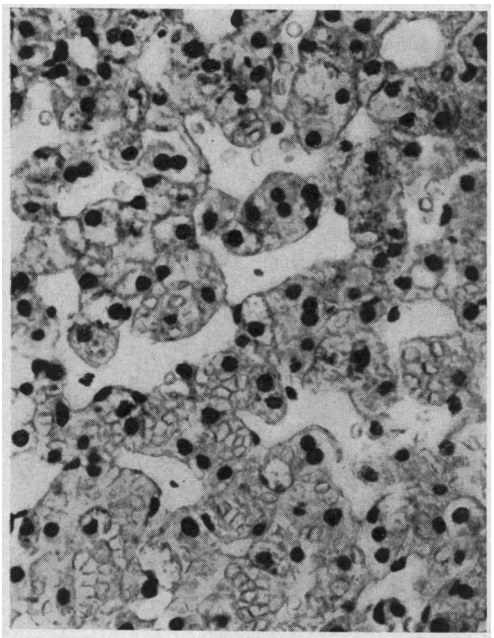

FIG. 2.—Biopsy of liver allograft in Case 1, showing degeneration of liver cells and intrahepatic haemorrhage. (×335.)

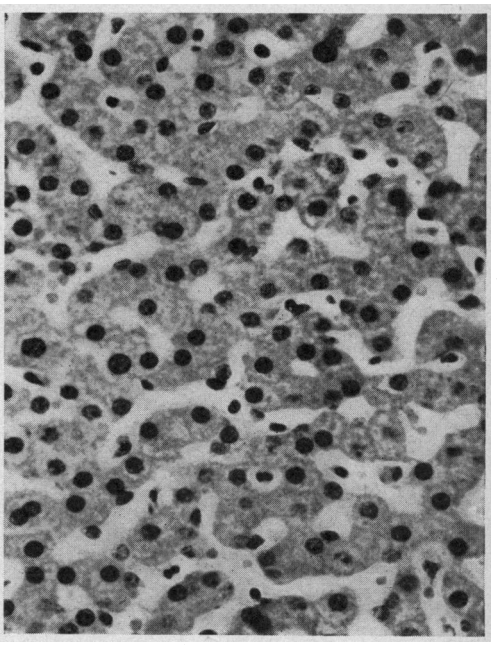

FIG. 8.—Operative biopsy of liver allograft of Case 4 at the time of transplantation, showing well-preserved liver parenchyma. (H. and E. ×335.)

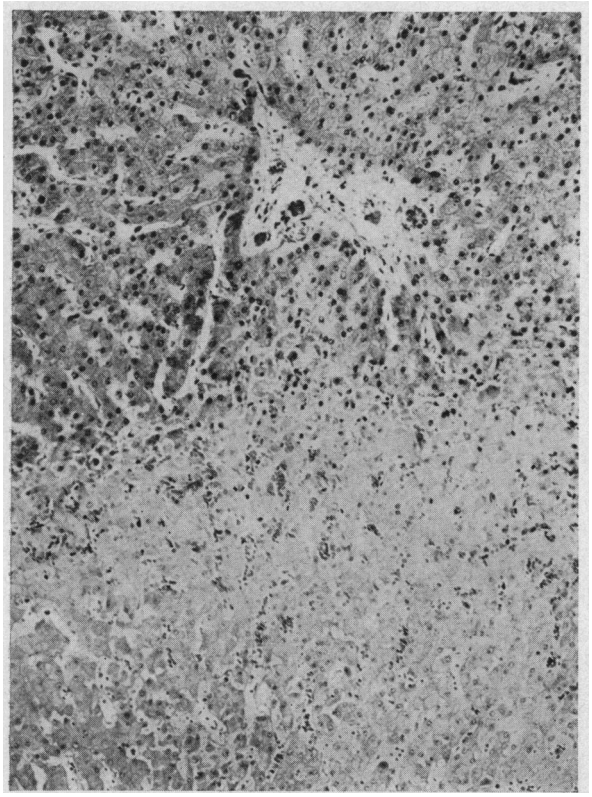

FIG. 2.—Case 3. Microscopical appearance of allografted liver at post-mortem examination, showing well-preserved liver parenchyma above with no evidence of mononuclear cell infiltration or other stigmata of rejection, while the lower area shows necrosis characteristic of infarction. No cellular infiltration in portal tract. (H. and E. ×100.)

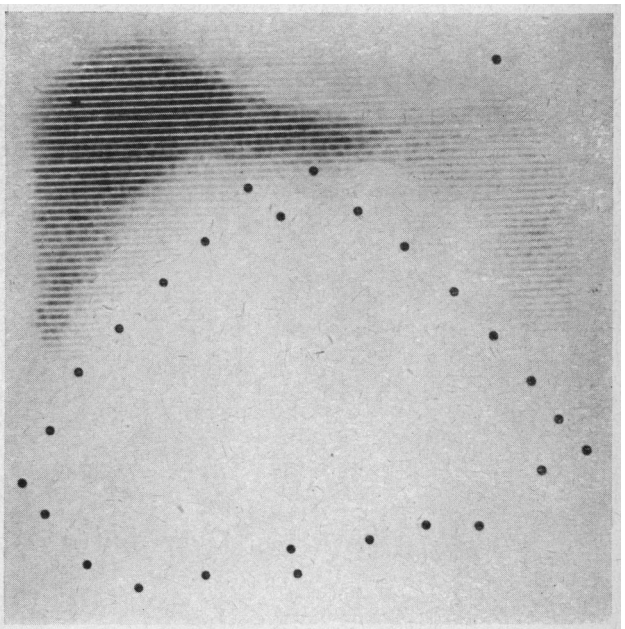

FIG. 3A

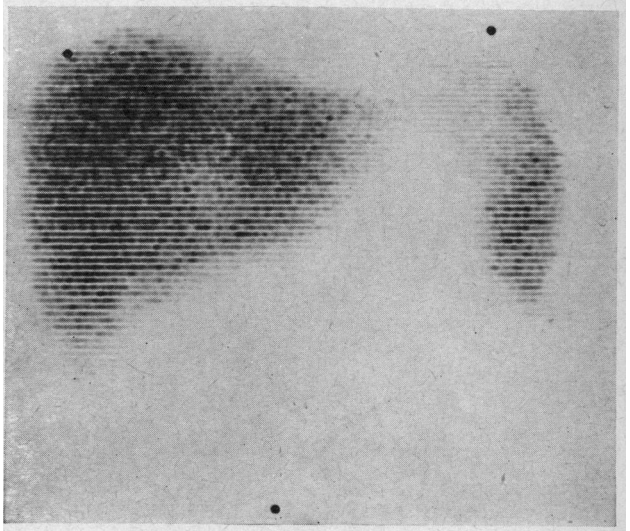

FIG. 3B

FIG. 3.—Case 4. 99m Tc sulphur colloidal scans. (A) Preoperatively, showing a small area of liver with normal activity lying above the large epigastric mass which does not take up the colloid; and (B) postoperatively, showing that the liver size, shape, and position are normal. Splenic activity is slightly increased. The dots mark the outline of the nipples, costal margins, xiphisternum, and liver edge as palpated.

