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Cytomegalovirus antibody production in renal transplant patients

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SUMMARY

Sera were examined from 50 patients on the renal transplant unit, Cambridge, for antibody against cytomegalovirus by complement fixation and by immunofluorescence for IgG and IgM antibodies.

The incidence of antibody on admission was 84% with a possible further 8% so that nearly all had been infected at some time by CMV.

43 (86%) patients showed evidence of active infection after admission, 39 by serology and four only from the examination of post-mortem material.

Twenty-one patients produced IgM antibody and production was prolonged for years in patients that survived. Antibody production was related both to transplantation and admission to hospital.

The evidence indicated that primary CMV infections were rare, that IgM antibody production was the result of active infection and that this could be attributed to reactivation without the need to invoke re-infection as the source although this type of patient is both susceptible and exposed to re-infection.

INTRODUCTION

The introduction of immunosuppression by the combination of corticosteroids and azathioprine, an analogue of 6-mercaptopurine, to reduce the rate of rejection of organ transplants was followed by an increase in the incidence of cytomegalovirus (CMV) infections in the recipients (Hedley-Whyte & Craighead, 1965).

It was soon found that other herpesviruses caused severe infections in renal transplant patients, e.g. varicella-zoster (Rifkind, 1966) and herpes simplex virus (Montgomerie *et al.* 1969) and as herpesviruses are known to produce latent infection the possibility of reactivation arose (Kanich & Craighead, 1966). However, immunosuppression also leads to increased susceptibility to infections of many kinds, including primary CMV infections (Bodey, Wertlake, Douglas & Levin, 1965) so that the problem arose of the part played by the two sources of virus, reactivated endogenous virus (Kanich & Craighead, 1966) compared with fresh infection (Craighead, Hanshaw & Carpenter, 1967). The demonstration that the post-perfusion syndrome (Kääriäinen, Klemola & Paloheimo, 1966) in open-heart surgery patients, not on immunosuppression, was due to CMV increased the likelihood that such CMV infections were exogenous, but even though infective

virus in donor blood was demonstrated by Kääriäinen *et al.* (1966) and others the relative parts played by reactivation and infection or re-infection continue to be the subject of discussion both in immunosuppressed (Andersen & Spencer, 1969; Armstrong *et al.* 1971) and other patients (Henle *et al.* 1970; Caul *et al.* 1971; Purcell *et al.* 1971).

As the work on renal transplant patients referred to above was based mainly on American studies it seemed of interest to compare a similar group in this country. The renal transplant unit in Cambridge has provided the opportunity and the work reported comprises a survey of the CMV antibody performance of 50 transplant recipients.

MATERIALS AND METHODS

The work described was based on the first 50 renal transplant patients from whom serum was obtained on admission, before transplantation and subsequently at intervals of about a month for an adequate period. This meant exclusion of patients that died in the early post-transplantation period before serological changes were expected to occur, so that apart from one who died after 5 weeks, but with significant serological change (M.McW.) the rest have been observed from 8 weeks to over 3 years. For simplification only the first transplant is considered, subsequent operations were not found to contribute greatly to the results obtained.

Complement fixation (CF) was performed in Perspex WHO trays by a micromethod based on Bradstreet & Taylor (1962). The antigen was the Rawles strain of CMV (Stern, Lambert & Shakespeare, 1963) grown in human embryo fibroblasts and supplied by Dr C. M. P. Bradstreet.

Fluorescent antibody (FA) staining was based on the sandwich technique used by Hanshaw, Steinfeld & White (1968), except that the Rawles strain was used instead of their A.D. 169 (Rowe *et al.* 1956) and grown in early passages of human embryo lung, the infected cells were stripped by 'Versene' 1/5000 for 5 min. followed by 0·025% trypsin for 2–3 min. then washed, transferred to slides and fixed. Twelve areas of cells per microscope slide were used so that titrations could be performed in parallel on the same slides. Sheep anti-human IgG and IgM from Wellcome Reagents Ltd. were used without absorption. Control positive and negative sera and uninfected cells were included in all titrations.

Rubella haemagglutination-inhibition (HI) titrations were done by the standard technique of Stewart *et al.* (1967) with removal of non-specific inhibitors by manganous chloride (Plotkin, Bechtel & Sedwick, 1968).

Post-mortem specimens and nephrectomy material were supplied by Dr P. D. Millard to whom I am also indebted for the histological data.

