Carbenicillin-Induced Coagulopathy

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SUMMARY

In high dosage, carbenicillin may interfere with the conversion of fibrinogen to fibrin and result in a haemorrhagic diathesis. The effect is dose-dependent and requires a high concentration of carbenicillin in plasma. Such a level may be attained in renal failure unless the dose of the drug is appropriately reduced. In such situations the screening coagulation tests may be prolonged and this should alert one to the development of a haemorrhagic diathesis due to the drug.

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Carbenicillin (α-carboxybenzylpenicillin, Pyopen) is a semi-synthetic penicillin which is of particular value in the therapy of infections caused by the *Pseudomonas aeruginosa* organism. It has been reported to be free from renal, haematological or neurological side-effects, even in high doses (30 g/day).[1] The majority of *Ps. aeruginosa* strains are inhibited by a carbenicillin concentration of 100 μg/ml.[2-4] To achieve this level in the serum of patients with normal renal function, it is necessary to administer 1 g carbenicillin intravenously every hour (24 g/day) together with probenecid.[2] In patients with severe renal failure a dose of 2 g intravenously every 4 hours (12 g/day) is recommended if the patient is on haemodialysis, or 2 g intravenously every 8 hours (6 g/day) if the patient is on peritoneal dialysis.[5]

A patient with severe renal failure who was receiving 24 g carbenicillin per day developed severe and persistent haemorrhagic episodes while taking the drug. Because of this, 2 more patients in severe renal failure and with *Ps. aeruginosa* infections, to whom carbenicillin was to be administered, were studied before, during and after withdrawal of the drug. This report documents the coagulation findings on the plasma of these patients, as well as *in vitro* studies on normal and uraemic plasma to which carbenicillin was added.

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CASE REPORTS

Case 1 (Fig. 1)

A 20-year-old male with end-stage renal failure and malignant hypertension caused by diffuse membranous glomerulonephritis (confirmed on renal biopsy) was placed on peritoneal dialysis because of uraemia, acidosis, oliguria and fluid overload. Dialysis was continued for 8 days, after which the blood urea was 65 mg/100 ml, the hypertension was controlled and the acidosis corrected. While on dialysis the patient developed peritonitis and *Ps. aeruginosa* was cultured from the peritoneal effluent. Treatment with carbenicillin (24 g/day) and gentamicin (40 mg/72 h) was begun 4 days after the peritoneal dialysis had been completed. At this time the patient's blood urea was 170 mg/100 ml and he was oliguric. Nine days later when the blood urea was 240 mg/100 ml, the serum creatinine level 22,5 mg/100 ml and the urinary output 240 ml/24 h a Scribner shunt was inserted. Haemodialysis. which commenced on the following day, was carried out every 48 - 72 h. Carbenicillin and gentamicin therapy was continued at the same dosage. Profuse bleeding occurred from the shunt site shortly after it had been inserted. Predialysis blood urea concentrations were approximately 120 mg/100 ml and postdialysis blood urea concentrations 80 mg/100 ml. After 13 days on carbenicillin and gentamicin therapy, melaena occurred. The patient remained pyrexial throughout the period of antibiotic therapy and evidence of peritoneal irritation persisted. After 23 days of antibiotic therapy the peritonitis appeared to have become localised to the right iliac fossa and a laparotomy was performed to evacuate a palpable mass in the right iliac fossa. This was found to be a partially organised retroperitoneal haematoma. Gentamicin was withdrawn 2 days after laparotomy but carbenicillin was continued for a further 7 days. During the postoperative period, the patient bled profusely from the abdominal wound, necessitating replacement transfusions. The bleeding ceased within 2 days after discontinuing the carbenicillin therapy.

Case 2 (Fig. 2)

In a 53-year-old female with end-stage renal failure caused by analgesic nephropathy and papillary necrosis, *Ps aeruginosa* was cultured from the urine. Carbenicillin therapy was instituted, 24 g/day intravenously for 9 days and 12 g/day for a further 5 days. The patient was not dialysed. The blood urea ranged from 145 to 300 mg/100 ml and urinary output from 125 to 1 650 ml/24 h. During this period the patient developed painful haemorrhagic excoriations on her lips and tongue.