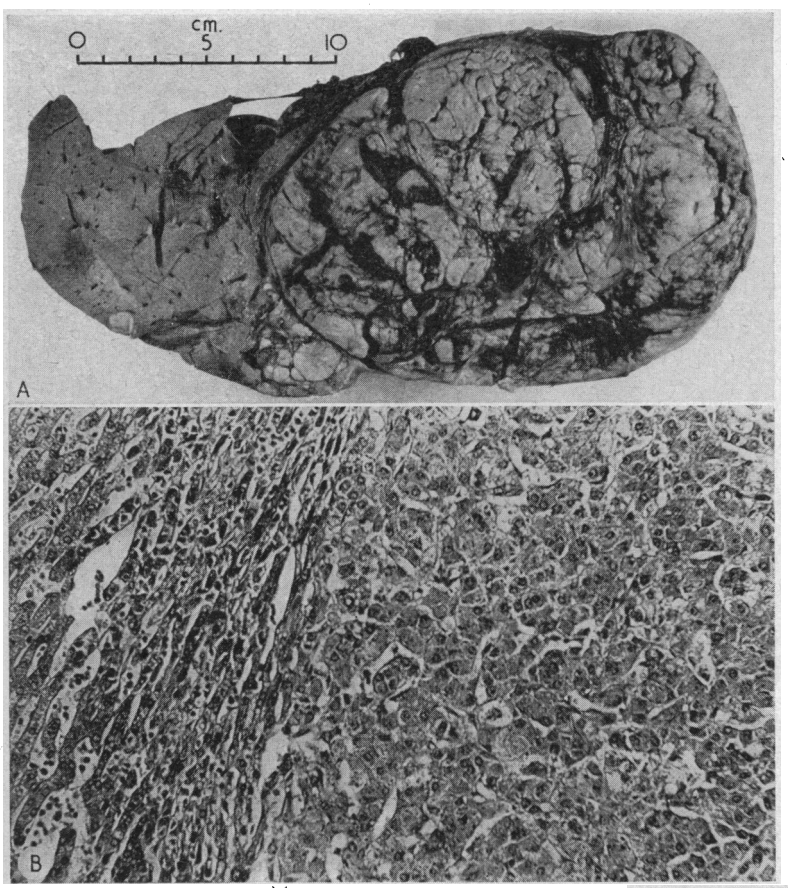

FIG. 4.—Case 4. (A) Coronal section through recipient liver to show lobulated tumour replacing left lobe and a small deposit of tumour in the right lobe which otherwise appears normal. (B) Section of recipient liver showing well-differentiated hepatocellular carcinoma on the right compressing adjacent normal liver. (H. and E. ×140.)

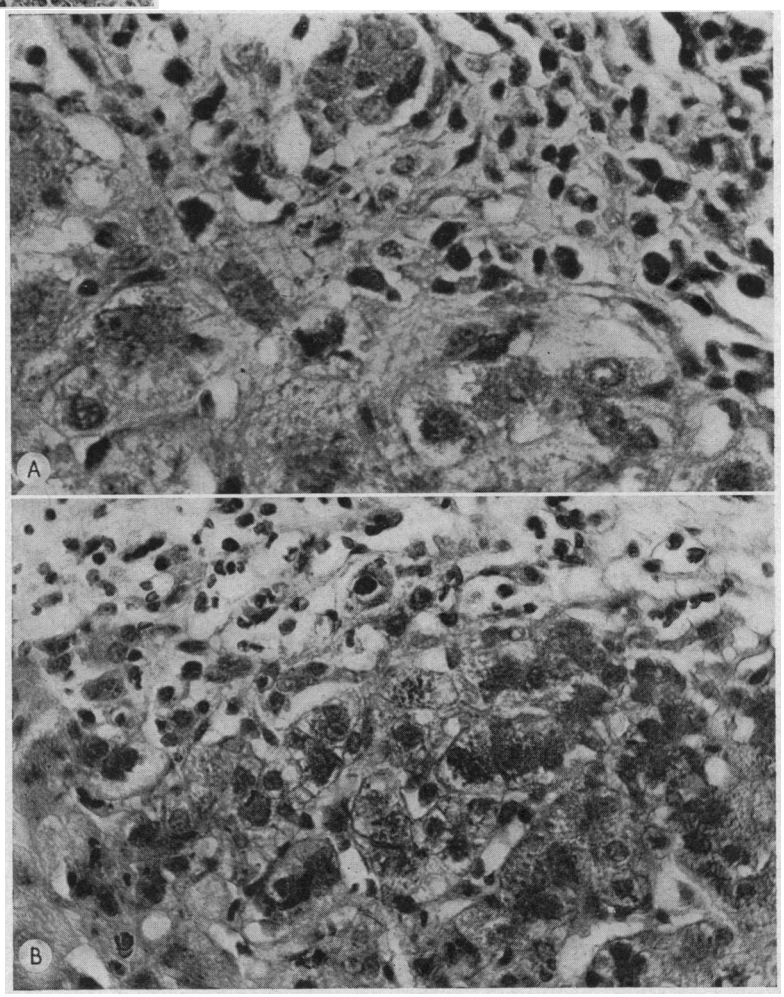

FIG. 6.—Case 4. Liver biopsy after transplantation. (A) Edge of portal tract showing infiltrate predominantly of plasma cells and eosinophil polymorphs. Note also swelling of hepatocytes. (H. and E. ×560.) (B) Focus of necrosis of hepatocytes near central vein. Note mixed inflammatory cell infiltrate; there is also a bile thrombus towards bottom centre of picture. (H. and E. ×350.)

