group (fig 3, left), largely because penicillin prevented leptospiruria (fig 3, right); after the start of treatment (or placebo) leptospires were isolated from 58% of placebo group patients but from only 13% of those in the treatment group ($p < 0.01$).

Discussion

This study clearly shows that intravenous penicillin benefits patients with severe leptospirosis, even when given late in the course of illness. 76% of the study patients had either renal or hepatic dysfunction, usually both, and only 1 patient had had symptoms for less than 5 days before treatment, yet every measurable aspect of disease improved with penicillin. Some animal studies have shown that penicillin exerts a beneficial effect only when given soon after infection, before jaundice appears,[9-12] but others have given different results. There were fewer deaths, and the course of the disease was less severe, among penicillin-treated jaundiced puppies than among untreated controls.[13] Leptospiraemia and leptospiruria were eradicated in the treated dogs. Hamsters severely ill with leptospirosis survived when given penicillin G[14] or the newer penicillins[2] 1 to 2 days before expected time of death. The many previous evaluations of penicillin in the treatment of human leptospirosis have also given conflicting results, but only four of these studies included concurrent controls; two showed no beneficial effect,[15,16] another slight benefit,[17] and the fourth considerable benefit.[18] However, these studies were neither double-blind nor adequately randomised, and penicillin was given in lower doses than that used by us and it was not given intravenously.

The beneficial effects of penicillin on affected kidneys have particular clinical relevance. Creatinine rises lasted only a third as long in the treatment group (fig 2) and leptospiruria diminished with penicillin (fig 3). These findings suggest that it is by eradicating leptospires that penicillin curtails the duration of renal dysfunction and thus shortens the period of susceptibility to complete renal failure and death.

Oral doxycycline has been shown to be beneficial[3] but its value in severe disease or when given after day 2 has not been established.[2] In addition, antibiotics must be given parenterally to patients with severe leptospirosis, many of whom are vomiting.[4,19] Parenteral doxycycline is expensive and also not available in many countries, including the Philippines.[20] For these reasons it was not chosen for our study. The beneficial effects of doxycycline in mild cases, although statistically significant, were small. Symptoms and fever were reduced by only 2 days.[3] Our randomised, placebo-controlled, well-controlled trial has shown that intravenous pencillin produced striking results in precisely those individuals in whom optimum therapy is most important—patients with serious, potentially fatal disease—even when therapy started late in the course of disease.

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SUCCESSFUL TREATMENT OF HOMOZYGOUS PROTEIN C DEFICIENCY BY HEPATIC TRANSPLANTATION

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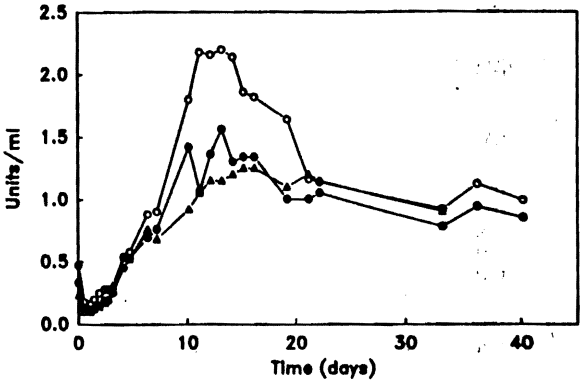
Summary A child with homozygous protein C deficiency was treated at age 20 months by orthotopic hepatic transplantation. Postoperatively there was complete reconstitution of protein C activity and resolution of the thrombotic condition.