Virus isolation was attempted only from tissues, no attempt was made to examine other sources as these have been well documented by others (e.g. Craighead, 1969).

Isolation was attempted in early passage human embryo lung fibroblasts and WI-38 cells, supplemented occasionally by other fibroblasts. Sera from patients were collected by Dr D. B. Evans.

RESULTS

To establish that the anti-IgM conjugate was free of anti-IgG and vice versa high titre sera were compared and the conjugates were found to be pure.

Comparison of the mean CF titres of undiluted initial sera which produced the same degree of fluorescence of infected cells with anti-IgG, with the individual titres of the same sera showed a good correlation (Fig. 1). In other words the degree of fluorescence was proportional to the CF titre, which confirms Hanshaw *et al.* (1968) and Lang & Hanshaw (1969) who considered the CF titre of CMV antisera to be due to IgG.

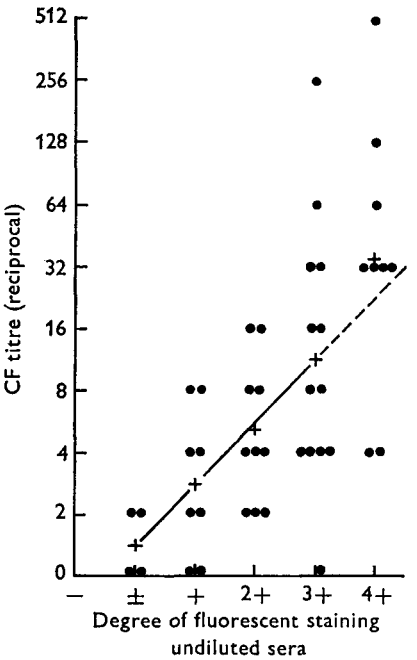

Fig. 1. Complement-fixation titre and fluorescent antibody titration of transplant patients' first sera. ●, Anti-IgG; +, mean CF titre of sera with same fluorescence.

The FA method for detection of IgG was, in general, slightly more sensitive than the CF test (Fig. 1) so that it was considered reasonable to regard a CF titre as low as 1/2 significant, provided that it was supported by specific IgG fluorescence (reading + or more on the Fig. 1 scale) and there was no anti-complementary activity. The use of CF titres of 1/2 is not usual because they can be unreliable, but because the detection of the lowest amount of antibody was essential for this study, to establish that previous infection had occurred, such titres were used with these safeguards. Earlier work in which titres of 1/2 were used include that of Craighead *et al.* (1967), Lang & Noren (1968), Andersen (1969) and Andersen & Spencer (1969).

Fluorescent antibody titres alone were considered significant provided control uninfected cells showed no fluorescence or, if they did, the titre with them was considerably less (i.e. at least eightfold) than with infected cells.

Incidence of antibody in patients

The number of patients with CMV antibody on admission was at least 42/50 (84%) and in all instances this was IgG. One patient only had a trace of IgM also. Four others had doubtful titres (Table 1).

This percentage is higher than Stern & Elek (1965) found by CF in the Greater London area in normal individuals (54%). It appears higher than in the Boston transplant patients examined by Craighead (1969) who found 41/63 (65%) with CF titres of 1/4 or more before transplantation. However if only CF titres of 1/4 or more are counted in the Cambridge patients this gives 33/50 (66%) positive which is exactly comparable, although CMV strain AD 169 was used for the American antigen. In the Boston patients the 30 to 39-year-old group was 86% positive and in our patients 84% (Table 1). Thus both series are practically identical, the main difference being a higher proportion in their under-20 years old group – 15 compared with our four patients.

Antibody titre changes

IgG antibody production. Because immunosuppression is essential after transplantation, azathioprine and prednisone were given to patients at a high dosage in the immediate post-operative period and adjusted later. This made it necessary to know if a change in titre was significant i.e. specific for CMV, or part of a general change with alteration of the immunosuppressive regime.

As a guide to general IgG production rubella HI antibody was selected because most of the patients were likely to possess it, production does not depend on the continued presence of virus, reactivation does not occur and titre changes due to transfusions were not likely to be significant.

Of the patients 47/50 (94%) had anti-rubella HI titres of 1/8 or more in their initial serum. This is similar to the incidence found in women of childbearing age in this area and as the ages of both groups are roughly the same it was felt that comparison of HI titres might show if the patients' titres were normal or not.