Liver Transplantation in Man—II, a Report of Two Orthotopic Liver Transplants in Adult Recipients

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[WITH SPECIAL PLATE]

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Summary: Two patients with primary hepatic malignancy were treated by hepatectomy and orthotopic liver transplantation. In both cases the donor liver was infused with cold solutions and kept chilled without continuous perfusion. There was immediate satisfactory hepatic function in both transplants.

The first patient died after 11 weeks from overwhelming bacterial and fungal infections probably secondary to hepatic infarction due to thrombosis of the recipient hepatic artery. The thrombus occurred at the site of the arterial clamp. In an attempt to control the growth before transplantation, the patient had been treated with large doses of chlorambucil, which resulted in extreme marrow depression and septicaemia.

The second patient developed cholestatic jaundice during the second and third weeks after transplantation, with histological evidence of mild rejection, which was controlled by increasing the dose of immunosuppressive agents. He is now well, having returned to work six weeks after the operation.

Though the first patient showed no evidence of rejection, it is concluded that patients receiving liver allografts should receive immunosuppressive therapy.

Introduction

Our experience of the technical and organizational aspects of liver transplantation in five clinical cases is discussed in the previous article (Calne and Williams, 1968). We report here details of two adult patients suffering from primary malignancy of the liver who received orthotopic liver allografts. In each case the outlook was hopeless, but there was no evidence, clinically or on investigation, of extrahepatic metastatic tumour spread. The patients realized the grave prognosis, and each wished to have the chance of a liver transplant operation. They were admitted to the collaborative liver transplant programme between Addenbrooke's Hospital, Cambridge, and King's College Hospital. These patients were Cases 3 and 4 of the report referred to above.

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Case 3

This 46-year-old woman developed jaundice in August 1967 while in Australia. A laparotomy in December showed a constricting lesion of the common hepatic duct extending to 1 cm. below the porta hepatis, biopsy showing an adenocarcinoma. On return to Britain in January 1967 she was treated at Addenbrooke's Hospital with chlorambucil and prednisone but without clinical improvement. A selective coeliac axis arteriogram showed an arteriovenous shunt in the region of the porta hepatis and a definite though small tumour blush in the later films.

When readmitted to hospital in April 1968 she was deeply jaundiced, with a large hard hepatic mass extending four finger-breadths below the costal margin. The white cell count by this time had fallen to 2,300/cu. mm. and over a further few days dropped to 600/cu. mm. Her haemoglobin was 7 g./100 ml. and platelet count 72,000/cu. mm. She had florid thrush in her mouth and fauces, and her sputum also contained *Candida albicans*. Chlorambucil was stopped and ampicillin and amphotericin B were given. Her white cell count and general clinical condition improved but the liver function tests became increasingly abnormal; the serum bilirubin rose to a maximum of 42 mg./100 ml. and the serum alkaline phosphatase to 159 King–Armstrong units/100 ml.

On 2 May orthotopic liver transplantation was performed, the donor being a 5-year-old child who had died of mumps encephalitis. Details of the operation are given elsewhere (Calne and Williams, 1968). The liver (weight 2,283 g.) was full of large deposits of carcinoma, but there was no evidence of growth outside the liver. Histological examination showed a mucin-secreting tumour lining and including the intrahepatic bile ducts with conspicuous cellular pleomorphism and connective tissue formation. The tumour was invading lymphatics and intrahepatic branches of the portal and hepatic veins. The overall appearances were those of a primary hepatic cholangiocarcinoma.

Subsequent Course

There were no immediate postoperative problems. The following day her haemoglobin was 14.7 g./100 ml. and W.B.C. 4,000/cu. mm. Clotting factors were virtually normal. The serum potassium was 3.6 mEq/l. and blood urea 60 mg./100 ml. Antilymphocytic globulin was given intramuscularly and hydrocortisone intravenously (Fig. 1).

There was a dramatic improvement in the liver function. On the first postoperative day the serum bilirubin was 24 mg./100 ml. and on the fourth day 6 mg./100 ml. Serum alkaline phosphatase had dropped to 24 K.A.u./100 ml. on the first postoperative day. The serum alanine aminotransferase (S.G.P.T.), which was 269 u./100 ml. on the first postoperative day, had fallen to 97 u. by the fifth day. A galactose clearance on the sixth day showed a slightly prolonged half-life of 22 minutes (normal up to 17 minutes).

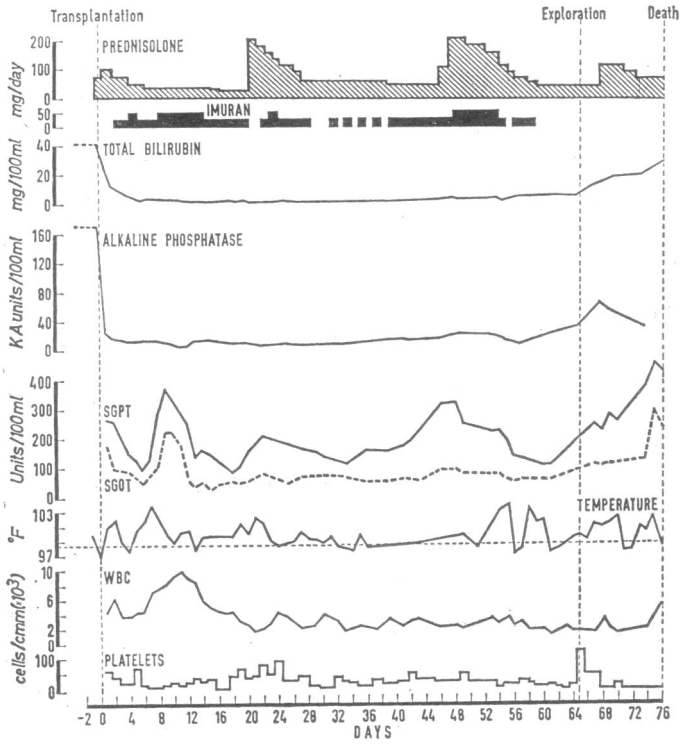

FIG. 1.—Case 3. The course after transplantation together with preoperative liver function tests and the final illness following a second operation for removal of a necrosed gall bladder.

There was a fall in the platelet count during the first few days, and on account of the known thrombocytopenic effect of antilymphocytic globulin it was discontinued. White cell counts remained at between 4,000 and 9,000 in the first postoperative week, and haemoglobin ranged from 9 to 13 g./100 ml. Occasional blood transfusions were given in order to maintain this level.