Case 3 (Fig. 3)

In a 35- year-old female with analgesic nephropathy and renal failure the blood urea was stable at 110 mg/100 ml, serum creatinine 10 mg/100 ml, urinary output 1 000-2 000 ml/24 h. The patient was not dialysed. Carbenicillin therapy (24 g/day for 5 days) was instituted for *Ps. aeruginosa* and *E. coli* urinary tract infections. During therapy the patient developed menorrhagia, haemorrhagic excoriations on her tongue, headache, and confusion.

MATERIAL AND METHODS

Clotting Tests on Patients' Plasma

The following standard techniques were used: prothrombin time;[6] kaolin cephalin clotting time (KCCT) (Diagnostic Reagents Ltd, Thame, Oxon.); prothrombin consumption index;[7] fibrinogen;[8] 2-stage prothrombin;[9] factor V;[10] factor VIII;[11] and factor X.[12]

In Vitro Clotting Tests

Various concentrations of carbenicillin were tested in the following clotting test systems: thrombin conversion of fibrinogen to fibrin; kaolin cephalin clotting time; and prothrombin time.

The ability of a fibrin clot to withstand lysis in 5M urea was tested, in the presence of carbenicillin. In addition, the effects of protamine sulphate and penicillinase on the carbenicillin-induced coagulation changes in normal plasma were studied.

Carbenicillin Assay

Concentrations of carbenicillin were determined by the cup-plate biological assay method, using *Ps. aeruginosa* NC TC 10490 as the test organism. Bacto-Antibiotic Agar No. 2, pH 6,5 - 6,6, was seeded with 0,05% of an overnight broth culture and poured into large rectangular assay plates using the layer technique.

Plugs of agar were removed to give holes 7 mm in diameter which were filled with the solutions to be assayed. The plates were then incubated overnight at 30°C.

Standard curves were drawn, using carbenicillin 2, 10, 20 and 50 μg/ml dissolved in pooled human serum, or 0,05M phosphate buffer (pH 7,0) for urine assays. The patients' serum specimens were diluted in pooled human serum to give levels within this range of concentration. Urine specimens were diluted in 0,05M phosphate buffer (pH 7,0).

RESULTS

The results of the clotting tests on the patients' plasma are shown in Figs 1 - 3

Each of the patients studied showed clotting abnormalities of the screening coagulation tests (prothrombin time, KCCT). Coagulation factor assays were, however, normal.

Case 1 (Fig. 1)

Coagulation studies were first carried out after 14 days of carbenicillin therapy (24 g/day).

The prothrombin time was 26,3 s (normal control 14,7 s). The KCCT was 93,9 s (normal control 51,4 s). Assays of factors common to both the extrinsic and intrinsic coagulation pathways, namely factors I, II, V and X were normal. The factor VIII assay gave a low result (4%). A repeat test 11 days later, when the patient was still receiving 24 g carbenicillin/day, showed similar abnormal screening tests (prothrombin time 24,5 s, normal control 12,5 s; KCCT 135 s, normal control 50,2 s) yet normal specific factor assays (fibrinogen 420 mg/100 ml, normal control 205 ml/100 ml; factor II 116%, factor V 90%; normal range 50 - 150%). The factor VIII assay on

