

# 263

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TRANSPLANTATION OF THE HUMAN LIVER

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## SERUM COMPLEMENT AFTER ORTHOTOPIC TRANS- PLANTATION OF THE HUMAN LIVER

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AND T. E. STARZL

*Departments of Surgery, Medicine and Pediatrics, University of Colorado School of Medicine  
and the Denver Veterans Administration Hospital, Denver, Colorado*

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### SUMMARY

In five patients with terminal liver failure, replacement of the diseased liver with a well functioning homograft restored markedly depressed total complement and C4 and C3 to normal levels. Concomitantly, C5 protein also increased. Post-operatively, two patients developed a marked drop in C4 and C3 probably in relation to homograft rejection. In contrast, serum hepatitis and biliary obstruction were not accompanied by significant changes. It is concluded that the liver is an important source of synthesis of C4, C3 and C5 and that complement assays might aid in otherwise equivocal diagnosis of hepatic homograft rejection.

### INTRODUCTION

The investigation of the human serum complement system after orthotopic liver transplantation must take into account a multiplicity of factors. First, the provision of a new homograft liver to a patient in hepatic failure makes it necessary to evaluate complement activity in relation to hepatic function. Secondly, postoperative events such as graft rejection, biliary tract obstruction, and serum hepatitis, all might influence complement levels by immunological or non-immunological mechanisms.

In this study the levels of total complement and several of its components were assayed in five hepatic homograft recipients. Excellent initial graft function was invariably obtained, but later all patients encountered at least one of the aforementioned postoperative complications.

### MATERIALS AND METHODS

#### *Case material*

The five recipients were 15-47 years of age; prior to surgery all were in terminal liver failure due to cirrhosis (OT39 and 40), chronic hepatitis (OT36 and 41), and Wilson's disease (OT42). Orthotopic hepatic transplantation was carried out with standard techniques

Correspondence: Dr Thomas E. Starzl, Veterans Administration Hospital, 1055 Clermont Street, Denver, Colorado, 80220, U.S.A.

(Starzl, 1969). In four of the five cases immunosuppression was provided with the triple drug regimen of azathioprine, prednisone and horse antilymphocyte globulin (Starzl, 1969); the fifth patient (OT42) was given cyclophosphamide instead of azathioprine (Starzl *et al.*, 1971).

Postoperatively, Au-antigen was looked for in the recipient sera two or three times weekly utilizing standard detection methods (Torisu *et al.*, 1971) as well as the complement fixation test (Sever, 1962; Shulman & Barker, 1969).

#### *Serum sampling*

Blood samples were collected once or twice before transplantation and at frequent intervals postoperatively. Following clotting at room temperature for approximately 1 hr, serum was separated by centrifugation and stored at  $-70^{\circ}\text{C}$  until used. All sera from an individual patient were analysed simultaneously.

#### *Serum haemolytic complement assays*

Total complement activity was measured in 50% haemolytic units (CH50) according to Mayer (1961) and with the immune adherence haemagglutination (IA50) method of Nishioka (1963). The activity of C1, C4, and C2 components were assayed by stoichiometric tube titration using  $\text{EAC}4^{\text{hu}}$ ,  $\text{EAC}1^{\text{hu}}$ , and  $\text{EAC}1^{\text{hu}}\text{C}4^{\text{hu}}$  cells at a concentration of  $1.5 \times 10^8$  cells per ml, respectively; the results were expressed in 50% haemolytic units (Nelson *et al.*, 1966; Borsos & Rapp, 1967). C3 activity was measured by immune adherence (Nishioka & Linscott, 1963) using  $\text{EAC}1^{\text{hu}}\text{C}4^{\text{hu}}\text{C}2^{\text{hu}}$  cells.

The C9 activity was titrated by immune haemolysis (Ruddy *et al.*, 1971). The cellular intermediate ( $\text{EAC}1\text{-}8^{\text{hu}}$  cells) was prepared by incubating  $\text{EAC}1^{\text{hu}}\text{C}4^{\text{hu}}$  cells in glucose gelatin Veronal buffer (Inoue & Nelson, 1965) with C2, 3, 5, 6, 7, 8 complex of human complement for 30 min at  $30^{\circ}\text{C}$  followed by washing in gelatin Veronal buffer (Mayer, 1961).