Many blood cultures were taken postoperatively whenever she developed a fever, but these all remained sterile. Her sputum, however, grew many coliform organisms, and staphylococci and coliforms were isolated from sutures removed from her abdominal wound on the ninth postoperative day. She had recurrence of moniliasis in the throat, and during the second week developed signs of a bronchopneumonia at the right base. Continued postoperative fevers were treated at various times with antibiotics, which included carbenicillin, kanamycin, trimethoprim, streptomycin, colistin sulphate, and cloxacillin as well as nystatin. Immunosuppression was continued with prednisolone and azathioprine (0.5–1 mg./kg./day). The donor had died of mumps encephalitis, so the patient was given three doses of gammaglobulin from patients convalescing from mumps.

By the tenth postoperative day the patient was clinically well, and pruritus had almost completely disappeared, though fever persisted.

She was discharged home on 7 June, and attended hospital for daily antibiotic injections. She remained well and the plasma pseudocholinesterase had risen to 41 Warburg units/ml. (normal 65–120) on 10 June. Subsequently there was a rise in the serum bilirubin and transaminase and she was readmitted to hospital on 25 June (Fig. 1). From the time that the wound stitches had been removed 10 days after liver transplantation she had had a biliary fistula which had continued draining throughout her outpatient period. A sinogram showed a cavity communicating with the common bile duct through into the duodenum.

On 6 July exploratory laparotomy was performed which revealed a necrotic gall bladder that had separated from the common bile duct of the recipient. Both these structures lay in a bile-containing cavity. The necrotic gall bladder was removed, the second part of the duodenum opened, and a T-tube inserted, the long limb entering the stomach emerging through a stab incision through the anterior abdominal wall. The short limbs of the T-tube were threaded superiorly into the common bile duct and inferiorly into the duodenum. The duodenum was then sutured to the liver capsule.

Postoperatively the patient's condition gradually deteriorated, and on 10 July her temperature rose to 105° F. (40.6° C.). Positive blood cultures were obtained growing *Klebsiella*. She was treated with streptomycin, Rifamide, and colistin sulphate to which the organism was sensitive. However, jaundice increased and on 17 July she became oliguric, finally lapsing into coma and dying on 19 July.

Necropsy Findings

The donated liver (23 by 14 by 10 cm.) was in a normal position and surrounded by dense adhesions. In the right lobe there were four greenish-yellow zones of necrosis (3–5 cm. in diameter) with irregular haemorrhagic margins and a few smaller necrotic zones. Some of the intrahepatic bile ducts were distended and contained extremely viscid green bile.

The portal vein anastomosis and the inferior vena caval-hepatic vein anastomosis were intact and patent. The anastomosis between the patient's hepatic artery and the donor's coeliac artery was also intact, but a firmly adherent thrombus (1.5 cm. long) either completely or almost completely occluded the patient's hepatic artery just proximal to the anastomosis. The distal end of the thrombus extended up to but not beyond the line of the anastomosis. This thrombus appeared to have developed at the site of clamping rather than at the anastomosis.

The orifice in the duodenal wall through which the T-tube had been inserted at the second operation communicated with an irregular cavity (about 10 by 12 cm.) containing bile-stained fluid lying below the right lobe of the liver and extending medially in front of the portal vein and hepatic artery and their respective anastomoses. The wall of the donated hepatic artery which formed part of the boundary of the cavity was friable and partly necrotic.

Microscopy.—Most of the liver substance was replaced by large and often sharply delineated zones of necrotic liver cells in which there were occasional surviving portal tracts and only a slight infiltrate of neutrophil polymorphs and lymphocytes. Some of these zones were more recent than others, with abundant nuclear debris, Gram-negative rods, and fungal hyphae with the characteristic morphological appearances of a *Candida* species. Smaller zones of necrosis were interspersed in some of the surviving liver lobules.

The surviving liver cells appeared morphologically normal, with no increase in mitotic rate and no increase in pleomorphism, and most contained some glycogen. Kupffer cells were not conspicuous; many contained haemosiderin. A few liver cells and Kupffer cells were enlarged, with large nuclei, and in one enlarged cell there was a definite mauve intranuclear inclusion body, separated from the nuclear membrane by a clear halo, characteristic of cytomegalovirus. A small number of portal tracts had slight to moderate increase in fibrous tissue with apparent dilatation of some of the branches of the portal vein. A single small artery was almost occluded by recent thrombus, but other arteries were patent and appeared normal. There was only a very slight infiltrate of lymphocytes and neutrophil polymorphs in some portal tracts, which were often adjacent to recent foci of liver cell necrosis (Special Plate, Fig. 2). The central veins appeared normal.

Bile Channels.—In the right lobe a few were dilated and contained Gram-negative rods, fungal hyphae, necrotic debris, and occasional neutrophil polymorphs and lymphocytes. Large sections of the walls of some of these channels were necrotic without recognizable epithelium. In necrotic and normal lobules there were apparently normal bile channels, though a few of these were surrounded by whorls of fibrous tissue. A few smaller bile channels and bile canaliculi contained bile plugs. The main bile duct was largely necrotic with its walls infiltrated by hyphae and bacteria, which were also present in the surrounding connective tissue that contained considerable deposits of bile.

Hepatic Artery.—In the recipient portion of the artery, adjacent to the anastomotic site, organizing thrombus filled the lumen, and the artery wall was necrotic with extensive haemorrhage. A large partly organized haematoma separated the media from the external elastic lamina. The internal elastic lamina was intact; there was no intimal thickening and no hyphae or bacteria were recognized.

In all sections of the donor portion of the artery adjacent to the anastomosis the wall was necrotic, though the lumen was patent and the internal elastic lamina was intact without intimal thickening. In some sections the surrounding connective tissue was necrotic and contained hyphae and Gram-negative rods. In the connective tissue and in two lymph nodes surrounding the recipient portion of the artery were islands of tumour cells, similar to the cholangiocarcinoma in the patient's removed liver.

Other Organs.—There was profound atrophy of the white pulp of the spleen with large areas of necrosis, and the lymph nodes showed virtually total loss of germinal centres with only a few small lymphocytes and some nuclear debris remaining. The vertebral marrow was cellular, with no significant abnormality. In both ventricles of the heart there were occasional small abscesses containing fungal hyphae; these were also present in the kidney and in the basal ganglia of the brain.