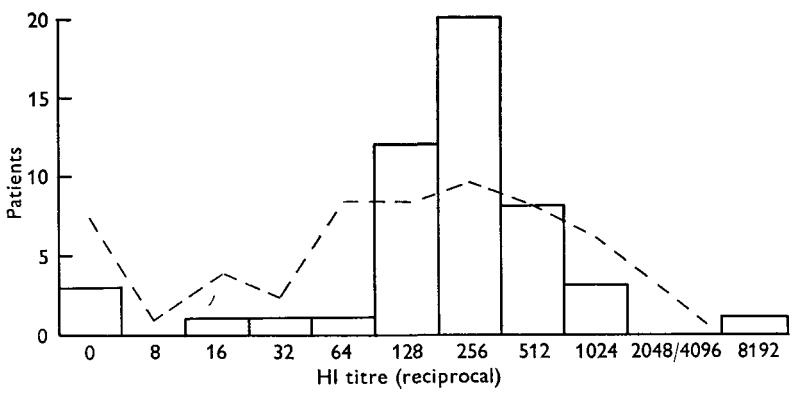

Fig. 2. Histogram of rubella HI titres of the transplant patients' initial sera. – – –, Profile of normal sera.

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Table 1. *Age distribution of antibody in initial sera*

Ages	Positive: CF and FA	Positive: FA only	Negative	Total	Positive (%)
10–19	2 (1)	[1]	0	4	50
20–29	11	1	2	14	84
30–39	15 (1)	2 [1]	2	21	84
40–49	5*	1	0	6	100
50–	5	0	0	5	100
	38 (2)	4 [2]	4	50	84

( ) = two patients with CF titre 1/2, FA negative.
[ ] = two patients with FA ± reading only with undiluted serum and CF < 1/2.
These four patients are not included in the 84 % total.
* = patient with trace of IgM.

Table 2. *Range of CF titres in the 42 positive patients and IgG FA*

CF titre (reciprocal)	Patients: FA positive	Patients: FA negative	Total
0	4	6	10
2	5	2	7
4	11	0	11
8	6	0	6
16	5	0	5
32	6	0	6
64	2	0	2
128	1	0	1
256	1	0	1
512	1	0	0
Totals	42	8	50

Table 3 (*A*). *Comparison of CMV CF and Rubella HI titre changes after transplantation in all patients*

		CMV–CF: Permanent	CMV–CF: Transient	Rubella–HI: Permanent	Rubella–HI: Transient	Rubella–HI: Terminal only
Change	Rise	32	6	5	4	1
	Fall	2	0	1	9	2
No change		10	.	28	.	.

Transient change = duration less than 3 months.
Terminal change = in sera during month patient died.

Table 3 (*B*). *Patients with synchronous titre changes*

(*a*) Steady increase in CMV antibody with transient initial HI rise	3 patients (K.B., B.B., T.H.)
(*b*) Steady increase in CMV antibody with permanent fourfold HI rise	1 patient (L.D.)
(*c*) Permanent parallel small increase in both CMV (0–4) and HI (256–1024)	1 patient (R.S.)
(*d*) Transient small rise (fourfold) in both	1 patient (A.L.)

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From Fig. 2 it can be seen that the initial HI titres of the patients do appear reasonably similar to those of the normal individuals.

The changes in CMV CF and rubella HI titres in the patients are shown in Table 3. Twenty-two patients had fourfold or greater HI changes but only five increases and one decrease lasted for more than 3 months. There were three patients with no detectable HI initially and two subsequently developed titres to a maximum of 1/64, the remaining patient had no detectable HI during 4 years observation. There were only six instances of parallel change in CF and HI titres (Table 3, *B*) and in five the CMV titre change was clearly significant and of a different kind to the HI. In only one patient (Table 3, *B*(*d*)) were the changes minor and equal so these were excluded from the data on titre changes.

From these results it was evident that the immunosuppressive treatment only caused minor variations in IgG production and they could readily be assessed from serial sera.

Cytomegalovirus antibody production

(*a*) *Extent of changes.* The CF changes in all 50 patients are summarized in Table 4 according to their initial IgG antibody levels. It will be seen that temporary production of a small amount of antibody was a feature of the group with little or no detectable antibody in their initial sera.