Introduction

PROTEIN C is a vitamin-K-depéndent anticoagulant glycoprotein which, once activated by thrombin, inhibits activated factors V and VIII and stimulates fibrinolysis.[1] Current evidence suggests that protein C deficiency is inherited as an autosomal codominant trait.[2,4] Patients heterozygous for protein C deficiency usually have about 50% of normal protein C activity. An increased incidence of serious thrombosis has been reported amongst the heterozygous relatives of several kindreds.[2,5,8] Patients with homozygous protein C deficiency typically present shortly after birth with purpura fulminans, retinal haemorrhage, and evidence of central nervous system or renal thrombosis.[9-17] Protein C activity levels have been undetectable in

G. WATT AND OTHERS: REFERENCES

1. Sanford JP. Leptospirosis. In: Petersdorf RG, Adams RD, Braunwald E, Isselbacher KJ, Martin JB, Wilson JD, eds. Harrison's principles of internal medicine. New York: McGraw-Hill, 1983; 1048–52.
2. Alexander AD, Rule PL. Penicillins, cephalosporins, and tetracyclines in treatment of hamsters with fetal leptospirosis. *Antimicrob Ag Chemother* 1986; 30: 835–39.
3. McClain BL, Ballou WR, Harrison SM, Steinweg DL. Doxycycline therapy for leptospirosis. *Ann Intern Med* 1984; 100: 696–98.
4. Bunnag D, Jaroonvesama N, Harinasuta T. A clinical study of leptospirosis: A comparison of jaundiced and non-jaundiced cases. *J Med Assoc Thailand* 1965; 48: 231–45.
5. Pappas MG, Ballou WR, Gray MR, Takafuji ET, Miller RN, Hockmeyer WT. Rapid serodiagnosis of leptospirosis using the IgM-specific Dot-ELISA: Comparison with the microscopic agglutination test. *Am J Trop Med Hyg* 1985; 34: 346–54.
6. Fletcher W. Recent work on leptospirosis, Tsutsugamushi disease, and tropical typhus in the Federated Malay States. *Trans R Soc Trop Med Hyg* 1928; 21: 265–87.
7. Johnson RC, Harris VG. Differentiation of pathogenic and saprophytic leptospires. 1. Growth at low temperatures. *J Bacteriol* 1976; 94: 27–31.
8. Feigin RD, Anderson DC. Human leptospirosis. *CRC Crit Rev Clin Lab Sci* 1975; 5: 413–65.
9. Larson CL. A comparison of the effect of penicillin and immune serum in the treatment of experimental leptospirosis in young white mice and hamsters. *Publ Hlth Rep* 1945; 60: 317–23.
10. Chang SL. Studies on *Leptospira icterohaemorrhagiae in vitro* and in leptospirosis in guinea pigs. *J Clin Invest* 1946; 25: 752–60.
11. Alston JM, Broom JC. The action of penicillin on leptospiral infections in guinea pigs. *Br Med J* 1944; 2: 718–19.
12. Augustine DL, Weinman D, McAllister J. Penicillin sodium therapy in experimental Weil's disease. *N Engl J Med* 1944; 231: 358–59.
13. Yoder HW, Bergman EN, Gleiser CA. Experimental canine leptospirosis IV. Evaluation of selection antibiotics in the therapy of acute experimental *Leptospira icterohemorrhagiae* infections in immature dogs. *J Infect Dis* 1957; 100: 257–67.
14. Alexander AD. In: Annual report 1970–71. Washington: Walter Reed Army Institute of Research, 1970; 2: 914–15.
15. Hall HE, Hightower JA, Rivera RD, Byrne RJ, Smadel JE, Woodward TE. Evaluation of antibiotic therapy in human leptospirosis. *Ann Intern Med* 1951; 35: 981–98.
16. Fairburn AC, Semple SJG. Chloramphenicol and penicillin in the treatment of leptospirosis among British troops in Malaya. *Lancet* 1956; i: 3–16.
17. Doherty RL. A clinical study of leptospirosis in North Queensland. *Aust Ann Med* 1955; 4: 53–63.
18. Kocen RS. Leptospirosis: A comparison of symptomatic and penicillin therapy. *Br Med J* 1962; i: 1181–83.
19. Manaloto CR, Alora BD. Leptospirosis: An analysis of twenty-nine cases. *Phil Soc Microbiol Inf Dis J* 1980; IX: 75–81.
20. Philippine Index of Medical Specialities 1986; V 15: no 3.