The presence of serum anticomplementary activity (ACA) was assayed as previously described (Shulman & Barker, 1969; Torisu *et al.*, 1971).

The normal values for the various assays in our laboratory are included in Table 1. CH50 and IA50 were measured in sera from 250 healthy blood donors, and C1, C4, C2, C3 and C9 were analysed in 100 of these specimens.

#### *Immunochemical assay*

Absolute weight concentration of the C1q subunit of C1 (Lepow *et al.*, 1963; Hanauer & Christian, 1967), C4,  $\beta_1\text{E}$  globulin (Müller-Eberhard & Biro, 1963), C3,  $\beta_1\text{C}$  globulin (Müller-Eberhard & Nilsson, 1960), and C5,  $\beta_1\text{F}$  globulin (Nilsson & Müller-Eberhard, 1965) were measured by single radial immunodiffusion (Mancini, Carbonara & Heremans, 1965; Kohler & Müller-Eberhard, 1967). Normal values (Kohler & ten Bonsel, 1969) are included in Table 1.

## RESULTS

#### *Changes with liver replacement*

Before transplantation, serum complement activity, as measured both with CH50 and IA50, was distinctly lower than normal in all five patients (Table 1).

TABLE 1. Levels of total complement and of the components before and 7-10 days after orthotopic hepatic transplantation, and normal ranges

Patient	Units/ml		Reciprocal of titres*							(Units/ml)*					(mg/ml)				
	CH50	IA50	IA50	C1	C4	C2	C3	C9	C1q	C4	C3	C5	C1q	C4	C3	C5			
OT 36	16.0/45.5	1.2/9.8	12.5/11.3	6.5/102.0	1.3/1.4	2.4/4.8	37.5/41.2	0.154/0.208	0.216/0.510	0.740/1.180	0.058/0.152								
OT 39	9.8/30.2	0.8/2.4	21.2/22.0	1.3/72.8	1.1/1.4	0.8/2.4	58.2/62.3	NT†	NT	NT	NT	NT							
OT 40	4.9/40.0	0.4/6.4	9.5/18.0	0.1/81.0	0.9/1.4	0.6/4.8	32.5/48.5	0.120/0.166	0.108/0.408	0.340/1.488	0.073/0.102								
OT 41	7.5/31.0	0.1/2.4	8.2/8.2	0.1/66.4	1.3/1.4	1.0/4.8	61.5/60.3	0.074/0.172	0.198/0.318	0.680/1.320	0.049/0.120								
OT 42	15.0/50.0	1.2/6.4	18.0/19.0	2.0/122.0	1.9/1.7	1.2/18.0	22.3/61.5	0.168/0.168	0.174/0.600	0.600/1.620	0.059/0.130								
Normal range‡	25.3-49.7	1.9-5.5	16.8-22.8	62.0-122.4	0.7-2.3	2.5-6.9	25.0-75.0	0.134-0.246	0.258-0.780	0.820-1.700	0.033-0.121								

\* In thousands. † NT = not tested. ‡ Mean ± 2SD.

Of the component activities, C4 and C3 were invariably low with C4 showing the most marked depression. C1 was subnormal in three of the patients. In contrast, C2 and C9 were within normal limits. Following replacement of the patients' diseased liver with a well functioning homograft, total complement, as well as C4 and C3 activities rose to normal within 1 week; C1 was less affected (Table 1, Fig. 1-3).

The immunochemical measurement of C4 and C3 showed changes which paralleled the haemolytic titres but the alterations were less extreme. In two patients a slightly subnormal preoperative C1q rose to normal following transplantation; two other patients had normal levels throughout. The C5 protein rose from low normal to high normal or slightly elevated values in all four studied cases (Table 1, Fig. 1-3).

Subsequently, normal graft function was accompanied by normal complement levels (Fig. 1, 2), except in one patient (OT42) who had supernormal total complement as well as C4 and C3 activities throughout the postoperative course (Fig. 3).

