Table). There may be a critical level of serum 5-H.T. beyond which features of the syndrome appear, related to a bulk of argentaffin tissue attained only by the development of multiple hepatic metastases. If the liver normally modified the amount of 5-H.T. passing from portal to peripheral blood it might be suggested that secondary growths situated in the liver could secrete large amounts of 5-H.T. directly into the systemic blood stream.

Cutaneous Vascular Phenomena

Some change in skin colour is reported in all cases, varying from patches of red, mauve, blue, and purple-grey to permanent cyanosis. If the skin changes depend on increased serum 5-H.T. they may indicate the age of the tumour. In our patient this would be at least five years. Experimentally, intradermal injection of 5-H.T. may cause a red, often morbilliform, reaction without itching or wealing (Reid and Rand, 1952).

The association of flushing with meals has been observed by several patients. Perhaps the flush signals a sudden increase in serum 5-H.T., the response of argentaffin cells to a humoral reflex following the entry of food to the stomach. It has been calculated that the serum 5-H.T. levels found by Pernow and Waldenström in patients during flushing indicate the instantaneous presence in the blood of up to 30 mg. of 5-H.T. In normal subjects, however, side-effects prevent the instantaneous injection of more than 1 mg. of 5-H.T., and experimental infusion of 5-H.T. in these subjects does not cause flushing (Page *et al.*, 1955).

Cyanosis in this syndrome may appear years before signs of congestive heart failure, and in no case has the haemoglobin exceeded 100%. The blue colour, therefore, cannot be attributed to pulmonary stenosis, because the cyanosis of pure pulmonary stenosis is a late feature associated with congestive heart failure, while the cyanosis of pulmonary stenosis with patent foramen ovale results from the shunt of blood from right to left auricles and is associated with anoxaemia and polycythaemia.

Other Effects

Dyspnoea.—The infusion of 5-H.T. in man may cause a short period of hyperpnoea with exaggerated respiratory movements and complaint of difficulty in breathing (Page). Three patients described by Thorson *et al.* had expiratory stridor and dyspnoea during flushing attacks.

Effects on the Heart.—Signs of an organic lesion are a late feature of the syndrome. One patient described by Thorson *et al.* had no heart murmur at first but a harsh systolic murmur 14 months later; another had a murmur and E.C.G. evidence of right heart strain, but no valve lesion was found at necropsy. When affected, the pulmonary valve usually showed narrowing of the valve ring and partial fusion and fibrosis of the cusps. Thorson *et al.* remark, however, that the degree of stenosis was slight and that they would not have expected it to offer much resistance in otherwise normal persons. Yet a common finding was dilatation and hypertrophy of the right ventricle. Tricuspid incompetence was another feature, and in the first patient described by Björck *et al.* the regurgitant flow was so great that a catheter could not be introduced into the right ventricle. Experimental infusion of 5-H.T. causes transient pulmonary vasoconstriction with high right ventricular pressure (Page). Repeated release of large amounts of 5-H.T. from argentaffin tumour tissue might be expected to cause repeated pulmonary vasoconstriction and secondary pulmonary hypertension. Whatever the cause of the heart lesions, the time over which it acts is evidently an important factor. Physical changes may follow the functional effects.

Abdominal Pain and Diarrhoea.—Before resection of a tumour in the bowel, pain and diarrhoea might result from recurrent subacute obstruction or from recurrent incipient intussusception. Alternatively, they might be caused before or after operation by high serum levels of 5-H.T. When subjected to barium-meal examination most of these patients have shown intestinal hurry. Normal individuals injected with 5-H.T. may complain of abdominal pain and a desire to defaecate (Page, 1954).

Diagnosis and Treatment

Diagnosis tends to be delayed. Even with a stenosing tumour of the small intestine, obstructive symptoms are late because the bowel content is fluid. Barium-meal examination confirmed the lesion in one patient described by Thorson

Summary of Recorded Cases

Author	Skin Colour	Flushing	Dyspnoea	Abdominal Pain	Diarrhoea	Histology	Primary Site	Hepatic Metastases	Heart Murmur	P.M. Pulmonary Valve Lesion
Cassidy (1930)	Red, mauve	Present	Present		Present	Columnar carcinoma	Not found	Present	Present	Stenosis
Arnett and Long (1931)	Bluish red		"		"	Adenocarcinoma	Pancreas	"	"	"
Scholte (1931)	Red, blue		"		"	Carcinoid	Ileum	"	"	"
Cassidy (1934)	Red	Present		"Discomfort"		Carcinoma	Stomach	"	None	Not reported
Currens <i>et al.</i> (1945)	Blue			Present	Present	"	Ileum	"	Present	Stenosis
Björck <i>et al.</i> (1952)	Red, blue	Present	Present	"	"	Carcinoid	"	"	"	"
Isler and Hedinger (1953)	" "		Present	"	"	"	Jejunum	"	"	"
	Blue			"	"	"	Ileum	"	"	"
Rosenbaum <i>et al.</i> (1953)	Purple-grey					"	"	"	"	"
	" "					Adenocarcinoma	Hepatic bile ducts	"	"	"
	Red	"Heat waves"	Present	Present	Present	Carcinoid	Ileum	Present	None	"
Thorson <i>et al.</i> (1954)	Red, blue	Present	"	"	"	"	Small intestine	"	Present	(Patient alive)
	" "	"	"	"	"	"	Not found	"	"	"
	" "	"	"	"Discomfort"	"	"	"	"	"	Not reported
Ratzenhofer and Lembeck (1954)	Cyanosis	"	Present	Present	"	"	Ileum	"	"	None found
Branwood and Bain (1954)	Red, blue	Present	"	"	"	"	Jejunum	"	"	Not reported (but "tricuspid incompetence")
Jenkins and Butcher (1955)	" "				"	"	Ileum	"	Present	Stenosis
	"Malar flush"		Present		"	Argentaffinoma	Caecum	"	"	None found
Fraser (1955)	Red, purple			Present	"	"	Ileum	"	"	Stenosis
Duncan, Garven, and Gibbons	Red, blue	Present	Absent	"	"	Carcinoid	Caecum	"	None	(Patient alive)
						Argentaffin-cell carcinoma	Ileum	"		