Preoperative and postoperative protein C levels in relation to factor X.

Levels of protein C activity (○) and antigen (●) are shown in relation to factor X levels (△) before (time 0) and after hepatic transplantation.

all reported cases, and the disease is invariably fatal if left untreated.

Although fresh frozen plasma (FFP), coumarin anticoagulants, and factor IX concentrates have been used successfully in the treatment of homozygous protein C deficiency, all existing treatments have substantial drawbacks. The biological half-life of injected protein C (< 8 h) necessitates unacceptably frequent administration of plasma products, and poor access to veins is often another limiting factor. The thrombotic tendency can be controlled with coumarin anticoagulants, but only in doses that severely restrict normal childhood activities and carry a risk of fatal haemorrhage.

Several lines of evidence suggest that the liver is the major site of protein C synthesis. First, other vitamin-K-dependent factors (II, VII, IX, and X) are synthesised in the liver; second, protein C levels are reduced in liver disease;[18] third, synthesis of soluble protein C by a human hepatoma line has been demonstrated in vitro;[19] and, fourth, cDNA inserts in a gt11 library prepared from human liver mRNA have been shown to code for protein C.[20] These observations led us to attempt orthotopic hepatic transplantation as primary therapy for a patient with protein C deficiency.

Case-report and Methods

The patient, born at full term, was the child of first cousins. Details of his clinical course and of protein C levels in the pedigree have been presented elsewhere.[17] Retinal and vitreous haemorrhages (ultimately resulting in complete blindness') were noted shortly after birth. Purpuric skin lesions were first observed at 24 h of age. Protein C antigen was present at 17% of normal levels, but protein C activity was undetectable. Therapy with FFP was instituted at 48 h of age, and was continued until 8½ months of age. Two attempts at withdrawal of FFP were associated with renal failure, microangiopathic haemolytic anaemia, and fibrinolysis. At age 8½ months, warfarin ('Coumadin') therapy was successfully instituted during phased withdrawal of FFP. At age 15 months an intracranial haematoma developed after a fall from bed. Warfarin was discontinued and treatment with FFP was reinstituted for six weeks. Warfarin was then resumed, and the haematoma resolved without neurological sequelae.

At age 20 months, the patient was transferred to the Children's Hospital of Pittsburgh for orthotopic hepatic transplantation. Before surgery, he had been receiving warfarin 0·5 mg/kg per day—3·0 mg in the morning and 2·5 mg at night. The last dose (2·5 mg) was administered 6 h before induction of anesthesia. At the time of induction the prothrombin and partial thromboplastin times were 25·6 s (control 11·8) and 57·2 s (control 25), respectively. 5 mg vitamin K_1 was given intramuscularly, followed by an intravenous infusion of 10 ml/kg FFP. The protein C antigen and activity levels immediately after the infusion were 29% and 24%, respectively. Maximum reduction of the prothrombin time was achieved 4½ h after induction, at which time the prothrombin time was 14·8 s with a partial thromboplastin time of 31·6 s. Revascularisation of the graft was complicated by hepatic artery and portal vein thrombosis. Heparin, 3 mg/kg as a single intravenous bolus, was administered immediately before thrombectomy of both vessels; however, thrombectomy of the portal vein failed, necessitating placement of a vein graft from the confluence of the splenic and superior mesenteric arteries to the hilum of the liver. A Roux-en-Y choledochojejunostomy was performed.