The patients that produced a considerable rise in titre did so to a ceiling of

Table 4. *CF titre changes in all 50 patients*

Maximum subsequent CF titre (reciprocal)	Initial CF titre (reciprocal)										
	0		2		4	8	16	32	64	128	256	512
1024	1				2	1	1	2				
512	1			1	1	2				1		1
256					1			1	2		1	
128		1	2	1	2		1	1				
64					1 (1)	1	2					
32	1							1				
16												
8	(1)					2		1				
4	1	(3)			3							
2				3								
0	1						1					
	FA−	+	−	+								

( ) = transient.

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1/128–1/1024 which explains observations that patients with high initial titres tend to produce smaller increases (Henle *et al.* 1970; Purcell *et al.* 1971; Caul *et al.* 1971).

(*b*) *Time of change.* To try to see which events might be associated with CMV antibody production the time of rise in titre of each patient was plotted against the time of the event. The main possibilities were: the start of peritoneal dialysis;

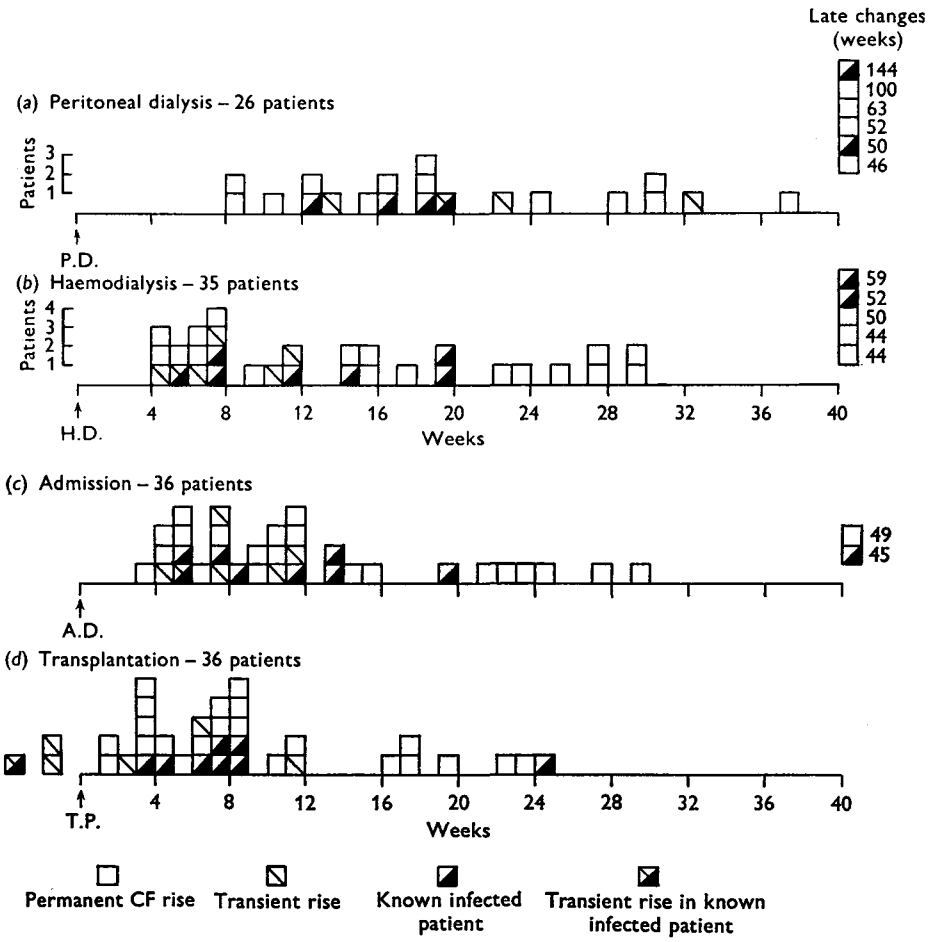

Fig. 3. Time of rise of CF titre in relation to peritoneal dialysis, haemodialysis, admission to hospital and first transplant.

haemodialysis; admission to hospital, and transplantation (Fig. 3*a–d*, Table 5). Blood transfusion was more difficult to assess, it occurred after admission and in some instances records are incomplete because of transfusions before patients were transferred to Cambridge. Peritoneal dialysis often preceded haemodialysis by weeks or months and was the first procedure in 32/37 patients. The appearance of Fig. 3(*a*) does not suggest that it was related to antibody changes. Haemodialysis was succeeded by a rise in titre after 4 weeks, an interval common to a number of reports dealing not only with renal transplantation (Craighead, 1969)

but post-perfusion (Henle *et al.* 1970; Caul *et al.* 1971) and post-transfusion (Purcell *et al.* 1971). It was associated with an earlier grouping of rises but they still extended for a long period. The relationship to admission was more definite as the majority of changes occurred 2–3 months earlier. Admission to hospital exposes a patient to infection from other patients, to the start of haemodialysis and blood transfusions and often precedes transplantation by only a day or two. It clearly could involve several factors. Transplantation (Fig. 3*d*) was preceded by three rises and three followed within 3 weeks afterwards. Transplantation could