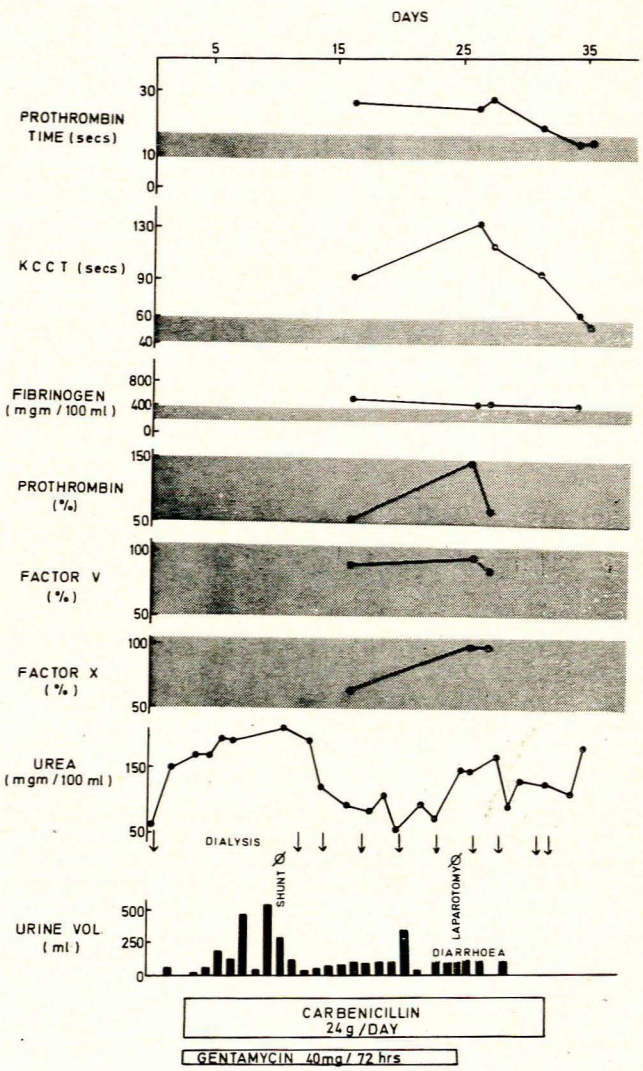

Fig. 1. Clinical course and coagulation studies on case 1.

this occasion was at the upper limit of normal (158%). Two days after the drug was withdrawn, the prothrombin time and KCCT were only slightly abnormal (prothrombin time 14,0 s, normal control 12,5 s; and KCCT 65,5 s, normal control 47,6 s). The KCCT returned to normal values one day later (56,9 s, normal control 51,7 s). The prothrombin time remained minimally prolonged (15,0 s, normal control 13,8 s).

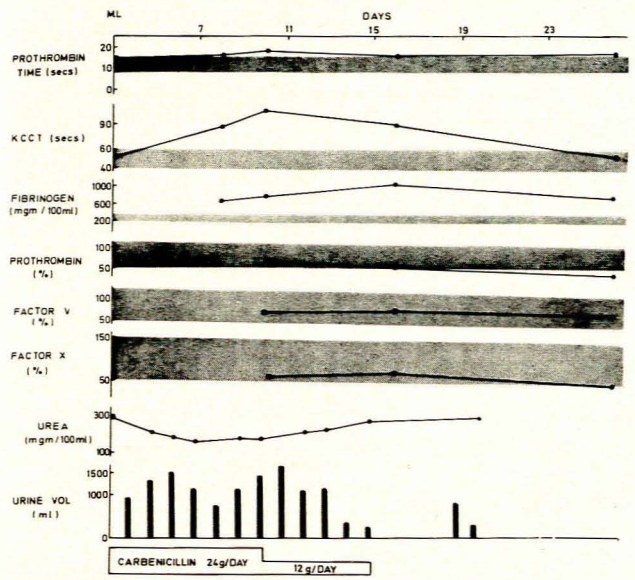

Fig. 2. Clinical course and coagulation studies on case 2.

Case 2 (Fig. 2)

Coagulation studies before carbenicillin therapy showed a prothrombin time of 14,5 s (normal control 12 s) and KCCT of 44,4 s (normal control 50,2 s). The fibrinogen level was 630 mg/100 ml (normal 200 - 400 mg/100 ml). Six days after the commencement of carbenicillin therapy at a dose of 24 g/day, the prothrombin time was 17,4 s (normal control 13,3 s) and the KCCT 88,2 s (normal control 51,0 s). The plasma fibrinogen remained elevated (630 mg/100 ml). After 8 days' carbenicillin therapy (24 g/day), the prothrombin time was 19,5 s (normal control 12,4 s) and KCCT 109,3 s (normal control 54,8 s). The fibrinogen level was then 716 mg/100 ml and factors II, V and X were normal. The factor VIII assay was normal (125%). Nine days after discontinuing the drug, the KCCT was normal (53,6 s, normal control 45,6 s), although the prothrombin time remained prolonged (22,1 s, normal control 12,4 s). The patient died 12 days after cessation of carbenicillin therapy from renal failure and septicaemia.

The serum carbenicillin level was measured one hour after the administration of a 6-g dose on the third day of the course of therapy. A serum carbenicillin level of 1 597 μg/ml was obtained. The patient's blood urea at this stage was 200 mg/100 ml. Nine days after cessation of carbenicillin therapy, a serum carbenicillin level of 62 μg/ml was obtained.