LEVELS OF ADDITIONAL COAGULATION FACTORS PREOPERATIVELY AND POSTOPERATIVELY*

Time	Factor								Protein
	II	V	VII	VIII	IX	X	XI	XII	S
Day 1 (preop)	0·29	0·76	0·33	2·70	0·39	0·23	0·69	0·96	
Day 1 (postop)	0·09	0·13	0·07	0·65	0·02	0·10	0·03	0·36	
Day 2	0·13	0·27	0·08	1·20	0·31	0·12	0·07	0·15	<0·12
Day 3	0·31	0·36	0·29	1·30	0·61	0·20	0·18	0·13	0·64
Day 4	0·37	0·46	0·32	1·60	0·78	0·31	0·27	0·13	
Day 5	0·56	0·74	0·42	2·25	1·10	0·54	0·42	0·32	1·37
Day 6	0·54	1·00	0·58	2·55	1·10	0·64	0·55	0·47	1·66
Day 8	0·54	0·80	0·64	2·75	1·05	0·68	0·72	0·69	1·37
Day 10	1·60	1·50	0·60	3·70	1·50	0·90	0·96	1·05	

*All assays are functional, with normal values of 50–150%, expressed as units/ml.

Postoperatively, the patient was maintained on aspirin 40 mg/kg once daily, dipyridamole 1 mg/kg orally twice daily, tapering doses of prednisone, and cyclosporin adjusted to maintain serum levels of approximately 500 ng/ml as measured by radioimmunoassay. Protein C levels improved steadily after reaching a nadir 24 h postoperatively (figure). In the second week after transplantation an episode of presumed rejection characterised by lymphocytic infiltration of the hepatic parenchyma and portal areas and mild increases in serum transaminases was treated with OKT3 monoclonal antibody. The liver enzymes fell, but four weeks after transplantation the patient became feverish and irritable, with atypical lymphocytosis and recurrent modest rises in transaminases. A cerebrospinal fluid mononuclear cell pleocytosis reached a maximum of 247/μl six weeks after transplantation; this remained unexplained and the patient improved spontaneously. He has been treated subsequently for two episodes of pneumococcal septicaemia without meningitis, the second within three weeks of administration of pneumococcal vaccine ('Pnu-immune'). He remains symptom-free with regard to his initial coagulation disorder. Protein C levels 7 months after hepatic transplantation are normal.

Laboratory Methods

Coagulation factors were assayed by published methods.[21-23] Protein C functional assay was done by the method of Bertina[4] and of Comp et al[24] (reagents from American Diagnostica Inc, Greenwich, CT). A venom derivative, 'Protac C', converts human protein C to the active protease (aPC) which is then measured on a chromogenic substrate, 'Spectrozyme-aPC'. Protein C immunological assay was done by the rocket technique in prepared plates from Helena Laboratories (Beaumont, TX). For both kinetic and immunological assays, the normal ranges were 65–145% of laboratory normal standard. Protein S immunological assay was likewise done with the rocket technique, antibody being purchased from American Diagnostica. The normal range was 50–150% of laboratory normal standard. The C4 bound protein was precipitated with polyethylene glycol at 3·75%. Crossed immunoelectrophoresis[25] was used if the result with rocket assay was low.

Results

The accompanying figure shows post-transplantation levels of factor X and functional and antigenic protein C, and the table gives results of additional coagulation assays. The time-course and pattern of protein C recovery did not differ noticeably from that of other vitamin-K-dependent

procoagulant factors. There were no thrombotic complications in the postoperative period.

Discussion

The successful reconstitution of protein C activity in this patient establishes hepatic transplantation as a useful treatment for an otherwise catastrophic illness. The results also unequivocally confirm that the liver is a major site of protein C synthesis. Preoperatively, there was cause for concern not only about the ability of the transplanted liver to synthesise protein C but also about the pattern of reconstitution. If protein C had been synthesised more slowly than the procoagulant proteins, for example, then an imbalance between procoagulant and anticoagulant activities might have led to postoperative thromboses. A similar imbalance may account for the episodes of skin necrosis that occur in heterozygotes for protein C deficiency who are receiving warfarin.[26,27] The explanation, presumably, is that protein C concentrations fall quickly after initiation of warfarin, because of the short half-life of the protein in vivo, whereas most of the procoagulant proteins have much longer half-lives. Fortunately, it seems that protein C is elaborated on a time-course similar to that of other vitamin-K-dependent factors.

