trinitrate biphosphate and placebo tablets, and the nitroglycine was supplied by the Key Corporation, Miami, Florida.

REFERENCES

- Dailheu-Geoffroy, P. (1951). *Clinique (Paris)*, **46**, No. 5, p. 27.
 David, A., and Rouleau, Y. (1957). *Canad. med. Ass. J.*, **76**, 616.
 Friend, D. G., O'Hare, J. P., and Levine, H. D. (1954). *Amer. Heart J.*, **48**, 775.
 Fuller, H. L., and Kassel, L. E. (1955). *J. Amer. med. Ass.*, **159**, 1708.
 Gilbert, N. C., and Nalefski, L. A. (1949). *J. Lab. clin. Med.*, **34**, 797.
 Gorlin, R., Brachfeld, N., MacLeod, C., and Bopp, P. (1959). *Circulation*, **19**, 705.
 Graham, D. M., Lyon, T. P., Gofman, J. W., Jones, H. B., Yankley, A., Simonton, J., and White, S. (1951). *Ibid.*, **4**, 666.
 Heller, E. M. (1956). *Canad. med. Ass. J.*, **74**, 197.
 Huppert, V. F., and Boyd, L. J. (1955). *Bull. N.Y. med. Coll.*, **18**, 58.
 Junkmann, K. (1940). Naunyn-Schmiedeberg's *Arch. exp. Path. Pharmacol.*, **195**, 175.
 Melville, K. I., and Lu, F. C. (1951). *Canad. med. Ass. J.*, **65**, 11.
 Merlen, J. F. (1951). *Thérapie*, **6**, 223.
 Riseman, J. E. F., Altman, G. E., and Koretsky, S. (1958). *Circulation*, **17**, 22.
 Russek, H. I., Zohman, B. L., and Dorset, V. J. (1955a). *Amer. J. med. Sci.*, **229**, 46.
 ———, Drumm, A. E., Weingarten, W., and Dorset, V. J. (1955b). *Circulation*, **12**, 169.

A METASTASIZING CARCINOID TUMOUR WITH UNUSUAL FEATURES

BY

ROGER WILLIAMS, M.B., M.R.C.P.

Formerly Medical Registrar,* Department of Medicine,
Postgraduate Medical School of London,
Hammersmith Hospital, London

Carcinoid tumours arise from specialized epithelial cells in the gastro-intestinal tract known as Kulchitzky cells, which in recent years have been shown to secrete 5-hydroxytryptamine. The presence of excessive quantities of this substance in the blood of patients with metastasizing carcinoid tumours may produce a characteristic clinical syndrome of cutaneous flushing, diarrhoea, asthma, and right-sided valvular disease of the heart. Not all metastasizing tumours produce this syndrome, and the following case, which shows many unusual features, was originally diagnosed 22 years ago as an inoperable carcinoma of the pancreas.

Clinical Record

A retired civil servant aged 62 had his first attack of jaundice in 1927 at the age of 30; this was transient and was attributed to infective hepatitis.

In 1937 jaundice recurred, but on this occasion it steadily deepened. The liver was enlarged five fingerbreadths below the costal margin and the gall-bladder was palpable. A laparotomy by Mr. R. M. Vick at St. Bartholomew's Hospital showed a mass $1\frac{1}{2}$ by 2 in. (3.8 by 5 cm.) in the head of the pancreas and a liver studded with secondary deposits. A palliative cholecystogastrostomy was performed. No biopsy was taken. After the operation the jaundice cleared, but the colour returned to the stools for a short time only. Subsequently these became loose, pale, and offensive, five or six a day, but his main complaint over the next few years was of recurrent attacks of severe abdominal pain probably pancreatic in origin.

In 1944 he developed oedema due to hypoproteinaemia (serum albumin 2.6 g./100 ml.). Treatment with a high-

protein low-fat diet and intravenous infusions of plasma resulted in a rise in serum albumin, disappearance of the oedema, and some improvement in the steatorrhoea. Shortly after discharge from hospital he had a further attack of jaundice, thought to be a serum hepatitis.