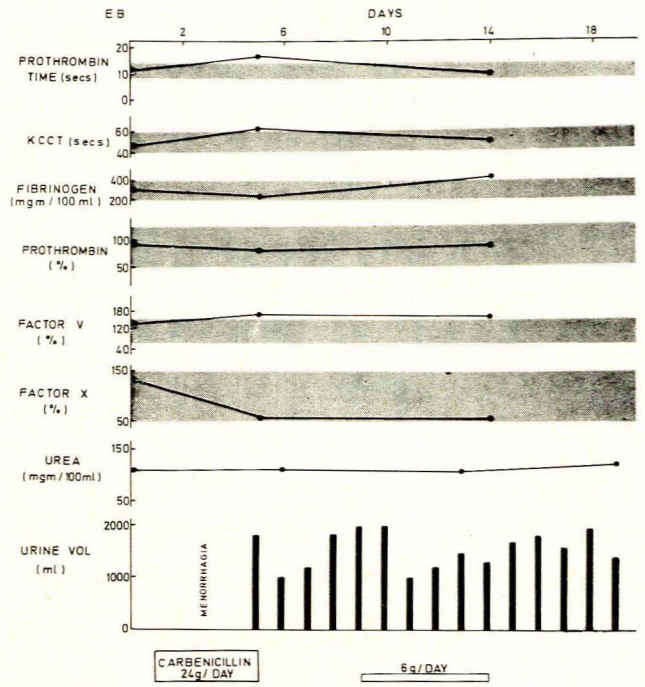

Fig. 3. Clinical course and coagulation studies on case 3.

Case 3 (Fig. 3)

This patient, too, was treated on 24 g carbenicillin/day for 5 days. Coagulation studies before administration of the antibiotic were normal. On the 3rd day of treatment, the prothrombin time was prolonged, 17,5 s (normal control 14,9 s) and the KCCT minimally prolonged, 62,9 s (normal control 56,7 s). A serum carbenicillin level one hour after administration of the drug was 3 194 μg/ml, and 1 597 μg/ml 5 hours after administration. The drug was then withdrawn, and therapy recommenced 3 days later at 6 g/day for 5 days. Repeat coagulation studies 1 day after the completion of the 6 g/day course of treatment were normal. Menorrhagia occurred after 2 days of 24 g/day carbenicillin therapy. Renal function was reduced to a creatinine clearance of 10 ml/min. Urine output was, however, adequate, and the patient was not dialysed.

Carbenicillin clearance was calculated on one occasion. A serum level of 12,5 μg/ml and urine level of 399 μg/ml were obtained, which gave a calculated carbenicillin clearance of 40 ml/min. Since the creatinine clearance done at the same time was 10 ml/min, a tubular secretion of the drug is suggested.

Results of in Vitro Tests

Thrombin times: Doubling and tenfold dilutions of carbenicillin in normal plasma were tested in a calcium-thrombin system. Prolongation of thrombin clotting times were noted at concentrations of carbenicillin of 6 250 μg/ml and greater (Fig. 4). No enhancement of the anticoa-

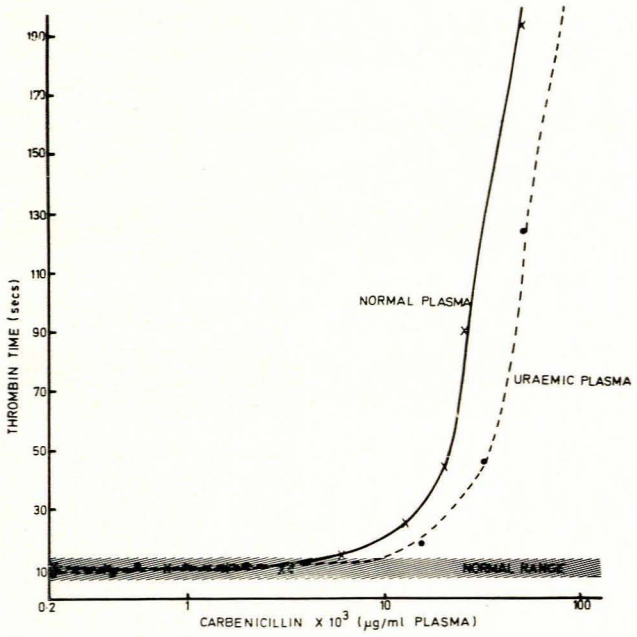

Fig. 4. Effect of increasing concentrations of carbenicillin on the thrombin times of normal and uraemic plasma.