Cultures.—Adenovirus, type 2, was grown for liver, lung, and colon contents. There was a heavy growth of *Candida albicans* from liver, lung, kidney, and brain; bacterial cultures of the same organs showed coliform bacilli and *Pseudomonas pyocyanea.*

Case 4

This 41-year-old man was admitted to Carshalton Hospital on 17 June 1968 with cramp-like abdominal pain in the right hypochondrium of 12 hours' duration. He had lost 1½ stone (9.5 kg.) in weight during the preceding two months. Apart from a partial gastrectomy for a duodenal ulcer in 1962 his previous health had been excellent. However, it was apparent that he had had some abdominal swelling for at least four years, and a photograph taken on holiday in the summer of 1967 showed considerable upper abdominal enlargement.

Laparotomy revealed a large vascular tumour arising from the lower surface of the liver with multiple small deposits scattered throughout both lobes. There was no underlying cirrhosis and no evidence of spread beyond the liver. Subsequently he was referred to Addenbrooke's Hospital, and the investigations done there and at King's College Hospital before the transplantation are given below.

Investigations

Haemoglobin 11.3 g./100 ml. White cell count 6,900/cu. mm. (neutrophils 66%, lymphocytes 33%, eosinophils 1%). Platelets 80,000/cu. mm. Liver function tests: serum total bilirubin 0.5 mg./100 ml., alkaline phosphatase 15 K.A.u./100 ml., serum aspartate aminotransferase (S.G.O.T.) 50 u./100 ml., serum albumin 3.7 g./100 ml., globulin 3.4 g./100 ml., electrophoresis showed a slight increase in the α_2 globulin.

Chest *x*-ray picture was normal, except for elevation of the right lobe of the diaphragm and an atelectasis at the right base. Cholecystogram: the gall bladder concentrated well and both the cystic and the common bile ducts appeared normal.

Liver Scan.—This showed a small area of liver with normal activity lying above the large epigastric mass which did not take up the colloid (Special Plate, Fig. 3 A). There was also a small filling defect in the downward extension of the functioning liver. The appearances were consistent with either an extrahepatic mass or a primary hepatoma arising from the lower border of the liver. Splenic activity was slightly increased but not sufficiently to support a diagnosis of portal vein block.

Coeliac Arteriogram.—A huge hepatic tumour was shown extending from the under-surface of the right lobe of the liver and continuing across the midline anteriorly. The hepatic artery and coeliac axis were displaced upwards and to the left, and other isolated areas of metastatic deposition were present within the liver. A diffuse pathological circulation was demonstrated around the periphery of the tumour, and the appearances suggested considerable central necrosis within the main mass.

Splenoportogram.—This revealed a diffuse anastomotic circulation around the spleen extending to the umbilical vein and involving a lateral lumbar and subphrenic plexus of veins. It appeared that there was complete obstruction of the portal vein in its most proximal part.

Operation and Histology of Resected Liver

Orthotopic transplantation of the liver was performed at King's College Hospital on 23 September, the donor being a 13-year-old boy who had died from head injuries. Full details of the operation have been given by Calne and Williams (1968).

The resected liver weighed 4,770 g. The left lobe had a soft yellow nodular surface, and numerous small yellow nodules measuring up to 0.5 cm. in diameter were present over the external surface of the right lobe. The cut surface of the left lobe showed it to be completely replaced by a yellow, partially necrotic, lobulated tumour measuring 18 by 15 by 10 cm., with areas of haemorrhage and firm grey fibrous tissue septa between the lobules (Special Plate, Fig. 4 A). Numerous nodules of similar tumour tissue were seen in the right lobe. Sections showed the tumour to be a well-differentiated hepatocellular carcinoma with a trabecular pattern which simulated normal liver tissue to a marked degree (Special Plate, Fig. 4 B). The tumour contained foci of haemorrhage, necrosis, and early organization. No vascular invasion was seen and the portal vein and lymph nodes from the porta hepatis were likewise free of tumour. The portal vein was fully patent and free of growth, despite the preoperative splenoportogram appearances.

A non-specific reactive hepatitis was present throughout the non-neoplastic liver parenchyma with focal necroses, parenchymal inflammation, and increased numbers of chronic inflammatory cells around portal tracts, but there was no evidence of cirrhosis.

Subsequent Course

After control of bleeding from the stab wound drain his clinical condition steadily improved and the blood pressure and pulse rate were maintained at normal levels. Urine output was satisfactory and the blood urea remained normal apart from a slight rise to 48 mg./100 ml. on the fifth day. The serum potassium dropped to 2.8 mEq/l. and the serum sodium to 128 mEq/l. on the third day, but both were easily corrected by intravenous potassium and sodium chloride.

Bile was draining from the T-tube at the end of the operation, and between 150 and 200 ml. drained daily, until the T-tube was clamped for increasing periods of time, starting on the tenth day. A T-tube cholangiogram on that day had shown only a small leak at the site of anastomosis. No leakage of bile occurred around the T-tube when it was completely clamped, but it is proposed to leave it as an internal splint for six months.

He was ambulant on the fourth postoperative day and was discharged home on the seventeenth day. He then gradually increased his activities and attended hospital by car daily for a blood count and infusion of antilymphocyte globulin. When this was discontinued in the sixth week he returned to work and is now working full-time and feeling very well.

Immunosuppression

At the end of the operation he was given 100 mg. of azathioprine (Imuran) and prednisone 100 mg. intravenously with subsequent daily doses as shown in Fig. 5. Antilymphocyte globulin (horse anti-human thoracic duct lymphocytes prepared by Professor W. Brendel) was started on the first postoperative day, this being given as an intravenous infusion in normal saline slowly over two hours. The subsequent daily dose for those three drugs is given in Fig. 5. During the first two weeks he had some shivering and rise in temperature to 101–102° F. (38.3–38.9° C.) following each antilymphocyte globulin infusion, but after that there were no reactions and the urine remained free of protein. Azathioprine was stopped on the sixteenth day to see whether it was contributing to the jaundice but was restarted again during the fourth week when the liver biopsy suggested rejection. Prednisone was increased to 200 mg. daily at this time, then reduced by 25 mg. every three days until a dose of 50 mg. daily was reached.

At the time of the maximal steroid dose he developed polyuria and heavy glycosuria, which required insulin for control. Some years previously he had attended the diabetic clinic for symptomless and transient glycosuria.

Changes in Liver Function and Biopsy

The serum total bilirubin and aspartate aminotransferase levels were raised on the second postoperative day and then fell to normal (Fig. 5). On the third postoperative day liver function tests, apart

from minimal rise of serum bilirubin at 1.8 mg./100 ml., were normal. Subsequently the serum bilirubin rose, initially sharply and then more slowly, on the 21st day reaching 13.4 mg./100 ml., which was the highest level attained.