#### *Changes with homograft rejection*

Two of the recipients developed postoperative liver function abnormalities which were thought to be due to homograft rejection. In a patient with postnecrotic cirrhosis (OT40), hyperbilirubinaemia began 2 weeks postoperatively and progressed until death in liver failure and pulmonary sepsis at day 32. No Au-antigen could be found in the patient's serum. During the latter half of the postoperative course, total serum complement, and C3 and C4 levels decreased progressively (Fig. 1).

The original disease of the other patient (OT41) was chronic aggressive hepatitis without demonstrable Au-antigenaemia. Liver dysfunction indicative of rejection occurred approximately 3 weeks after transplantation and was accompanied by a marked temporary drop in CH50, IA50, C4 and C3 and a moderate decrease in C2 (Fig. 2). Later, persistent hyperbilirubinaemia in spite of anti-rejection therapy prompted re-exploration. At operation a biliary tract obstruction was diagnosed and surgically corrected but the patient succumbed 16 days later. Before death, the complement levels again decreased with C4 being the most affected component. It was interesting that the liver had only minor histopathologic signs of rejection.

In both patients the immunochemical studies revealed changes in the C4 and C3 components mimicking the changes in the haemolytic titres.

#### *Changes with Au-antigen positive hepatitis*

One patient who was transplanted during active chronic Au-positive hepatitis (OT36) first converted to Au-negative but later became positive again concomitant with the appearance of liver dysfunction. The postoperative 'hepatitis' ran its total course from onset to full recovery from post-transplantation days 60 to 140 as fully reported elsewhere (Torisu *et al.*, 1971). The maximum derangements of liver function included a bilirubin that rose to 14 mg%, S.G.O.T. of 5000 IU, and total protein depression to less than 5 g%.

When the post-transplantation liver dysfunction was most severe, a minor transient decrease in the complement levels occurred. Four weeks earlier, while graft function and complement levels were still normal, the anti-complement activity had risen to 1:256 and at the time of the appearance of liver injury a titre of 1:8 remained.

The patient treated for Wilson's disease (OT42) developed Au-antigenaemia and abnormal

