they could conceivably have an extrarenal cause for the hypertension. It is well known that renin, angiotensin I, and angiotensin II are measurable in the blood of many anephric patients (Medina *et al.*, 1972).

From the data presented here it would seem reasonable to conclude that the presence of diseased kidneys alone is sufficient to sustain hypertension in some transplant patients in the presence of a normally functioning transplant. This would suggest that the normal kidney does not produce a depressor substance but the diseased kidneys in spite of their minimal blood flow are still able to produce a pressor effect.

It is of interest that the mean renal function in all our non-nephrectomized recipients was better than in the nephrectomized patients compared six months after transplantation. This could possibly be related to improvements in technique, as the patients who retained their kidneys were transplanted more recently.

Finally, in non-nephrectomized recipients, if hypertension persists despite good transplant function, if rejection and renal artery stenosis are excluded, bilateral nephrectomy may be indicated. In the four patients in this series who were bilaterally nephrectomized for hypertension after transplantation (table IV), hypertension became significantly easier to control in two, both of whom had good renal function. In the other two patients hypertension was ameliorated only temporarily by removal of their original kidneys. Five months after the bilateral nephrectomy irreversible decline in transplant function occurred in one patient who had become severely hypertensive again. Histology of this transplant showed severe obliterative arterial lesions. As none of the transplants in the other three patients

were biopsied the possible contribution of vascular lesions in the kidney to their hypertension remains conjectural.

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Immunological Function in Dystrophia Myotonica

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Summary

Humoral and cellular immunity have been investigated in 15 patients with dystrophia myotonica. No abnormalities in total serum levels of the five major immunoglobulin classes were found but there was a rise in the mean serum level of β_1A complement. Altogether, 54% of patients failed to make antibody to tetanus toxoid as compared with 1% of controls: 13% of patients failed to make antibody to *Salmonella typhi* H antigen as compared with no failure of this function in control subjects. There was a reduced uptake of tritiated thymidine by whole blood lymphocyte cultures spontaneously, while in the presence of phytohaemagglutinin (PHA) and both autologous and fetal calf serum the uptake was normal. It is suggested that there may be a wider derangement of immunological function in dystrophia myotonica than previously thought.

Introduction

Reduced serum levels of gammaglobulin have been found in many patients with dystrophia myotonica by some workers (Lowenthal and van Sande, 1956; Kuhn and Weicker, 1957) but not by others (Oppenheimer and Milhorat, 1961). These reduced levels were observed to be associated with a reduction in the half life of ^{131}I -labelled gammaglobulin (Zinneman and Rotstein, 1956). With the delineation of the various immunoglobulin classes it was found that there were reduced serum levels and increased catabolism of immunoglobulin (Ig) G (Wochner *et al.*, 1966), though a more recent report suggested that serum levels of IgM may also be reduced (Bundey *et al.*, 1970). These abnormalities prompted a more comprehensive investigation of immunological function in this disease.

Patients and Methods

Altogether, 15 patients were studied, including two pairs of siblings and one mother and daughter. Each patient had characteristic clinical features and showed the classical electromyographic changes of dystrophia myotonica. It is difficult to be certain of the duration of clinical symptoms, but the average was 10 years and ranged from five to 16 years. There were eight men and seven women, with ages ranging between 30 and 71 years (see table II). Two patients were in institutions, the rest living at home. Two patients were receiving digoxin and diuretics for congestive cardiac failure and one was receiving bronchodilators for chronic bronchitis. One patient suffered with rheumatoid arthritis but was receiving no therapy, while

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another had peripheral vascular disease. Three were taking respectively quinine bisulphate, isopropamide iodide, and an aspirin-codeine mixture. Patients were questioned about their previous immunization history. Most believed that they had been immunized with tetanus toxoid though none remembered being immunized with typhoid vaccine.

Immunological measurements were made according to a variation of a previously described regimen (Forbes, 1971). Serum levels of immunoglobulins IgG, IgA, and IgM, and β_1A complement were measured using Behringwerke immuno-diffusion plates. IgD and IgE were measured by radioactive single radial diffusion using the method described by Rowe (1969). The IgE standard was from a batch of pooled human serum, 69/204, supplied by the World Health Organization. IgD Standard (Behringwerke) was referred to British Research Standard No. 67/37.