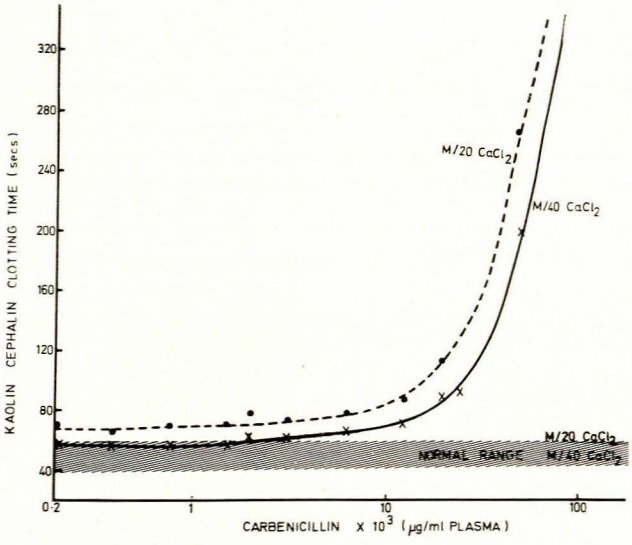

Fig. 5. Effect of increasing concentrations of carbenicillin on the KCCT of normal plasma using M/40 $CaCl_2$ (0,025M) and M/20 $CaCl_2$ (0,05M).

gulant effect of carbenicillin was detected when the drug was added to uraemic plasma.

As a result of the prolongation of the thrombin clotting time by carbenicillin, all tests dependent on this final stage of fibrin formation were abnormal. Thus the KCCT (Fig. 5), thromboplastin generation test, and recalcification time of plasma were abnormal, provided the concentration of carbenicillin in the test system was high enough.

Effect of penicillinase (Table I): A concentration of carbenicillin sufficient to cause an abnormal thrombin time (10 000 µg/ml) was added to normal plasma. To this was added a penicillinase concentration theoretically capable of neutralising 10 000 µg penicillin. No reduction in the abnormal thrombin clotting times was detected at 0 min and after 60 min incubation of the plasma mixtures at 37°C.

Effect of protamine sulphate (Table II): The effect of protamine sulphate on normal plasma containing carbenicillin (25 000 µg/ml) or heparin (1 unit/ml) was studied. No neutralisation of the abnormal KCCT was observed when a range of protamine sulphate concentrations was added to normal plasma containing the carbenicillin. Heparinised plasma was neutralised by 1 mg protamine sulphate, causing a reduction of the KCCT from 300 s to 147 s.

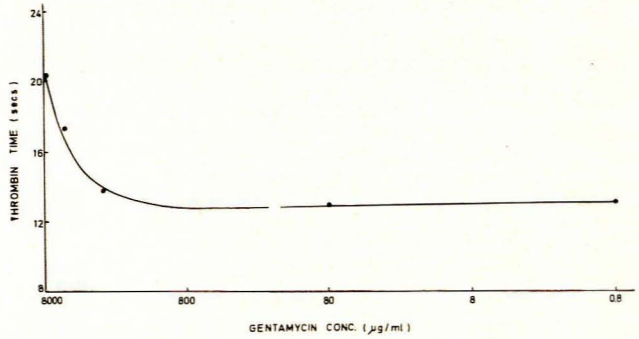

Fig. 6. Effect of increasing concentrations of gentamicin sulphate on the thrombin time of normal plasma.

Effect of gentamicin sulphate and carbenicillin on the thrombin time of normal plasma (Fig. 6 and Table III): Since patient 1 was receiving gentamicin sulphate as well as carbenicillin, the effect of increasing concentrations of gentamicin on the thrombin time of normal plasma was tested. This was compared with the anticoagulant effect of carbenicillin alone and carbenicillin together with gentamicin. Concentrations of gentamicin from 0,8 µg/ml to 2 000 µg/ml had no anticoagulant effect. At concentrations of 8 000 µg/ml gentamicin was found to have an additive effect on the anticoagulant properties of carbenicillin.

DISCUSSION

Carbenicillin has been found to be a safe and effective preparation for the therapy of *Ps. aeruginosa* infections.[1] Plasma levels inhibitory to these organisms appear to be in the region of 100 µg/ml.[2-4] At this plasma concentration, no haemorrhagic side-effects have been noted. The dosage recommended to achieve this level is 24 - 30 g daily. In renal failure, however, lower dosages are recommended (6 - 8 g daily) to achieve the same therapeutic level.