Table 5. *Time of rise of CMV CF titre in relation to start of four procedures*

Procedure	Number of patients	Number with titre rise (including transient rises)	Number with rise within 12 weeks	Range of times to rise in weeks
Peritoneal dialysis	37	26	3	9–144
Haemodialysis	47	35	16	5–59
Admission	50	36	23	3–49
Transplantation	50	36	29	<4–25

Table 6. *Time between admission and transplantation, and start of haemodialysis and transplantation, arranged according to number of weeks after transplantation to rise of CF titre*

Weeks to CF titre rise after transplant	Weeks between admission and transplant			Weeks between start of haemodialysis and transplant		
	Patients	Average time	Range	Patients	Average time	Range
0–4	8	3·5	1–6	8	19	1–52
5–8	10	3·7	0–12	9	15	1–52
9–12	7	2·8	0–5	7	10·5	0–52
13–16	1	7	.	1	9	.
17–20	4	7	4–10	4	8	4–10
21–	3	8	0–20	3	18	4–27

not be the cause of the first three and may not have been the cause of all the second three changes as they are earlier than is usual. The distribution of rises in general was less extended than after admission so that transplantation appears to be a significant factor.

All three rises before transplantation were transient i.e. of less than 3 months duration, in patients with no detectable CF antibody and the titres produced were only 1/4 (2) and 1/8 (they are discussed further in the paragraph on patients without CF antibody). All had haemodialysis and transfusions in the weeks preceding their rises and these may have been the cause.

The three patients with rises within 3 weeks after transplantation all had IgG initially and two had a CF titre of 1/4. They produced CF rises to 1/64 (transient), 1/256 and 1/1028, with IgM also in the first and last, so they were similar to the majority of responses seen and may have been just slightly earlier than usual.

As haemodialysis preceded transplantation in this group of early risers the effect of haemodialysis on the whole group of patients was compared with the effect of admission (Table 6). It was found that the average time haemodialysis began before transplantation was 4 weeks later for each successive month that rises occurred in for the first 3 months after transplantation and the range of times before haemodialysis began was 0–52 weeks. This is not what would be expected if haemodialysis was a frequent precipitating factor.

The time from admission to transplantation was much more constant and the range less so that it was felt that the cause of the rises was related to admission.

This left admission and transplantation as the most important factors. Admission could lead to infection by cross-infection and by blood transfusions. Transplantation could lead to infection from the implanted kidney and from reactivation by the immunosuppressive treatment.

IgM antibody production

Twenty-one of the 50 patients produced CMV-specific IgM antibody, 20 after transplantation and one, who had a trace on admission, in increased amount. Nineteen of them produced IgG at the same time, either for the first time or in increased quantity, and in 12 the increase in titre of the two antibodies coincided. The relationship of IgM production to the initial CF titre is shown in Table 7.

Table 7. *IgM production and initial CF titre*

	Initial CF titre (reciprocal)										
	0	2	4	8	16	32	64	128	256	512	Total
Number of patients	10	7	11	6	5	6	2	1	1	1	50
IgM produced	6	3	2	3	1	4	1	0	0	1	21
IgM + CF rise	6 (2)	3	2 (1)	3	1	3	1	0	0	0	19 (3)
Total number with CF rise	10 (4)	4	8 (1)	4 (1)	4	4	2	1	0	0	37 (6)

( ) = proportion of patients with transient rise in titre.

When the patients were divided into two groups with highest initial CF titres in one and lowest in the other there was no difference in frequency of IgM production. A patient with a low or absent initial CF titre was therefore no more or less likely to produce IgM than a patient with a high initial CF titre.

Half of the patients with transient CF rises produced IgM so that a transient CF rise may be as significant as a longer lasting one.

The production of IgM in relation to the time of CF rise after admission and transplantation is shown by Fig. 4 to be evenly distributed. The duration of IgM production appeared to be prolonged and was frequently found to last for 2 years or more (Table 8), the longest duration recorded so far is 136 weeks and cessation of production has not yet been noted.