Patients were immunized with tetanus toxoid (Commonwealth Serum Laboratories (C.S.L.)) 0.5 ml subcutaneously and typhoid vaccine (C.S.L.) 0.1 ml subcutaneously and blood was collected two weeks later. Haemagglutinating antibodies to tetanus antigen and precipitating antibodies to *Salmonella typhi* antigen were measured before and after treatment with mercaptoethanol.

Delayed hypersensitivity reactions to intradermal injections of 0.1 ml of candida (C.S.L.), mumps (Eli Lilly), and Varidase (Lederle) were measured at 48 hours.

Phytohaemagglutinin (PHA) induced uptake of tritiated thymidine by lymphocytes: cultures contained 0.2 ml whole blood, 3.0 ml HEPES-buffered medium 199 (C.S.L.), 0.02 ml PHA (Wellcome, Reagent Grade), and 0.4 ml of fetal calf serum (C.S.L.) or autologous serum. Triplicate cultures were incubated for four days at 37°C. At 92 hours 2.5 μ Ci tritiated thymidine (specific activity 500 mCi/mmol) were added and incubated for another four hours. Cultures were kept at 4°C for half an hour, centrifuged at 250 g for 10 minutes, washed with 3% acetic acid, 0.9% saline, and 5% trichloroacetic acid in 0.45% saline, centrifuged at 1,000 g for 20 minutes with 5% trichloroacetic acid and twice with methanol. The residue was dissolved in 0.5 ml Soluene (Packard) for liquid scintillation counting. The results were expressed as disintegrations/culture tube/minute.

The spontaneous uptake of tritiated thymidine by cultures—that is, in the absence of PHA—was assessed by incubating 0.2 ml of whole blood in 3.8 ml of medium with 2.5 μ Ci of tritiated thymidine at 37°C for four hours and treated as above.

Each patient was age and sex matched by taking the first appropriate person from a bank of controls. This bank was comprised of normal people together with a few patients with vascular and psychiatric disease.

FIG. 1—Titres of antibody to tetanus toxoid before and after immunization. Each line represents an individual patient, with dot on left being pre-immunization titre and that on the right representing postimmunization titre. Titre is $\log_2$ of reciprocal of highest dilution at which haemagglutination was observed. Results were highly significant ($P < 0.002$, Fisher's exact test).

Results

Serum immunoglobulin levels were not significantly different in patients and control groups (table I). Serum β_1A complement levels in the patient group were higher.

TABLE I—Levels of Serum Immunoglobulins and β_1A Component of Complement

	No.	Mean*	S.D.	Probability†
IgG:				
Patients ..	15	1,120	343	N.S.
Controls ..	15	1,200	327	
IgA:				
Patients ..	15	231	68	N.S.
Controls ..	15	216	69	
IgM:				
Patients ..	15	118†	60	N.S.
Controls ..	15	104†	80	
IgD:				
Patients ..	15	36†	39	N.S.
Controls ..	15	15†	66	
IgE:				
Patients ..	15	87†	50	N.S.
Controls ..	15	99†	65	
β_1A complement:				
Patients ..	15	152	35	<0.05
Controls ..	15	124	35	

*IgG, IgA, IgM, and β_1A complement expressed as mg/100 ml. IgD and IgE expressed as units/ml.

†Geometric mean.

‡Student's *t* test.

N.S. = Not significant.

Antibody responses at two weeks after immunization are shown in figs. 1 and 2; no antibody to tetanus was detectable, in eight subjects, while two patients failed to make antibody to *Salm. typhi*. Mercaptoethanol treatment of serum reduced

FIG. 2—Titres of antibody to *Salm typhi* H antigen before and after immunization, plotted similarly to fig. 1. Titre is reciprocal of highest dilution at which agglutination was observed. Zero point actually indicates no agglutination at a dilution of 1 in 20. Results were not statistically significant.

typhoid antibody titres to zero or very low levels, while leaving tetanus antibody titres relatively unchanged. There is considerable overlap when the ages of the tetanus responders and non-responders are compared (table II).

Delayed hypersensitivity skin reactions to the three antigens are shown in table III. The two patients who failed to make a reaction against any antigen were brothers.

Spontaneous uptake of tritiated thymidine was lower in dystrophic patients (table IV). PHA-induced uptake of tritiated thymidine by lymphocytes was normal in the presence of both fetal calf serum and autologous serum.