The occurrence of intraoperative thrombosis in this patient suggests that perioperative management of his protein C abnormality could have been improved upon, although thrombosis of the hepatic arteries and portal veins has also been seen in liver-transplant patients without protein C deficiency. Perhaps continued anticoagulation during the operative procedure will turn out to be the best course despite the added risks. Alternatively, higher doses of FFP before and during the operation might provide enough protein C to prevent thromboses.

Severe homozygous protein C deficiency joins the growing list of metabolic disorders including alpha-1-antitrypsin deficiency,[28] Wilson's disease,[29] hereditary tyrosinaemia,[30] type 1 and 4 glycogen storage[31,32] disease, Crigler-Najjar syndrome,[33] porphyria,[34] familial hypercholesterolaemia,[35] factor VIII[36,37] deficiency, and primary hyperoxaluria[38] that are correctable by hepatic transplantation. Yet, protein C deficiency is distinguished from all but the last three in that hepatic failure is not a primary feature of the disease. In fact, to our knowledge, protein C is the first coagulation defect in which liver transplantation has been done to correct the specific disorder and not end-stage liver disease related to previous blood product therapy. Removal of the need for continuous warfarin therapy or frequent infusions of plasma products has resulted in a sustained improvement in the quality of life for this child, despite the need for continuing immunosuppression.

Further observation of this and other patients will be necessary before liver transplantation can be declared the treatment of choice for protein C deficiency. Future difficulties with this approach may be encountered. For example, protein C, like many other proteins, may exhibit polymorphisms.[4,39,40] Therefore, protein C synthesised by donor livers may differ from that formerly synthesised by the recipient's liver. As a consequence, the patient's immune system might respond by forming antibodies against the foreign protein C. In the future, other treatment methods will need to be pursued—in view of the inherent risks of liver transplantation and the fact that early morbidity such as blindness cannot conceivably be prevented by transplantation. Ultimately, a genetic approach is likely to be most successful, either through carrier detection in high-risk populations or through gene therapy.