After some earlier fluctuations the serum transaminase rose sharply from the 16th day, reaching a maximum level of 233 u./100 ml. on the 22nd day. The serum alkaline phosphatase remained normal until the ninth day. Subsequently there was a slow rise which paralleled the increase in serum bilirubin level, reaching 35 K.A.u./100 ml. on the 24th day.

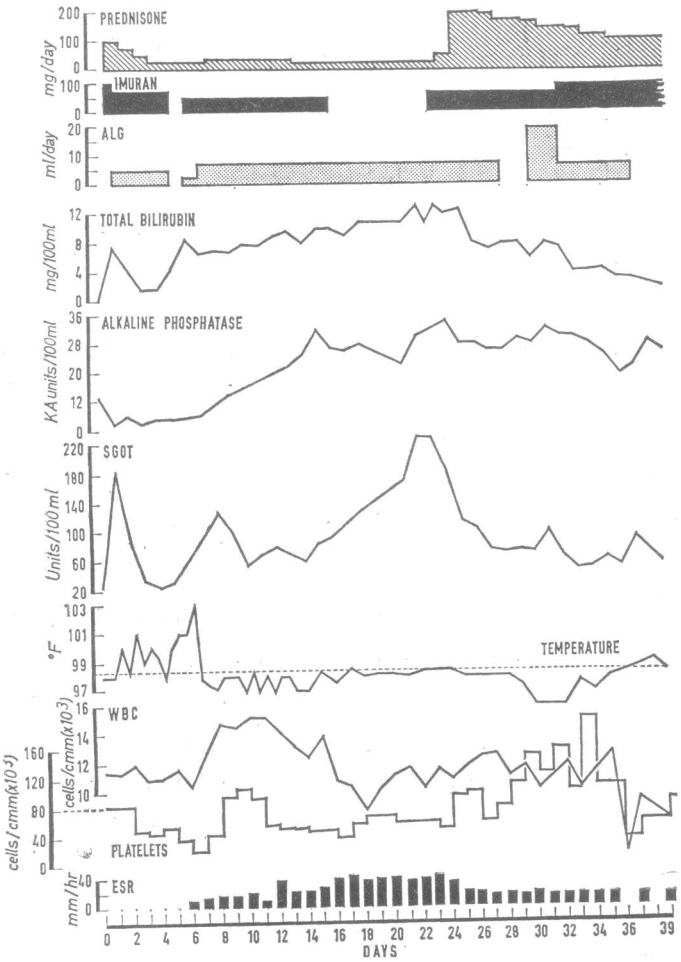

FIG. 5.—Case 4. Course during the first 40 days after hepatic transplantation.

With treatment of the probable rejection episode by the resumption of azathioprine, increased dose of prednisone, and antilymphocyte globulin, there was a sharp fall in serum bilirubin and transaminase and a slower fall in serum alkaline phosphatase. When antilymphocyte globulin was discontinued the serum bilirubin was 2.2 mg./100 ml., transaminase 59 u./100 ml., and alkaline phosphatase 28 K.A.u./100 ml.

Serum albumin was 4.12 g./100 ml. before operation and 3.5 g./100 ml. on the 30th day, with corresponding gammaglobulin levels of 1.23 and 1.90 g./100 ml.

Liver Scan (Special Plate, Fig. 3 B).—This was repeated on the 16th day. Liver shape, size, and position were normal. The absence of definite filling defects implied that there were no marked regional variations in blood flow, but the peak count rate over the liver was low normal, and, though splenic size was the same as on the preoperative scan, splenic activity had increased, suggesting an increase in the spleen/liver blood flow ratio.

Liver Biopsy (Special Plate, Fig. 6).—This was performed on the 24th day. Sections showed foci of reticulin collapse predominantly around central veins. There was marked centrilobular cholestasis and foci of necrosis of hepatocytes, especially around central veins. Kupffer cells were prominent, and many of them contained bile. Portal tracts were not oedematous but contained moderate numbers of neutrophil polymorphs, lymphocytes, and plasma cells; similar cells were associated with necrotic hepatocytes in the liver parenchyma. The overall histological appearances were thought to be those of mild rejection, an opinion with which Professor K. A. Porter concurred.

Haematological Changes

The haemoglobin level following the second operation and haemostatic control was 13 g./100 ml. Subsequently the level fluctuated between 10 and 12 g./100 ml., the lowest levels being obtained during the second and third weeks.

The total white cell count of about 11,000/cu. mm. during the immediate postoperative period showed a sharp rise to 14,000/cu. mm. during the second week (Fig. 5). The count fell during the third week and remained thereafter at 5,000–6,000/cu. mm. Neutrophils constituted 70–80% of the total count. The total lymphocyte count was always below 1,000/cu. mm. and usually below 500 except on a day when antilymphocyte globulin was not given. The eosinophil count rose to 1,000/cu. mm. on the fifth and sixth days, at a time when the patient was experiencing rigors and an allergic maculopapular rash following each antilymphocyte globulin injection.

The platelet count, which was low before operation (80,000/cu. mm.), remained so during the immediate postoperative period. A slight rise was then followed by a sustained fall coincident with the period of rejection. A subsequent rise to 100,000–150,000/cu. mm. paralleled the improvement in liver function, and has been maintained.

The E.S.R. rose from a maximum of 15 mm./hour during the first week to 43 mm./hour during the period of rejection.

Blood Coagulation Studies

Standard methods were used for the one-stage and two-stage prothrombin time, partial thromboplastin time with kaolin, one-stage assay of factor VIII, and gravimetric measurement of plasma fibrinogen (Hardisty and Ingram, 1965). Plasminogen was estimated by the method of Alkjaersig *et al.* (1959) and fibrinogen and fibrin degradation products in serum by an immunological method (Merskey *et al.*, 1966); direct estimates of plasminogen activator were made by testing plasma euglobulin fractions on bovine fibrin plates (Flute, 1964). Preparation of the ^{125}I-labelled fibrinogen has been described by Flanc *et al.* (1968).

The changes fall into two phases (Fig. 7). The rapid bleeding of the first 12 hours was accompanied by slight prolongation of the prothrombin and partial thromboplastin times, a factor VIII level only 49% of normal, and a fall in plasma fibrinogen. These represent the accelerated loss of clotting factors produced by the acute bleeding, and occurred even though fibrinogen concentrate, fresh frozen plasma, and fresh as well as stored blood were being transfused. The changes were probably no greater than might be expected from bleeding of this magnitude due to any cause.