et al., but was not helpful in other reported cases. The estimation of urinary 5-H.I.A.A. or serum 5-H.T. is not a routine measure, but raised values either before operation or without it would confirm a diagnosis of argentaffin carcinoma. A surgeon possessing this information might attempt more radical treatment than would be justifiable for widespread adenocarcinoma.

The slow progress of some of these cases makes it seem reasonable to resect the primary tumour and accessible metastases, to relieve obstruction, and to reduce the amount of tissue producing 5-H.T. One patient of Thorson *et al.* was still alive eight years after such a resection. Experimentally the vascular response to 5-H.T. is modified by drugs. Lysergic acid diethylamide (L.S.D.) specifically inhibits the effects of 5-H.T. on blood vessels of perfused rabbit's ear in concentrations of less than one in ten million; dihydroergotamine methane sulphate is another active antagonist (Gaddum *et al.*, 1955), and anti-5-H.T. effects are claimed also for chlorpromazine (Gyermek, 1955). In man, doses of L.S.D. around 30 μ g. cause intoxication with traits suggestive of schizophrenia (Stoll, 1949), but L.S.D. or other antagonists might have therapeutic value if they were found to prevent flushing attacks and pulmonary hypertension.

Summary

A case of argentaffin carcinoma of the ileum with hepatic metastases and a serum 5-hydroxytryptamine level amounting to at least ten times the normal is described. 5-H.T. originates in the argentaffin cells of gastro-intestinal mucosa, is a normal constituent of the blood platelets, and appears in the urine as 5-hydroxyindoleacetic acid. It is produced in excess by tumours of argentaffin cells, and may be associated with a syndrome comprising flushing attacks, cyanosis, dyspnoea, abdominal pain, and diarrhoea, and secondary pulmonary hypertension.

APPENDIX

Serum 5-Hydroxytryptamine.—The patient's serum, which had been frozen for transport, was stored at -17° C., and three days later was thawed at room temperature. Duplicate samples of 2 ml. were placed in clean dry flasks. A third sample of 1.5 ml. of serum was added to 1 μ g. of 5-H.T. base in 0.2 ml. of saline in a third flask.

Extraction.—Twenty volumes of acetone was added to each sample, which was shaken and then kept at 5° C. for one hour before filtering. Each precipitate was washed twice with 10 ml. and once with 5 ml. of 95% acetone; then the acetone filtrate and washings were evaporated almost to dryness, under reduced pressure at 35° C. To each sample 1 ml. of water was added before successive extractions with 15 ml. and 10 ml. of petroleum ether to remove lipids. The aqueous residues had a creamy yellow colour. They were evaporated to dryness and stored overnight at -17° C.

Assay.—The isolated atropinized oestrous uterus of the rat was used for assay of the specimens in terms of 5-H.T. base, with a sample of 5-H.T. creatinine sulphate supplied through the courtesy of Upjohn Laboratories, Kalamazoo, Michigan. The duplicate extracts were estimated to contain 3.2 μ g. and 2.8 μ g. of 5-H.T. base per ml. of serum respectively, and from the third sample 90% of the added 5-H.T. was recovered.

Specificity Test.—The uterus preparation was treated with L.S.D., which is a specific antagonist for 5-H.T. on this tissue (Gaddum, 1953). The contractions subsequently caused by the serum extracts and by 5-H.T. were similarly reduced, so the activity of the serum extracts was attributed to 5-H.T.

Urinary 5-Hydroxyindoleacetic Acid.—The sample was collected two weeks after operation and was stored in a frozen state for three months before analysis. The total volume was 815 ml.

Method.—A 400-ml. aliquot was extracted by the method of Erspamer (1955), and semi-quantitative estimation of the 5-H.I.A.A. content was made by comparison of the intensity of coloured spots given by the extract and by known quantities of 5-H.I.A.A. on paper chromatograms sprayed with *p*-dimethylaminobenzaldehyde in acid ethanol solution (1 g. of *p*-D.M.A.B.A. dissolved in 30-ml. mixture of equal volumes of concentrated hydrochloric acid and ethanol). Separate chromatograms were developed with *n*-butanol: 30% methylamine (8:3), *n*-butanol saturated with $\bar{N}$ -HCl, and the organic phase of a mixture of *n*-butanol:acetic acid: water (4:1:5).

ADDENDUM.—In a recent publication Snow *et al.* (1955) have described three patients presenting with flushing and hepatomegaly. Increased amounts of blood 5-H.T. and urinary 5-H.I.A.A. were demonstrated in each case. In one the primary tumour was found in a Meckel's diverticulum, and in another in the stomach. Dosage with L.S.D., "Bol 148" (a derivative of L.S.D.), and ergotamine tartrate did not prevent flushing.

In another patient Goble *et al.* (1955) have shown that blood from the pulmonary artery contained more 5-H.T. than peripheral blood. The primary tumour was in the ileum, and there were signs of tricuspid stenosis. 5-H.T. antagonists were given without benefit, but temporary relief of flushing and dyspnoea was achieved by intravenous administration of colloidal radioactive gold, which is concentrated in the liver.

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