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REFERENCES

1. Clouse LH, Comp PC. The regulation of hemostasis: The protein C system. *N Engl J Med* 1986; 314: 1298–1304.
2. Griffin JH, Evatt B, Zimmerman TS, Kleiss AJ, Wideman C. Deficiency of protein C in congenital thrombotic disease. *J Clin Invest* 1981; 68: 1370–73.
3. Broekmans AW, Veltkamp JJ, Bertina RM. Congenital protein C deficiency and venous thromboembolism. *N Engl J Med* 1983; 309: 340–44.
4. Bertina RM, Broekmans AW, Krommenhoek-van Es C, Van Wijngaarden A. The use of a functional and immunologic assay for plasma protein C in the study of the heterogeneity of congenital protein C deficiency. *Thromb Haemostas* 1984; 51: 1–5.
5. Horellou MH, Conard J, Bertina RM, Samama, M. Congenital protein C deficiency and thrombotic disease in nine French families. *Br Med J* 1984; 289: 1285–87.
6. Pabinger-Fasching I, Bertina RM, Lechner K, Noessner H, Korninger C. Protein C deficiency in two Austrian families. *Thromb Haemostas* (Stuttgart) 1983; 50: 810–13.
7. Broekmans AW. Hereditary protein C deficiency. *Haemostasis* 1985; 15: 233–40.
8. Marlar RA. Protein C in thromboembolic disease. *Semin Thromb Hemo* 1985; 11: 387–93.
9. Branson HE, Marble R, Katz J, Griffin JH. Inherited protein C deficiency and coumarin-responsive chronic relapsing purpura fulminans in a newborn infant. *Lancet* 1983; ii: 1165–68.
10. Seligsohn U, Berger A, Abend M, Rubin L, Attias D, Zivelin A, Rapaport SI. Homozygous protein C deficiency manifested by massive venous thrombosis in the newborn. *N Engl J Med* 1984; 310: 559–62.
11. Estellés A, Garcia-Plaza I, Dasi A, Azner J, Durant M, Sanz G, Perez-Requejo JL, Espana F, Jimenez C, Abeledo G. Severe inherited "homozygous" protein C deficiency in a newborn infant. *Thromb Haemostas* 1984; 52: 53–56.
12. Sills RH, Marlar RA, Montgomery RR, Deshpande GN, Humbert JR. Severe homozygous protein C deficiency. *J Pediatr* 1984; 105: 409–13.
13. Marciniak E, Wilson HD, Marlar RA. Neonatal purpura fulminans: a genetic disorder related to the absence of protein C in blood. *Blood* 1985; 65: 15–20.
14. Yuen P, Cheung A, Lau HJ, et al. Purpura fulminans in a Chinese boy with congenital protein C deficiency. *Pediatrics* 1986; 77: 670–76.
15. Wehinger H, Geiger E, Freudenberg V, Schurmann J, Alexandrakis E, Witt I. Schwerer hereditarer protein-C-mangel bei einem neugeborenen mit purpura fulminans—erfolgreiche behandlung mit phenprocoumon. *Klin Pädiat* 1985; 197: 116–20.
16. Rappaport ES, Speights VO, Helbert B, Trowbridge A, Koops B, Montgomery RR, Marlar RA. Protein C deficiency. *South Med J* 1987; 80: 240–42.
17. Peters C, Casella JF, Marlar RA, Montgomery RR, Zinkham WH. Homozygous protein C deficiency: observations on the nature of the molecular abnormality and the effectiveness of warfarin therapy. *Pediatrics* 1988; 81: 272–76.
18. Mannucci PM, Vigano S. Deficiencies of protein C, an inhibitor of blood coagulation. *Lancet* 1982; ii: 463–66.
19. Fair DS, Marlar RA. Biosynthesis and secretion of factor VII, protein C, protein S, and the protein C inhibitor from a human hepatoma cell line. *Blood* 1986; 67: 64–70.
20. Foster D, Davie EW. Characterization of a cDNA coding for human protein C. *Proc Natl Acad Sci USA* 1984; 81: 4766–70.
21. Lewis JH. Coagulation defects. *JAMA* 1961; 178: 1014–20.
22. Lewis JH. Hemostasis and hemorrhage. *Sci Clin* 1971; 1: 1–66.
23. Lewis JH, Spero JA, Hasiba U. Diagnostic methods: laboratory tests. In: Bleeding disorders: discussions in patient management. Garden City, NY: Medical Exam Publishing 1978: 22–34.
24. Comp PC, Nixon RR, Cooper MR, Esmon CT. Familial protein S deficiency is associated with recurrent thrombosis. *J Clin Invest* 1984; 74: 2082–88.
25. Comp PC, Doray D, Patton D, Esmon CT. An abnormal plasma distribution of protein S occurs in functional protein S deficiency. *Blood* 1986; 67: 504–08.
26. Broekmans AW, Bertina RM, Loeliger EA, Hofmann V, Klingemann HG. Protein C and the development of skin necrosis during anticoagulant therapy. *Thromb Haemostas* 1983; 49: 251.
27. McGehee WG, Klotz TA, Epstein DJ, Rapaport SI. Coumarin necrosis associated with hereditary protein C deficiency. *Ann Intern Med* 1984; 101: 59–60.
28. Hood JM, Koep LJ, Peters RL, et al. Liver transplantation for advanced liver disease with alpha-1-antitrypsin deficiency. *N Engl J Med* 1980; 302: 272–75.
29. Groth CG, Dubois RS, Corman J, et al. Metabolic effects of hepatic replacement in Wilson's disease. *Transplant Proc* 1973; 5: 829–33.
30. Van Thiel DH, Gartner LM, Thorp FK, et al. Resolution of clinical features of tyrosinemia following orthotopic liver transplantation for hepatoma. *J Hepatol* 1986; 3: 42–48.
31. Malatack JJ, Finegold DN, Iwatsuki S, et al. Liver transplantation for type I glycogen storage disease. *Lancet* 1983; i: 1073–74.
32. Gordon RD, Shaw BW Jr, Iwatsuki S, Esquivel CO, Starzl TE. Indications for liver transplantation in the cyclosporine era. *Surg Clin N Am* 1986; 66: 541–56.
33. Kaufman SS, Wood RP, Shaw BW Jr, et al. Orthotopic liver transplantation for type I Crigler-Najjar syndrome. *Hepatology* 1986; 6: 1259–62.