By the third day the patient had passed into a hypercoagulable phase. The expected postoperative rise in fibrinogen did not occur; instead the concentration fell to subnormal values. Other evidence—namely, the short partial thromboplastin time, high factor VIII, and thrombocytopenia—suggested that intravascular coagulation was accelerated, and this was confirmed by finding an increased turnover of radioactive fibrinogen, with a half-life of 20 hours in place of the normal around 80 hours. The low plasminogen and raised fibrin degradation products in the serum indicated increased fibrinolysis, another sign of accelerated intravascular coagulation. Direct estimates of circulating plasminogen activator were always within the normal range.

Decreased hepatic synthesis of fibrinogen could also have accounted for the hypofibrinogenaemia. However, this is a late sign of liver failure usually preceded by a decreased synthesis of prothrombin. Though prothrombin was reduced to 29% of normal on the tenth day, it then rose progressively, and the return of fibrinogen values to normal, despite the increased turnover, further supported the view that hepatic synthesis of these and other clotting factors was adequate.

Tissue Typing

This was performed by means of a microcytotoxic test (Joysey, in preparation). Sera available were anti-LA1, anti-LA2, and sera of specificities similar to 4a, 4b, 4c, and 7c.

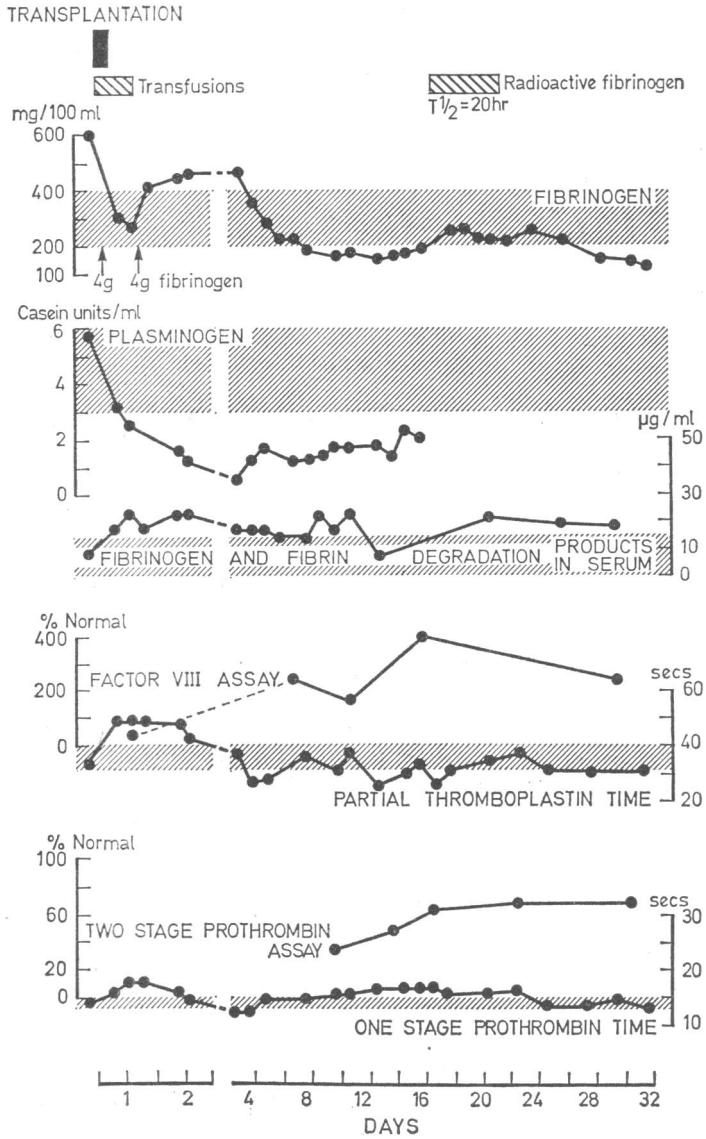

FIG. 7.—Case 4. Results of blood coagulation studies.

Case 3.—The recipient's blood group was A and that of the donor O. Major incompatibilities were LA1 and 4C; minor incompatibility was LA2.

Case 4.—Both recipient and donor were blood group O. Major incompatibilities were LA1, 4C, and 7C.

Discussion

In these two patients excellent initial function of the allografted liver was demonstrated in spite of total ischaemia periods of 141 and 106 minutes. Warm ischaemia times were, however, short, and the preservation techniques with the simple perfusion methods described elsewhere (Calne and Williams, 1968) were therefore quite adequate. Both the excretory and the synthetic functions of the transplanted liver were satisfactory. Indeed, the decrease in jaundice in Case 3 during the first few days after operation was dramatic, as was the rapid fall in serum alkaline phosphatase. The almost normal plasma pseudocholinesterase in this patient by the third week, and the maintenance of normal serum albumin level in Case 4, together with the normal clotting factors in both cases, all testify to the satisfactory capacity of the transplanted liver for synthesizing proteins.

The early jaundice in Case 4 on the second and third postoperative days could have been due to a number of factors, including the effects of prolonged surgery, anaesthesia, and massive blood transfusion. The jaundice had largely cleared by the fourth day. The subsequent increase in serum bilirubin level from the sixth day onwards may have resulted from a rejection process which was partially but not completely controlled by the immunosuppressive therapy. Certainly, by the 21st–24th day, when the serum bilirubin and transaminase levels were at their highest, there was evidence in the liver biopsy of a rejection process. It is interesting to speculate whether the stopping of azathioprine was responsible for the increase in the severity of this process. It was stopped because azathioprine is hepatotoxic, and when patients with active chronic hepatitis or cirrhosis are treated with conventional doses of 100–150 mg. daily there is a high incidence of toxic reactions, including jaundice, coma, leucopenia, and thrombocytopenia (Mistilis and Blackburn, 1967). Patients with cirrhosis, however, may tolerate 50–75 mg. daily with good effect. The donor liver probably suffers some damage during the removal, even with short periods of warm ischaemia as in the present cases, so that it may be more susceptible to toxic damage from azathioprine. Little is known also about the possible toxic effects of high doses of intravenous antilymphocyte globulin on the liver under these circumstances.