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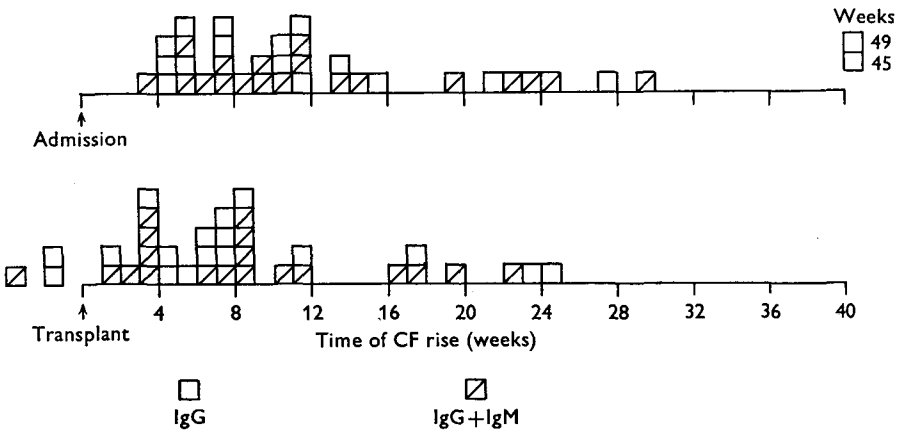

Fig. 4. IgM producers in patients with CF antibody rise, relationship to times of admission and transplantation.

Table 8. *Duration of IgM production*

	Observations still in progress	Patient died or transferred
0–1 year	7	5
1–2 years	3	3
2–3 years	3	0

Table 9. *Comparison of titre changes in 15 infected patients with the rest*

CF and IgG titres	Known infected patients IgM		Remainder of patients IgM	
	Positive	Negative	Positive	Negative
No change	1	3	1	6
Transient rise	1	0	3	2
Permanent rise	4	5	11	11
Fall	0	1	0	1
Total	6	9	15	20

Infected patients

To see if IgM production was mainly found in those patients with evidence of infection by virus isolation or histology these were compared with the rest of the patients (Table 9).

Fifteen patients were found to be infected; nine by examination of 15 post-mortems; one at post-mortem and own kidney at nephrectomy 3 weeks earlier; two from allografts removed for rejection (4 and 24 weeks after transplantation) and three nephrectomy specimens of the patients' own kidneys. The kidney of one of these was removed 11 weeks *before* transplantation.

The absence of IgM did not mean the absence of infection because it was not found in nine of the 15 infected patients.

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Various tests were carried out to discover the fate of the oxalate when milk was admixed with both distilled water infusions and calcium-loaded infusions in the ratio of milk : infusion = 1:5. Following domestic practice, the mixtures were prepared either by pouring the infusions onto the milk, or vice versa, and stirring briefly. The mixtures were then allowed to stand for 10 min. Determination of the oxalate content of the mixtures showed that more than 90 % of the oxalate remained within the body of the mixture. Further checks on the oxalate content both of the undisturbed bottom layers of mixtures held in tea-cups for 10 min., and also of any material deposited on the surfaces of the cups, confirmed that less than 10 % of total oxalate had settled out of the mixtures.

Phytic acid and phosphate

Using a method capable of detecting phytate if present at concentrations above 5 mg./15 g. leaf, no measurable phytate was found in 0·5 N-HCl extracts of four teas (A, C, E, G). This result indicates that, in the process of calcium uptake by the leaf in calcium-loaded infusions, deposition of calcium phytate would not account for more than 1 mg. Ca/15 g. dry leaf. Determinations of the phosphate content of distilled water and calcium-loaded infusions carried out for one tea sample (A) showed that the presence of calcium was without influence on the total phosphate extracted (phosphate content of infusion: 25·0 mg. P/l.).