References continued at foot of next page

POSTOPERATIVE POSITIVE NITROGEN BALANCE WITH INTRAVENOUS HYPONUTRITION AND GROWTH HORMONE

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Summary 11 patients having major gastrointestinal surgery were allocated at random to receive either biosynthetic human growth hormone (BSHGH) 0·1 mg/kg or placebo daily for the first 7 postoperative days. All patients received the same intravenous feeding regimen, which containd 2·09 MJ glucose, 1·88 MJ fat, and 7 g N daily. Patients receiving BSHGH were in positive nitrogen balance throughout the study (mean 1·8 [SEM 0·4] g N/day) and those receiving placebo were in negative nitrogen balance (mean −0·9 [0·7] g N/day). Resting energy expenditure progressively increased in the patients receiving BSHGH (115·7% [14·8] on day 7) but remained unchanged in patients receiving placebo (99·35% [1·4]). Fat oxidation was nearly three times higher in the patients on BSHGH (4·09 [0·38] MJ/day) than in controls (1·38 [0·50]). Carbohydrate oxidation remained about the same in both groups. Whole-body protein turnover, synthesis, and breakdown were increased in the patients receiving growth hormone.

Introduction

AN inevitable accompaniment of the metabolic response to trauma is negative nitrogen balance. Although the administration of large quantities of intravenous nutrients (18 g N and 12·55 MJ) (1 MJ = 239 kcal) after operation can produce a positive nitrogen balance,[1] smaller quantities (10 g N and 2·09 MJ) only partly reverse the negative nitrogen balance.[2] Growth hormone improves nitrogen balance after thermal injury,[3-6] major gastrointestinal surgery,[7] and in volunteers during intravenous nutrition.[8] We have investigated the effect of hyponitrogenous hypocaloric intravenous nutrition and biosynthetic human growth hormone (BSHGH) on postoperative nitrogen, protein, and energy metabolism.

Patients and Methods

Patients having major gastrointestinal tract surgery were allocated at random to receive either BSHGH ('Somatonorm', KabiVitrum) 0·1 mg/kg daily or placebo by intramuscular injection for the the first 7 postoperative days. Patients with diabetes, or who were on corticosteroids, or who had evidence of sepsis or non-localised malignant disease were excluded.

A complete nutrient mixture ('Vitrimix', KabiVitrum) was infused continuously through an intravenous cannula inserted into a forearm vein. The cannula was changed every 48 h or earlier if thrombophlebitis occurred. This regimen provided 7 g N, 2·09 MJ as carbohydrate, and 1·88 MJ as fat ('Intralipid', KabiVitrum) daily.

TABLE I—DIAGNOSIS, AGE, AND BODY WEIGHT FOR THE PATIENTS STUDIED

—	Age (yr)	Diagnosis	Initial wt (kg)
Placebo			
1	76	Anal carcinoma	45·6
2	58	Diverticular stricture	60·0
3	79	Diverticular disease	42·3
4	60	Gastric carcinoma	46·0
5	73	Gastric ulcer and diverticular disease	63·0
BSHGH			
6	71	Rectal carcinoma	75·0
7	70	Caecal carcinoma	84·0
8	69	Diverticular disease	53·0
9	70	Gastric carcinoma	65·0
10	48	Chronic pancreatitis	62·0
11	73	Reconnection of Hartmanns	61·0

Venous blood samples were taken on the preoperative (control) day and postoperative days 1, 3, 5, and 7 and analysed for blood glucose, plasma free fatty acids, serum insulin, and serum somatomedin-C. Free fatty acids, insulin, and somatomedin-C were measured by radioimmunoassay.