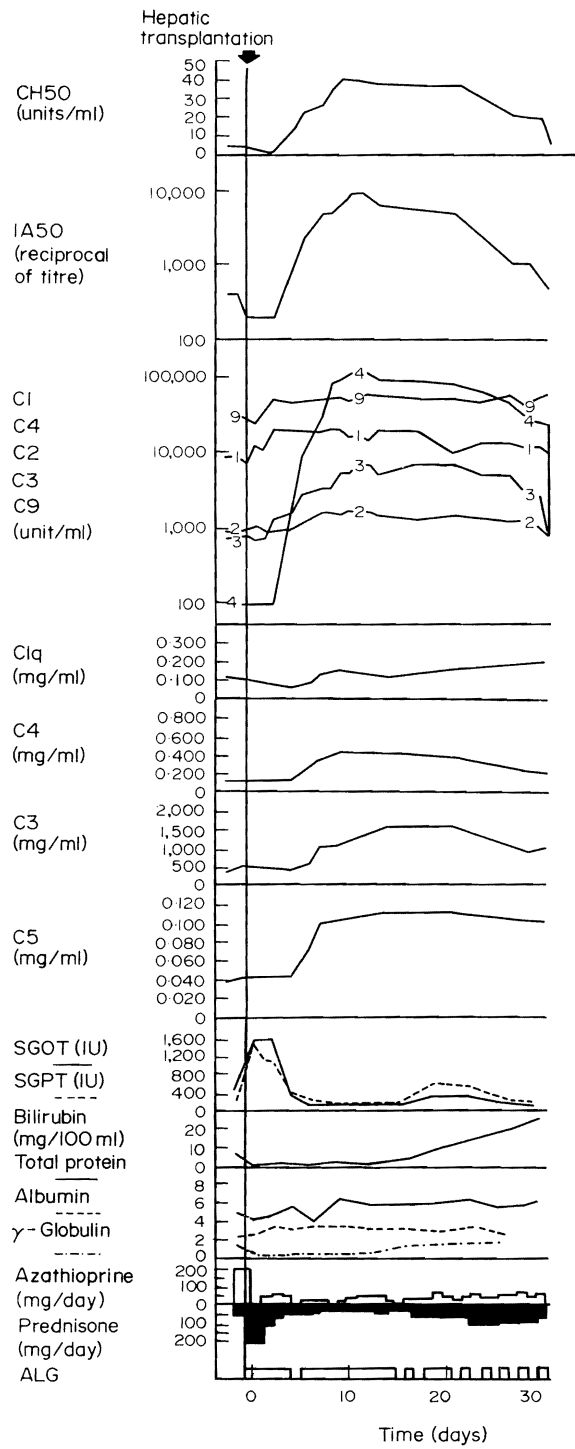


FIG. 1. Complement levels, liver function tests, and immunosuppressive treatment in a patient (OT40) who died of homograft failure caused by rejection (ALG—antilymphocyte globulin).

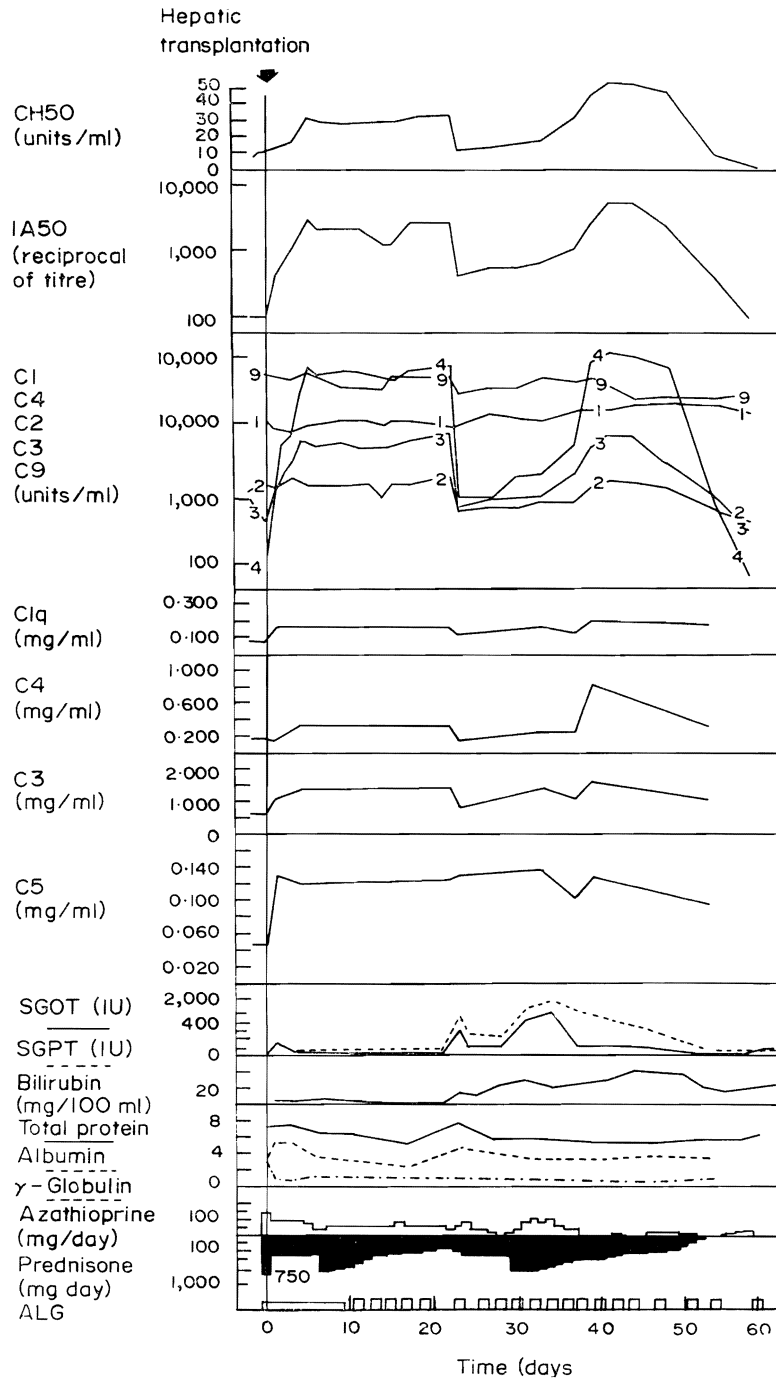


FIG. 2. The course in a patient (OT41) who developed liver dysfunction 3 weeks after operation which was thought to be due to rejection. Subsequently, biliary tract obstruction was diagnosed. Note the marked drop in total complement and several components concomitant with the rejection.