In 1952 he became jaundiced again, the serum bilirubin rising over three weeks to 32.5 mg./100 ml. A laparotomy was performed by Professor I. Aird at Hammersmith Hospital, the operation note being as follows: "The liver was grossly enlarged, with about a dozen hard white nodules characteristic of a carcinoma deposit. In the head of the pancreas and related to the termination of the common bile duct there was a stony-hard tumour encircling not only the common duct but also the lower part of the portal vein. The remainder of the pancreas presented to palpation and vision the characteristic appearances of chronic pancreatic obstruction. The common bile duct was grossly distended and there was only a tiny fistulous opening between the gall-bladder and duodenum. This was closed and a Roux-en-y choledochojunostomy performed. Biopsies were taken from the liver and from the pancreatic mass through the lumen of the common bile duct. The appearances of the liver were exactly those described at the first operation."

Histological report (Dr. E. D. Williams): "Naked-eye examination of the liver biopsy showed one main nodule of tumour tissue (0.8 cm. diameter) and two smaller ones. On microscopy these consisted of clumps and strands of closely packed polygonal cells embedded in a meshwork of loose fibrous tissue. In some areas there was a tendency to an acinar structure and in one part there were well-formed tubules. The tumour nuclei were remarkably uniform in size and shape, and only occasional mitoses were seen. The clear vacuolated cytoplasm contained a few granules which did not stain with the Masson silver technique or with the diazo reagent. The pancreatic biopsy was badly distorted and consisted mainly of dense fibrous tissue, but in one area at the edge of the specimen there was similar tumour tissue to that described above. Despite the absence of specific granules, the histological appearances were typical of a carcinoid tumour."

The patient made a good recovery from the operation, though the steatorrhoea and recurrent bouts of abdominal pain continued. He also complained of nocturnal polyuria and cramp in the hands and feet, suggestive of tetany, but never had any attacks of flushing or cyanosis.

Results of Examination

In 1958 he was readmitted for further assessment. He was thin with a dry, scaly skin and slight, pitting oedema of the ankles. Trousseau and Chvostek signs were negative. The liver edge was hard but regular, and was palpable three fingerbreadths below the costal margin. There was no splenomegaly or ascites. The cardiovascular system was normal apart from a soft systolic murmur at the apex.

Investigations.—Haemoglobin, 12.9 g./100 ml.; M.C.H.C., 30%; white-cell count, 6,000, with a normal differential; sternal marrow, normal. Serum electrolytes: sodium 130, potassium 4.1, chloride 99, bicarbonate 25, calcium 4.0, phosphate 1.9 mEq/l. Serum proteins: albumin 3.6 g., globulin 3.0 g./100 ml.; serum amylase 170 units. 5-hydroxyindole acetic acid excretion, 10.5, 10.7, and 9.2 mg. per 24 hours (normal range 3–13 mg.). Liver-function tests: serum bilirubin, 0.3 mg./100 ml.; thymol turbidity, 0 unit; alkaline phosphatase, 21 units; serum G.O.T. 25 units/ml., serum G.P.T. 25 units/ml. Bromsulphthalein test, less than 10% dye retention at 30 minutes. Prothrombin time, 14.5 seconds (normal 10–11 seconds). Liver biopsy, only normal tissue obtained. Intestinal absorption tests: stool examination, numerous undigested meat fibres; no fat-forming organisms on culture. Glucose tolerance, normal. Starch tolerance, abnormal; no significant rise in blood sugar after 50 g. of starch by mouth. Fat balance (75 g. intake): first three days, average daily excretion 64.7 g.; second three days, average daily excretion 38 g. Radioactive fat studies (labelled with

*Now at the Medical Unit, Royal Free Hospital, Gray's Inn Road, London.

iodine-131): impaired triolein, normal oleic acid absorption. Radioactive vitamin B₁₂ absorption impaired. Folic acid clearance and absorption tests showed folic acid deficiency with impaired intestinal absorption.

A straight x-ray film of the abdomen showed pancreatic calculi, and a barium follow-through examination revealed a dilated small intestine consistent with steatorrhoea.