Three patients with renal failure were treated with high doses of carbenicillin. In 1 patient severe bleeding

TABLE I. EFFECT OF PENICILLINASE ON THE THROMBIN TIME OF NORMAL PLASMA WITH AND WITHOUT ADDED CARBENICILLIN

Incubation at 37°C (min)	Thrombin time (s)			
	Control	Carbenicillin (10 000 μg/ml)	Penicillinase	Carbenicillin + penicillinase
0	9,0	63,8	10,0	58,0
60	9,6	63,5	9,8	66,2

0,2 ml 0,025M $CaCl_2$ (4 parts)/thrombin 50 units/ml (1 part) was added to a mixture of 0,4 ml normal plasma, 0,1 ml carbenicillin dilution, and the clotting times recorded. The mixtures were tested at 0 min and after 60 min incubation at 37°C. As a control, 0,2 ml buffer was added to the normal plasma instead of the carbenicillin and penicillinase solutions.

TABLE II. EFFECT OF PROTAMINE SULPHATE ON THE KCCT OF NORMAL PLASMA WITH ADDED HEPARIN OR CARBENICILLIN

Protamine concentration (mg)	KCCT (s)	
	With heparin	With carbenicillin
1,0	300	300
0,1	300	300
0,01	117	146
0,001	300	86
0,0001	300	86
—	300	86

KCCT of plasma without added heparin or carbenicillin = 69,1 s. 0,2 ml kaolin/cephalin suspension was added to 0,1 ml plasma containing heparin 1 μg/ml or carbenicillin 25 000 μg/ml, and 0,1 ml protamine sulphate concentration. The mixture was incubated for 2 min, then recalcified with 0,1 ml 0,025M $CaCl_2$, and the clotting times recorded.

TABLE III. EFFECT OF GENTAMICIN SULPHATE AND CARBENICILLIN ON THE THROMBIN TIME OF NORMAL PLASMA

Reaction mixture		
Gentamicin (μg/ml)	Carbenicillin (μg/ml)	Thrombin time (s)
40	—	13,0
8 000	—	20,6
—	6 250	18,2
—	12 500	30,0
40	6 250	18,4
8 000	12 500	68,0
—	—	14,2

0,2 ml 0,025M $CaCl_2$ (4 parts)/thrombin 50 units/ml (1 part) was added to a mixture containing 0,3 ml normal plasma, 0,1 ml gentamicin dilution and 0,1 carbenicillin dilution and the clotting times recorded. As a control 0,2 ml buffer was added instead of the antibiotic solutions.

occurred from the bowel, Scribner shunt site, retroperitoneal space and abdominal wound. The bleeding ceased when the carbenicillin was withdrawn. Two further patients developed abnormalities of their screening clotting tests when carbenicillin was administered. These returned to normal when the drug was withdrawn. In all 3 patients the screening coagulation tests of the intrinsic and extrinsic systems were abnormal (prothrombin time and KCCT), yet assays of specific factors common to both pathways were normal. This suggested an anticoagulant effect which was detected by the screening tests but not by specific factor assays. These latter assay procedures utilise diluted plasma, whereas the prothrombin time and KCCT procedures use relatively concentrated plasma. If an anticoagulant were present in the plasma it could therefore interfere with the screening coagulation tests, but its effect might be diluted out in the factor assays.

The anticoagulant effect was demonstrated *in vitro* by adding increasing concentrations of the drug to normal plasma. The greater the concentration of the drug in plasma, the greater was the anticoagulant effect. This effect could be reduced by reducing the concentration of the drug or by increasing the volume of the plasma.

The anticoagulation was found to be due to an interference in the conversion of fibrinogen to fibrin. The mode of action of this interference has not been established. If the concentration of carbenicillin in plasma is high enough, all tests dependent on the conversion of fibrinogen to fibrin are abnormal.

The serum level obtained in the second patient studied (1 597 μg/ml), is far in excess of that required for therapy of *Pseudomonas* infections (100 μg/ml). Thus it is unlikely that such levels will be attained, provided the therapeutic dose is reduced in the presence of renal insufficiency. If not, a bleeding diathesis may ensue, which may present a difficult clinical and laboratory diagnostic problem.

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