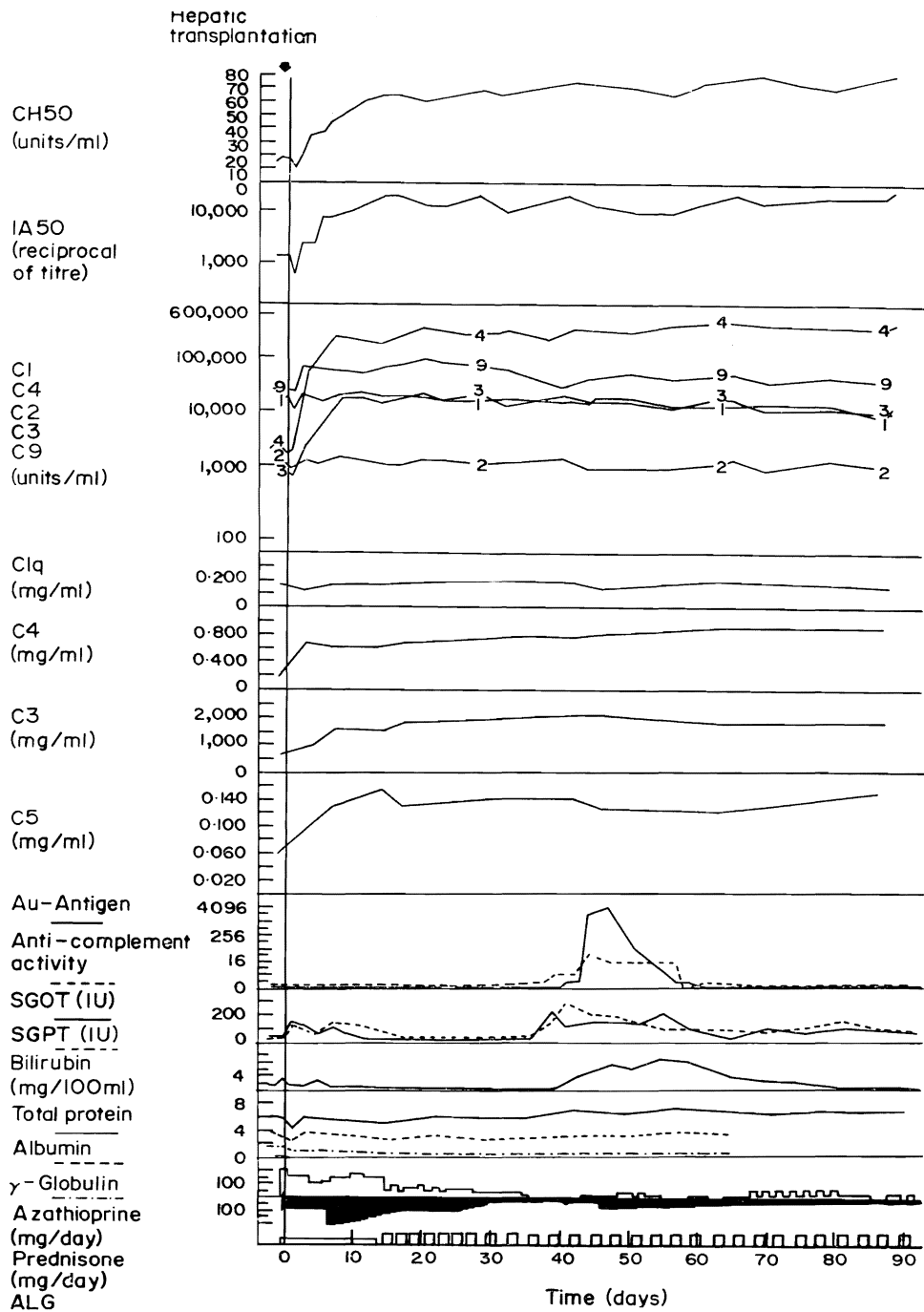


FIG. 3. Complement levels, Au-antigen assayed with complement fixation test and anti-complement activity (reciprocals of titres), liver function tests and immunosuppressive treatment in a patient (OT42) who developed Au-antigenaemia 6 weeks after transplantation. The deterioration in liver function was accompanied by a rise in anticomplement activity while complement levels were unaffected.



liver function tests 6 weeks after transplantation. At this time, complement levels remained unaffected while anticomplement activity rose significantly (Fig. 3).