Treatment

The patient was given large doses of pancreatin, but, though the stools were thought to be less fatty, there was no change in fat absorption as measured by radioactive triolein. On the supposition that an abnormal bacterial flora might be contributing to the steatorrhoea a course of antibiotics (chlortetracycline, chloramphenicol, and succinylsulphathiazole consecutively, each for five days) was given but with no improvement either clinically or in fat excretion. He was discharged on pancreatin and a low-fat diet. Since then there has been no change in his condition. Random fat analyses done as an out-patient have shown a fat excretion varying between 70 and 90 g. daily.

Discussion

Kultschitzky cells have been described in the efferent ducts of the pancreas, and Willis (1940), in reporting two cases of metastasizing carcinoid tumour of the small intestine, postulated that some or all of the deposits in the pancreas might have been multiple primary formations. No proved case of a primary carcinoid tumour of the pancreas has, however, been reported, and it is much more likely in the present case that the tumour arose in the duodenum. This is a rare site for carcinoid tumours; they usually arise in the first part, within 1 in. (2.5 cm.) of the pylorus, but a few in the region of the ampulla of Vater have been reported. These, as in the present case, presented with obstructive jaundice (Brentano, 1920; Warren and Coyle, 1951; Mrazek *et al.*, 1953), but in one of them (Brunschwig and Childs, 1939) the common bile duct opened separately in the duodenum below the ampulla of Vater and the presenting feature was a severe pancreatic steatorrhoea.

The term "carcinoid" was applied to these tumours in the belief that they were benign despite the histological resemblance to carcinoma. Subsequent experience has shown this to be untrue and they are now regarded as slowly growing malignant tumours and will probably all metastasize eventually. The commonly occurring tumours in the appendix tend to be diagnosed early and consequently are removed before they have time to metastasize, whereas in the small intestine they can persist for much longer before producing symptoms. Even when metastases are present the prognosis may be measured in years. In occasional cases prolonged survival may occur, the longest reported being that of a patient who died of an unassociated lobar pneumonia 20 years after a laparotomy had shown extensive retroperitoneal metastases (Mallory, 1940). At necropsy these metastases did not appear to have increased in size.

One of the most remarkable features of the case here recorded was the lack of any signs or symptoms of the carcinoid syndrome such as flushing, asthma, or right heart lesions, despite the presence of hepatic metastases for 22 years. Diarrhoea, oedema, and hypoproteinaemia may also occur in this syndrome, but in the present case they were clearly due to the severe pancreatic deficiency. Nevertheless, the impairment of absorption

of both vitamin B₁₂ and folic acid suggests some additional functional or structural lesion of the intestinal wall which might be related to the carcinoid tumour. Occasionally, however, malabsorption of both these substances occurs in pure pancreatic steatorrhoea (McIntyre *et al.*, 1956; Chanarin *et al.*, 1958). It is now believed that 5-hydroxytryptamine is chiefly destroyed in the liver (Thorson, 1956; McKusick, 1956), hence the importance of hepatic metastases in the pathogenesis of the flushing syndrome, for when this substance is produced in the liver it will escape destruction and reach the systemic circulation in high concentrations. In support of this hypothesis Thorson (1958) found that in 40 cases of metastasizing carcinoid tumours without the flushing syndrome all the deposits were drained by the portal system, whereas at least 11 out of 12 cases with flushing had deposits in the liver, ovary, or abdominal wall, which drained direct to the inferior vena cava without passing through the liver parenchyma.

Not only were there no signs of the carcinoid syndrome but there was also no biochemical evidence of increased 5-hydroxytryptamine formation such as an increased excretion of 5-hydroxyindole acetic acid—its principal breakdown product—in the urine. This is even more surprising, for Thorson (1958) found that, apart from a doubtful exception reported by Knauff (1956), all the carcinoid tumours of the small intestine with metastases that had been investigated biochemically had shown evidence of increased 5-hydroxytryptamine formation. Not all these cases showed clinical signs of the carcinoid syndrome, and there appears to be a wide individual variation in the body's ability to deal with and respond to excess 5-hydroxytryptamine. There may be a considerable daily variation in the output of 5-hydroxytryptamine acetic acid (Hanson and Serin, 1955; Snow *et al.*, 1955), and occasionally it may even drop to normal, but in the present case it was within the normal range on three widely separate occasions.