The jaundice at the time of rejection in Case 4 had many features of an intrahepatic cholestasis with a conjugated hyperbilirubinaemia, raised serum alkaline phosphatase, and only a moderately raised serum transaminase, though at no time did he have skin-itching. This picture of a cholestatic jaundice has also been described in the dog during rejection of the liver (Moore, 1967), though one might have expected a more diffuse hepatitic type of reaction.

There is a marked species difference with regard to rejection of the liver. The dog rejects a transplanted liver as quickly as a kidney, whereas rejection of the liver by the pig is a very slow and mild process and protects against the rejection of kidney or skin grafts done simultaneously (Calne *et al.*, 1967). It is too soon to say whether man will consistently resemble the dog or pig in this respect, for in Case 3 there was no histological evidence of rejection either at necropsy or in a liver biopsy taken during the course of the second operation. However, Starzl *et al.* (1968a, 1968b) observed the histological appearances of rejection, with lymphocytic and mononuclear cell infiltration, some with pyroninophilic cytoplasm, in liver tissue which had been removed surgically from patients developing hepatic lobar gangrene. In another paper (Fulginiti *et al.*, 1968) there is an account of a clinical episode of rejection developing on the sixth day, which was controlled by immunosuppressive therapy.

At present, therefore, it would appear that patients undergoing liver transplantation should be given immunosuppressive therapy. The schemes of immunosuppression used in the present two patients were based on experience gained from renal transplantation, in which prednisone and azathioprine are of proved value. Early and intensive therapy with antilymphocyte globulin may enable the dose of prednisone and azathioprine to be reduced during the early postoperative phase, when these patients are most susceptible to infection. Rejection may be less of a problem when the donor and recipient can be matched by tissue typing, but it is impossible to comment further at this early stage in the development of hepatic transplantation. In the present patients typing was done retrospectively and the results showed a relatively poor match in both cases.

The cause of the hypercoagulable phase in Case 4, which developed on the third day and has continued, is uncertain. It may be related to the formation of fibrin clots during the rejection phase, though on histological examination the small hepatic arterial vessels in the portal tracts were patent. It is of interest that during the period of rejection there was a significant decrease in platelet count. Mowbray (1968) has shown that during rejection of the kidney in man platelet thrombi form in the arterioles and capillaries, and that immediately preceding an episode of clinical rejection there is a fall in the blood platelet count. Starzl *et al.* (1968a, 1968b) observed marked thrombocytopenia in three cases with a prolonged survival, which developed during the second and third weeks, with a rise to normal or near-normal levels during the ensuing one to three months. The cause of the leucocytosis between days 7 and 14 in our patients is uncertain. This has been observed at a similar time in patients with renal transplants.

The ultimate cause of death in Case 3 was overwhelming bacterial and fungal infection. The extensive hepatic necrosis found at necropsy was almost certainly ischaemic in origin, resulting from the thrombosis of the hepatic artery. The thrombus, which involved only the recipient part of the hepatic artery, was probably due to injury to the endothelium from the application of a clamp during the transplantation operation. This was probably at the root of many of the complications which later developed, including gangrene of the donated gall bladder, the biliary fistula, and hepatic ischaemia. This, together with the diminished resistance with the immunosuppression, was the basis for the recurrent and finally massive infection.

Infections have also been a major problem in the cases done by Starzl and his group (Fulginiti *et al.*, 1968). Of the eight patients treated before July 1967 all died with a variety of infections within 34 days. Gram-negative bacilli, *Candida albicans*, and cytomegalovirus were all implicated. Of nine cases operated on between then and May 1968, five were also troubled by infections. In each case these were superimposed on a syndrome of hepatic lobar gangrene and hepatic arterial thrombosis, which was attributed to inadequate fixation of the liver to the diaphragm. In the four most recent patients, who had survived for periods of up to five and a half months at the time of the report and in whom the liver had been fixed to the diaphragm, there had been only minor episodes of sepsis.

Patients having hepatic transplantation must be carefully screened for infection, and daily skin and throat swabs and cultures of urine, faeces, and blood are essential. Cytomegalovirus is particularly important, because it may cause a hepatitis with cholestatic features (Toghill *et al.*, 1967). Serum should be examined for a rise in complement-fixing antibodies, and examination of the urine should be done at regular intervals. The virus may not be excreted until four to six weeks after the initial infection of the liver. Whether a patient should have prophylactic antibiotics before operation and whether the gastrointestinal tract should also be sterilized are debatable. It does not prevent superimposed infections in other circumstances and may indeed contribute to the emergence of resistant strains. Prompt treatment of the specific infection by the appropriate antibiotic is to be preferred.

The prolonged survival of the two patients reported here—in particular, the return to work of Case 4 and his excellent general health and liver function at present—provide us with considerable hope that orthotopic transplantation of the liver will have much to offer in the treatment of patients with primary hepatic malignancy or advanced cirrhosis.

We have already expressed our indebtedness to the many colleagues and the departments concerned in these transplantations.

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Absorption and Utilization of Polyglutamyl Forms of Folate in Man

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Summary : Some three-quarters of folate compounds in a normal mixed diet have a chain of seven glutamic acid residues (polyglutamates). The extent to which these forms of folate are absorbed and utilized by man is of considerable nutritional importance. These studies indicate that the polyglutamate forms were absorbed and utilized to about one-third of the extent of simpler (monoglutamate) forms, as judged by the serum folate levels after oral equimolar doses, by the amount incorporated into red cells in long-term studies, by the amount excreted into the urine, and by their capacity to initiate haematological responses in patients with folate-deficient megaloblastic anaemias.

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Introduction

Folate compounds may be divided into two groups, the one having a single glutamic acid residue such as pteroylglutamic acid (PteGlu) and the other a chain of seven glutamic acid residues—that is, pteroylheptaglutamate ($PteGlu_7$) (Binkley *et al.*, 1944 ; Pfiffner, Calkins, Bloom, and O'Dell, 1946). The simpler forms, with the addition of a form having three glutamic acid residues (pteroyltriglutamate), are able to support the growth of microbiological assay organisms such as *Lactobacillus casei*, and these forms have been collectively termed "free" folates. These forms are readily absorbed from the gut (Anderson, Belcher, Chanarin, and Mollin, 1960), and they constitute some 25% of the folates in a normal cooked diet (Butterworth, Santini, and Frommeyer, 1963 ; Chanarin, Rothman, Perry, and Stratfull, 1968). The remaining 75% of dietary folate is not detected by microbiological assay until the