DISCUSSION

The 'hardness' of drinking waters is due to their content of calcium and magnesium ions. In the majority of waters in the United Kingdom calcium is the principal component of 'hardness', the calcium content ranging from less than 1 mg. Ca per litre in very soft waters to about 150 mg. Ca per litre in the hardest of waters normally supplied (Skeat, 1961). An important fraction of the calcium in most waters is present as calcium bicarbonate ('temporary hardness'), and during the boiling of waters for the preparation of tea infusions some loss of calcium from solution, by deposition as carbonate, will generally occur for waters containing more than about 10 mg. calcium per litre. An indication of the extent of this loss is provided by Hollins (1965) who found that, when solutions containing equivalent amounts of calcium and bicarbonate ions were heated to boiling over a 5 min. period and boiled for a further 3 min., between 10 and 20 % of the calcium was deposited from solutions which had starting concentrations of 50 and 100 mg. calcium per litre. The present studies show that a further substantial loss of calcium occurs from drinking-waters during the preparation of tea infusions due to calcium uptake by the tea leaf. The general pattern of this uptake over the range of calcium concentration normally found in drinking-waters is indicated by the curve in Fig. 1. As outlined in the Introduction, our primary concern here is with the influence of the calcium on the composition of the infusions, and for the present purpose it will suffice to note that calcium uptake was shown in similar degree by all the teas examined and that the uptake process was essentially unaffected by the presence of any of the other principal ions normally found in drinking-waters.

Group 2. The remaining six patients failed to produce appreciable CF antibody. In two (D.B., M.McW) perhaps because the sera were collected within a month of death, a further rise might have occurred had they survived; in one the trace of CF antibody was maintained for months afterwards, but in the other three all the rises were pre-transplantation and transient.

One of these (M.O'M.) died with widespread CMV infection and could have been a primary infection, unable to produce more IgG because of immunosuppression, although she produced some IgG as judged by FA, before and after transplantation. Her antibody titres are shown in Fig. 5.

No post-mortem specimens were available from the other patient who produced IgM (D.B.). He may also have been a primary infection.

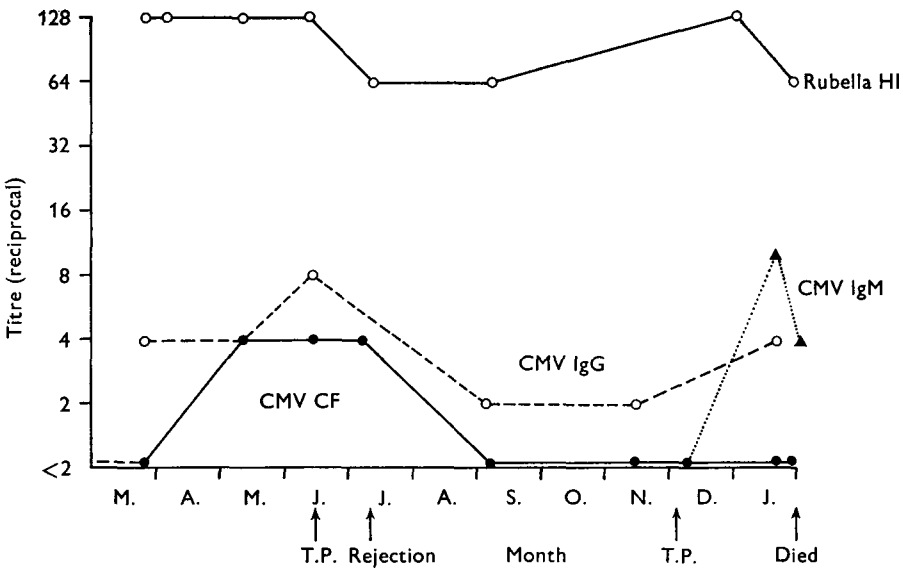

Fig. 5. Antibody production in patient M.O'M.

DISCUSSION AND CONCLUSIONS

In the work described the FA method was a useful supplement to CF and together they provided evidence that the incidence of previous CMV infection was at least 84% and possibly up to 92%. The antibody production by some CF-negative patients with no IgG or only a doubtful trace by immunofluorescence suggested that they may also have been previously infected so that the incidence of infection before transplantation was probably higher still.

The absence of CF antibody is inadequate evidence that previous CMV infection has not occurred which Weller (1970) has emphasized. The technique is not highly sensitive and the neutralization test is probably better. It may well be significant that wherever similar groups are examined before surgery the maximum incidence of CF-positives, which occurs usually in the group aged 30 years and over, is in the region of 80–86%. In Cambridge we found 84%, in Boston there

were 86% (Craighead, 1969), in Washington 81% (Rowe *et al.* 1956) and in Bristol there were 86% (Caul *et al.* 1971). When younger patients are included in the groups the total is again similar – Cambridge 66%, Boston 65%, Bristol 64%, in blood donors in Rumania 64% (Diosi, Moldovan & Tomescu, 1969) and in Finland 66% (Klemola, von Essen, Paloheimo & Furuhjelm, 1969).