The question then arises whether the lack of activity might be correlated with the absence of specific granules on histological examination. Although the majority of tumours associated with the flushing syndrome are reported as argentaffin-positive there are several (Snow *et al.*, 1955; Duncan *et al.*, 1955) in which cytoplasmic granules could not be demonstrated. Thorson (1958) described two cases, both with advanced signs of a functioning tumour, one of which was strongly argentaffin, whereas the other with exactly the same technique was negative, though both tumours contained large quantities of 5-hydroxytryptamine. Occasionally one portion of a tumour may be negative and another strongly positive, and metastases from argentaffin primary tumours are not always positive.

It is noteworthy that carcinoid tumours in other sites, such as the rectum and bronchus, rarely show granules, and Warner and Southren (1958) described two cases of metastasizing bronchial tumours with the flushing syndrome in which no granules could be demonstrated. The granules are now believed to be artifacts produced by the interaction of 5-hydroxytryptamine and the formol-saline used in fixation. For their demonstration not only must this be used but also the material must be fixed soon after removal from the body. Nevertheless, these considerations do not always explain the variable staining properties of carcinoid cells, and it is possible that the agranular tumours arise from the pre-enterochrome cells described by Erspamer (1939) as

the non-granular precursors of the normal Kultschitsky cells in the intestinal crypts.

Summary

An unusual case of a metastasizing carcinoid tumour is described in which, despite the presence of hepatic metastases for 22 years, there was no clinical or biochemical evidence of increased 5-hydroxytryptamine formation. The site of origin was probably in the second part of the duodenum, and the main clinical features were obstructive jaundice and severe pancreatic steatorrhoea.

I thank Mr. R. M. Vick, Dr. C. M. Fletcher, and Professor I. Aird, who in turn have looked after this patient, for permission to quote from their original notes. I am indebted to Dr. C. C. Booth and Dr. I. Chanarin for the studies on vitamin B₁₂ and folic acid, and to Dr. E. D. Williams for the histological report.

REFERENCES

- Brentano, A. (1920). *Zbl. Chir.*, **47**, 547. Quoted by F. Christopher, *Surg. Gynec. Obstet.*, 1933, **56**, 202.
 Brunschwig, A., and Childs, A. (1939). *Amer. J. Surg.*, **45**, 320.
 Chanarin, I., Anderson, B. B., and Mollin, D. L. (1958). *Brit. J. Haemat.*, **4**, 156.
 Duncan, D., Garven, J. D., and Gibbons, J. L. (1955). *Brit. med. J.*, **2**, 1586.
 Erspamer, V. (1939). *Z. Anat. Entwickl.-Gesch.*, **109**, 586.
 Hanson, A., and Serin, F. (1955). *Lancet*, **2**, 1359.
 Knauff, H. G. (1956). *Zbl. allg. Path. path. Anat.*, **94**, 545.
 McIntyre, P. A., Sachs, M. V., Krevans, J. R., and Conley, C. L. (1956). *A.M.A. Arch. intern. Med.*, **98**, 541.
 McKusick, V. A. (1956). *Bull. Johns Hopk. Hosp.*, **98**, 13.
 Mallory, T. B. (1940). *New Engl. J. Med.*, **222**, 686.
 Mrazek, R. G., jun., Godwin, M. C., and Mohardt, J. (1953). *Surg. Gynec. Obstet.*, **96**, 661.
 Snow, P. J. D., Lennard-Jones, J. E., Curzon, G., and Stacey, R. S. (1955). *Lancet*, **2**, 1004.
 Thorson, A. H. (1956). *Amer. Heart J.*, **52**, 444.
 — (1958). *Acta med. scand.*, Suppl. No. 334.
 Warner, R. R. P., and Southren, A. L. (1958). *Amer. J. Med.*, **24**, 903.
 Warren, K. W., and Coyle, E. B. (1951). *Amer. J. Surg.*, **82**, 372.
 Willis, R. A. (1940). *Med. J. Aust.*, **2**, 400.