Each parameter was compared in tetanus responders and non-responders. No significant differences were found.

TABLE II—Age and Tetanus Responsiveness

					Ages
Tetanus responders	..	..	..	..	30, 38, 47, 49, 53, 56, 60
Tetanus non-responders	..	..	..	..	41, 45, 54, 55, 55, 65, 65, 71

TABLE III—Skin Test Response

	Responders	Non-responders	Percentage of Responders	Probability*
Patients	13	2	87	} N.S.
Controls	15	0	100	

*Fisher's exact test.
N.S. = Not significant.

TABLE IV—Tritiated Thymidine Uptake

	No.	Geometric Mean*	S.D.	Probability†
Spontaneous uptake:				
Patients	15	262	168	} <0.05
Controls	15	382	150	
PHA uptake—autologous serum:				
Patients	15	56,500	41,200	} N.S.
Controls	15	69,700	45,000	
PHA uptake—fetal calf serum:				
Patients	15	39,700	29,400	} N.S.
Controls	15	45,100	24,000	

*Disintegrations/culture/minute.
†Student's *t* test.

Discussion

Dystrophia myotonica is a multisystem disorder. Reports of abnormalities in IgG serum levels and metabolism suggested it would be worth investigating other parameters of immunological function.

Contrary to several reports, no significant differences in the serum level of IgG were found. Oppenheimer and Milhorat (1961) also failed to find reduced IgG levels. In series which reported low levels of IgG in the dystrophic group, many individual patients had values which fell within the normal range. This suggests that measurement of serum immunoglobulin levels is a crude index of immunological abnormality in these patients. No significant differences were found with the other immunoglobulin classes. Waldman *et al.* (1972) also found no change in IgE levels. The mean level of the β_2A complement was raised in dystrophic patients. The significance of this is obscure.

Perhaps the most striking finding of this investigation was the failure of about 50% of patients to make antibody to tetanus antigen. It should be stressed that this represents not merely a failure to increase antibody titre after immunization but inability to produce any detectable tetanus antibody. Most South Australians have previously been exposed to tetanus toxoid, and the absence of change in titre after treatment of the serum with mercaptoethanol, is consistent with this immunization producing an IgG secondary response. Many patients with other diseases who failed to make tetanus antibody have been re-immunized and a proportion produced tetanus antibody (Forbes, 1971). We chose not to submit these patients to further testing. Measurement of antibody response to tetanus toxoid at two weeks is probably a relatively sensitive test of humoral immunological capacity. It seems unlikely that the failure to make

antibody is related to age, as the ages of the group responding to tetanus immunization are similar to those of the group which did not respond. Moreover, all patients were age matched with controls, all of whom did respond. There is no evidence that failure to respond could be attributed to intercurrent infection or drug therapy. The measurement of a specific IgG antibody response may be a more sensitive indicator of impaired IgG production than the measurement of total serum IgG levels.

Failure of two subjects to make antibody to *Salm. typhi* is additional evidence of impairment of humoral immune responses. In our experience *Salm. typhi* is a potent antigen, and failure to make this antibody is always associated with clinically apparent disease.

No statistical indication of impaired cellular immunity was found in this study, though two patients, brothers, failed to make delayed hypersensitivity skin reactions. The uptake of tritiated thymidine by PHA-stimulated lymphocytes was normal in these two patients, as in the group as a whole.

The spontaneous uptake of tritiated thymidine was reduced. It is probable that this parameter is a measure of the activity of circulating immunoreactive cells (Crowther *et al.*, 1969; Horwitz *et al.*, 1970). The same phenomenon of reduced spontaneous uptake has been observed in asthmatic patients who failed to respond to tetanus immunization (Grove and Forbes, 1973). It has previously been shown that the catabolism of IgG is increased in dystrophia myotonica (Wochner *et al.*, 1966). These authors concluded that the synthesis of IgG was normal. Reduced spontaneous uptake of tritiated thymidine together with impaired response to tetanus immunization suggests, however, that synthesis of specific antibody may be reduced in this condition.

Dystrophia myotonica is inherited as an autosomal dominant trait and often does not present until later life (Walton, 1969). It may be informative to study the capacity of young members of afflicted families to produce specific antibody. It is possible that such a failure may antedate clinical presentation of the disease.

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