It is known that neutralizing antibody is more common than CF antibody in the normal population (Carlstrom, 1965) and that the CF antibody titre can fall to undetectable levels in some children 2 or 3 years after infection (Starr, Calafiore & Casey, 1967; Lang & Noren, 1968; Andersen, 1969) so it seems highly probable that at least some of the 14% or so of adults without CF antibody are individuals in whom infection has occurred and the CF titre has subsequently fallen. This is supported by Andersen & Spencer (1969) who found neutralizing antibody in seven of their 10 renal transplant patients who had no CF antibody in their initial sera. The figure of 86% may represent a population maximum for the CF method.

The ability of the renal transplant patients to produce IgG was not greatly affected by immunosuppressive therapy when they had had their primary infection before immunosuppression started, as was the case with rubella, and this result is in agreement with the work of Rowley, Mackay & McKenzie (1969) who examined the immune response to a bacterial antigen in Melbourne patients on a comparable immunosuppressive regime to ours.

The production of CMV IgG was not greatly affected by immunosuppression in most patients, which is what would be expected. Immunosuppression was a possible reason for the absence of production in some of the patients with no detectable initial CF antibody who may have been primary infections or may have had practically no residual capacity for CMV IgG production.

The production of CMV IgG was as common in the patients not known to be infected as it was in those found to be infected from culture or histology of nephrectomy and post-mortem specimens, which suggested that they were equally infected. Not all of the infected patients produced IgM, perhaps because there was insufficient antigenic stimulus, perhaps related to circulating IgG. However, when IgM was produced it continued to be formed steadily for long periods, perhaps for as long as the patient survived because none have so far become negative and the longest recorded period of synthesis is 136 weeks.

This is also compatible with the observations of Rowley *et al.* (1969) who found that repeated antigenic stimuli under immunosuppression could cause primary type responses with IgM predominating, because this immunosuppressive technique is less effective for the inhibition of IgM than it is against IgG. Lang & Hanshaw (1969) cite Uhr & Finkelstein (1967) for the demonstration that repeated small doses of antigen can produce an IgM response alone in patients not on immunosuppression.

Thus IgM production implies constant or repeated production of antigen and therefore active infection.

In patients that are not immunosuppressed CMV IgM production is taken as evidence of primary infection, especially when there are no demonstrable initial

antibodies (e.g. Lang & Hanshaw, 1969; Caul *et al.* 1971) and in immunosuppressed patients evidence of primary infection was suggested from the observations of Craighead *et al.* (1967).

It is important to stress that in this group of immunosuppressed patients IgM synthesis does only mean active infection. It cannot be taken further and considered evidence for primary infection because nearly all have evidence of having had their primary infection in the past.

It is not possible to say that any were unequivocal primary infections in this series. There were no patients without CMV antibody initially who then produced IgM during immunosuppression, but little or no IgG (patients D.B. and M.O'M. in Table 10 are the nearest, with four others as possibles, M.McW., R.S., J.R. and M.S.).

Herpesviruses are known to be capable of reactivation, especially during immunosuppression (Rifkind, 1966; Kanich & Craighead, 1966; Montgomerie *et al.* 1969) and this could explain the active CMV infections seen in this group without the need for re-infection to have occurred (Carlstrom, 1965; Anderson & Spencer, 1969). Kanich & Craighead (1966) did not find evidence of CMV infection in those renal transplant patients not given immunosuppressives.

However, immunosuppressed patients are rendered susceptible to fresh infection as is only too well known and their environment contains infective CMV (Craighead, 1969) as well as do many of the kidneys transplanted and at least 5% of units of fresh blood they may be given (Diosi *et al.* 1969; Henle *et al.* 1970; Klemola *et al.* 1969). The situation is more complex than post-perfusion for open-heart surgery where primary infections have been described (Henle *et al.* 1970; Lang & Hanshaw, 1969; Paloheimo *et al.* 1968) yet even here reactivation is thought to be a factor by some (Klemola *et al.* 1969; Caul *et al.* 1971) whilst others maintain reactivation is the main factor (Purcell *et al.* 1971).

To resolve this argument detailed comparison is required between the primary infecting CMV strain and virus recovered subsequently. There are likely to be a range of antigenic differences between strains (Weller, Hanshaw & Scott, 1960; Dreesman & Benyesh-Melnick, 1967; Krech & Jung, 1969) but it does appear from the preceding observations that reactivation could be a major factor in the series described.

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