VALUE OF PLASMA PEPSINOGEN ESTIMATION

BY

KATHLEEN EDWARDS, B.Sc.

R. P. JEPSON, F.R.C.S.

Professor of Surgery, University of Adelaide; Formerly
 Professor of Surgery, University of Sheffield

AND

K. F. WOOD, F.R.C.S.

Senior Surgical Registrar, Sheffield Royal Infirmary
 From the Department of Surgery, Sheffield Royal Infirmary

It has long been recognized that there is some relationship, but certainly not a direct one, between the rate of secretion of gastric pepsin and the excretion in the urine of uropepsinogen. It is also known that patients with peptic ulceration have as a group a higher uropepsinogen than non-ulcer patients, and that the amount excreted is reasonably constant for any one individual; nor does this amount fluctuate as does gastric pepsin under nervous, psychic, and chemical stimuli. Clinical interest in uropepsin excretion has declined because of the considerable overlap in values, derived from normals and patients with peptic ulceration, and because of the considerable practical difficulties that attend any measurement made on 24-hour collections of urine.

It is generally agreed that there is a need for some test to supplement tubal aspiration analysis or ion-exchange resins as indices of gastric function or gastric cell mass. To this end we have used the level of plasma pepsinogen in a single sample of 5 ml. of venous blood. Mirsky *et al.* (1952a) demonstrated that its mean coefficient of variation in any one individual from hour to hour in any day is 8% (range 2–22%), and that the daily fluctuation over a month was 12–18%. Hoar and Browning (1956), who repeated Mirsky's work, also found a reasonably constant plasma level with a coefficient of variation of 4%. We ourselves substantially agree with these findings, and have noted, with previous authors, that the plasma level is little affected by food, exercise, or mental stress. Accordingly we consider it permissible for clinical purposes to examine a single plasma sample which will, within fairly narrow limits, characterize an individual's plasma pepsinogen level.

Method

The method used is a modification of that described by Mirsky *et al.* (1952b). A 2.5-ml. sample of plasma from an oxalated specimen of the patient's blood is added to 10 ml. of human plasma substrate at pH 2 (1 ml. contains 20 mg. of dehydrated plasma), the pH of the resultant mixture being readjusted to pH 2 with 2 N HCl, and the volume made up to 15 ml. with distilled water and mixed thoroughly. An aliquot (6 ml.) of this mixture is incubated in a water-bath at 37° C. for 24 hours, after which time the reaction is stopped by the addition of 10 ml. of 4% trichloroacetic acid. After filtration of the protein precipitate the concentration of acid-soluble "tyrosine-like" substances is determined by Folin and Ciocalteu's method (A). In this method 2 ml. of filtrate and 3 ml. of the colour reagent are added to 20 ml. of 0.25 N NaOH, and the mixture is examined in a photoelectric absorptiometer ("Spekker") at room temperature. By the similar analysis of 6 ml. of the mixture added to 10 ml. of trichloroacetic acid the concentration of chromogenic substances present in the mixture without previous incubation is determined (B): subtraction gives the amount of chromogenic substances produced during incubation. As chromogenic substances also appear after prolonged incubation of the plasma substrate itself, two controls (C and D), each of plasma substrate (4 ml.) and water (2 ml.), are treated similarly to the plasma-digestion mixtures, A and B. From these readings the amount of chromogenic substance (largely tyrosine) liberated by the proteolytic actions of 1 ml. of plasma can be calculated, as in the following example:

	Optical Density
Plasma substrate+water, incubated	= 40 (C)
Plasma substrate+water, non-incubated	= 30 (D)
∴ colour produced by incubation of substrate	= 10
Plasma-digestion mixture incubated	= 100 (A)
Plasma-digestion mixture, non-incubated	= 45 (B)
∴ colour produced by incubation of plasma-digestion mixture	= 55
But 10 units of this is due to incubation of plasma alone	
∴ colour produced by the proteolytic action of plasma	= 45

In this paper, plasma pepsinogen is reported in terms of the amount of tyrosine liberated by 1 ml. of plasma—that is, micrograms (or units) of tyrosine released from 80 mg. of dehydrated plasma at 37° C. in 24 hours by