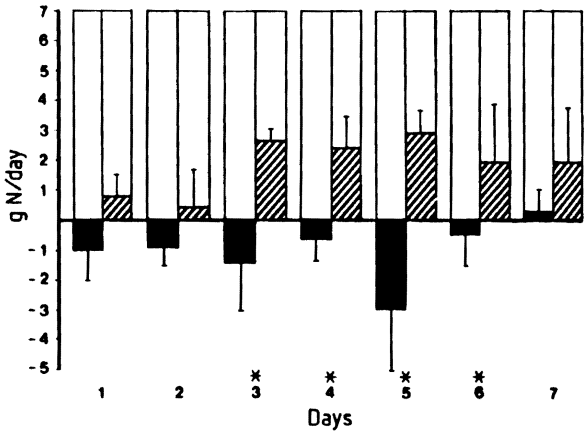
Urine was collected throughout the study and nitrogen content measured by Kjeldhal's technique. In calculating nitrogen balance we did not take account of either faecal or integumentary losses.

Whole-body protein metabolism measurements were made on the 4th or 5th study days. Nitrogen turnover was calculated from the isotope enrichment in a sample of urine taken from the second 12 h of a 24 h primed continuous intravenous infusion of ^{15}N-labelled glycine.[10] Protein synthesis and breakdown were derived by subtraction of nitrogen excretion and input, respectively, from turnover. Indirect calorimetry, with a continuous flow system, was done on the control and 7 postoperative study days. The system has been validated by alcohol combustion and gas dilution.[11] Resting energy expenditure and substrate oxidation rates were calculated as described by Frayn.[12]

Results are expressed as mean and SEM. Statistical analysis was by the one-tailed Mann Whitney U test. The study was approved by the district ethics committee and informed consent was obtained from all patients.

Results

Of the 11 patients investigated 6 received BSHGH and 5 placebo. Table I shows the details of the patients. The patients receiving growth hormone were significantly heavier (median weight 61·5 kg, range 53–84) than those receiving placebo (median 46, range 42–63; $p < 0.05$).



Nitrogen balance in patients on BSHGH and placebo.

Hatched columns = BSHGH; black columns = placebo; unshaded columns = nitrogen intake. *$p < 0.05$.

J. F. CASELLA AND OTHERS: REFERENCES—*continued*

34. Wells MM, Golitz LE, Bender BJ. Erythropoietic protoporphyria with hepatic cirrhosis. *Arch Dermatol* 1980; 116: 429–32.
35. Starzl TE, Bilheimer DW, Bahnson HT, et al. Heart-liver transplantation in a patient with familial hypercholesterolaemia. *Lancet* 1984; i: 1382–83.
36. Lewis JH, Bontempo FA, Spero JA, Rajni MV, Starzl TE. Liver transplantation in a hemophiliac. *N Engl J Med* 1985; 312: 1189–90.
37. Bontempo FA, Lewis JH, Gorenc TJ, et al. Liver transplantation in hemophilia A. Blood 1987; 69: 1721–24.
38. Watts RWE, Calne RY, Koffler K, et al. Successful treatment of primary hyperoxaluria type I by combined hepatic and renal transplantation. *Lancet* 1987; ii: 474–75.
39. Comp PC, Nixon RR, Esmon CT. Determination of functional levels of protein C, an antithrombotic protein, using thrombin-thrombomodulin complex. *Blood* 1984; 63: 15–21.
40. Barbui T, Finazzi G, Mussoni L, et al. Hereditary dysfunctional protein C (protein C Bergamo) and thrombosis. *Lancet* 1984; ii: 819.