Both the last mentioned patients are still alive 12 and 4 months after transplantation with excellent liver function and normal and supernormal complement levels, respectively.

The fifth patient (OT39), who suffered from Laennec's cirrhosis, had excellent homograft function until death from cerebrovascular insufficiency on the 26th postoperative day, although Au-antigenaemia was detected 5 days before demise. During the last days of life complement levels fell with C4 being the most affected component. Anticomplement activity was elevated (titres 1:4 and 1:32) throughout the postoperative course.

## DISCUSSION

The site of synthesis of the specific proteins constituting the human complement system has been obscure. In the 1950s, two investigators using now abandoned methods reported subnormal (Jordan, 1953) and normal (Mandel & Lange, 1955) complement levels, respectively, in patients with cirrhosis. More recently, Inai *et al.* (1967) found markedly depressed CH50 and C4 in three out of thirty cirrhotic patients and speculated on a hepatic origin of C4.

The extremely low preoperative complement levels in our five patients would be an indication of severely decompensated liver disease if a hepatic synthesis of complement could be proved. Circumstantial evidence that the liver is in fact a main site of synthesis was provided by the invariable restoration to normal levels of total complement, IA50 and some complement components after provision of a well functioning liver homograft. The C4 and C3 components were most markedly elevated and C5 also showed a significant increase. The fact that the changes in C4 function were more striking than those in the protein content could be attributed to some activation during handling. In contrast, no relation seemed to exist between hepatic function and the C1, C1q, C2 and C9 levels. Previously, a hepatic origin of C3 had been suggested by the observation of Alper and his associates (1969) who studied a liver transplant recipient whose C3 allotype changed to that of the donor. Moreover, the recent findings of Johnson, Alper, Rosen & Craig (1971) were consistent with a hepatic origin of C4.

After the first postoperative week, several factors in addition to hepatic function came into prominence of which the most important appeared to be rejection. It has been shown by Austen & Russell (1964) and others (Guiney, Austen & Russell, 1964; Carpenter *et al.*, 1967; Levine *et al.*, 1970) that renal homograft rejection is accompanied by a fall in complement. The data from two of our recipients of hepatic homografts showed the same pattern although interpretation in one of the cases was complicated by the fact that there was accompanying biliary tract obstruction.

In patients rejecting renal homografts, there has been little argument that falls in complement levels are due to an increased consumption by the immunologic reaction. This mechanism undoubtedly applies with hepatic homografts. However, an aggravating factor would be the coincident deterioration of hepatic function, if the liver were a site of complement synthesis as discussed above. Under these circumstances, the decline of complement components manufactured in the liver would be predicted to be considerably more drastic than during a comparable phase of renal homograft rejection. On the basis of limited observations this appeared to be the case, since the falls in total complement and C4 in patients

OT40 and OT41 were more severe than those reported in our kidney recipients (Yokoyama *et al.*, 1971).

Assay of the complement system, and particularly C4, might be a sensitive diagnostic aid in differentiating hepatic homograft rejection from other forms of hepatic dysfunction. Normal complement has been reported to occur in acute viral hepatitis (Inai *et al.*, 1967), unless the disease is complicated by arthritis (Alpert *et al.*, 1971), a finding borne out in one of our recipients. Moreover, biliary obstruction that developed in another patient did not cause significant complement depletion. Finally, no essential artefact is introduced into the complement analysis by post-transplantation immunosuppressive therapy, as pointed out by Austen & Russell (1964) and confirmed in our own recent studies of renal transplant recipients (Yokoyama *et al.*, 1971).

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