

## Preliminary Communications

### Lymphocyte Transformation in Thymectomized and Nonthymectomized Patients with Myasthenia Gravis\*

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Nastuk *et al.* (1960) and Simpson (1960) first suggested an autoimmune basis for myasthenia gravis. It is now well established that about one-third of myasthenics have circulating antibodies which cross-react with skeletal muscle and thymus (Osserman and Weiner, 1965; Strauss *et al.*, 1965), and that there is a high incidence of antinuclear factor (White and Marshall, 1962; Sturgill *et al.*, 1964) and of antibodies directed against tissues other than skeletal muscle and thymus (Adner *et al.*, 1964; Downes *et al.*, 1966).

The reported depression of serum complement level during clinical exacerbations (Nastuk *et al.*, 1960) suggests that an antigen-antibody reaction is taking place, but so far there is no evidence that the circulating antibodies to skeletal muscle and thymus play any pathogenic part. The fact that they are absent in most patients and that they have been found in 12 out of a group of 51 patients with thymomas but not suffering from myasthenia (Strauss *et al.*, 1966) makes such a role unlikely. The presence of lymphorrhages in skeletal muscle (Russell, 1953), however, suggests that the immunological reaction could be lymphocyte-mediated. Therefore we have tested the response of peripheral blood lymphocytes from myasthenic patients to homogenates of muscle and thymus. We have also compared the capacity of lymphocytes of thymectomized and nonthymectomized myasthenics to react *in vitro* to phytohaemagglutinin and various antigens to detect possible impairment of immunological competence secondary to absence or dysfunction of the thymus.

#### MATERIAL

*Patients.*—Six patients were investigated, all with generalized myasthenia gravis; three had been thymectomized 3, 6, and 18 years previously; all had normal peripheral white blood cell counts. Clinical details are given in Table I.

TABLE I.—Details of Patients

Case No.	Age	Myasthenic Status	Thymic Status	Muscle Anti-body Titre	Other
1	29	Improving	Thymectomy in 1960; hyperplastic	1 in 20	
2	57	Static	Thymectomy in 1963; hyperplastic	0	
3	49	"	Thymectomy in 1948; ? histology	0	Rheumatoid arthritis.
4	59	"	Not thymectomized; normal chest x-ray	1 in 20	Antinuclear factor Hypergammaglobulinaemia
5	31	"	"	1 in 20	
6	28	Deteriorating	"	1 in 20	Antinuclear factor

*Control Subjects.*—Five healthy adult laboratory staff (three male, two female) aged 21 to 33 (mean 27 years) were all tuberculin (P.P.D.)-positive.

*Phytohaemagglutinin* (P.H.A.). Wellcome brand; 0.06 ml. added per 3 ml. culture.

\* From the Department of Experimental Pathology, Medical School, Birmingham 15.

*Purified protein derivative* (P.P.D.). Ministry of Agriculture, Food and Fisheries, Weybridge, England; 10 µg./ml. cell suspension.

*Streptolysin O reagent* (S.L.O.). Difco Laboratories, Detroit, Michigan, U.S.A. 0.25 ml. (1 in 25 dilution) per 3 ml. culture.

*Staphylococcal filtrate* (S.F.), supplied by Dr. N. Ling, Department of Experimental Pathology, University of Birmingham, 0.5 ml./3 ml. culture.

*Polystyrene latex*. Dow Chemical Company, Midland Michigan, U.S.A., diluted 1 in 100 with 0.15 M NaCl, 0.033 ml./3 ml. culture.

*Eagle's Medium*.

*Normal Thymus homogenate* (N.T.H.). Portion of normal thymus, removed from 6-year-old boy undergoing cardiac surgery, homogenized in the cold with an equal volume of 0.15 M NaCl in a Vertis blender and stored at -20° C. 0.3 ml. added per 3 ml. culture.

*Myasthenic thymus homogenate* (M.T.H.) and *normal muscle homogenate* (N.M.H.) prepared in the same fashion from operation specimens.

*Tritiated thymidine* (TdRH<sup>3</sup>), the Radiochemical Centre, Amersham, England. Specific activity 3 Ci/mM.

*Whole live muscle* (W.L.M.). Biopsy material, less than one hour old, cut up finely with scissors. 0.3 g. added per 3 ml. culture.

*Thymus culture supernatant* (T.C.S.). Supernatant from 6-day-old cultures of normal thymus cells, 2 × 10<sup>6</sup>/ml. in 20% autologous plasma and 80% minimal essential medium, stored at -20° C.; 0.5 ml./3 ml. culture.

*Immunofluorescent Test for Skeletal Muscle Antibodies.*—Indirect method using 4-µ frozen sections of rat abdominal wall muscle and fluorescein-conjugated rabbit specific anti-human IgG serum. Occasional faint striational staining seen with normal sera up to 1 in 10 dilution. Generalized staining at serum dilutions greater than 1 in 10 taken as positive.

#### METHODS

*Preparation of Leucocyte Cultures.*—50 ml. of blood was withdrawn and anticoagulated with 500 units of heparin. The red blood cells were gravity-sedimented at 37° C. at an angle of 15 degrees from the horizontal for one to two hours and the white-blood-cell-rich plasma was aspirated. Differential counts revealed 50% or more lymphocytes. 3-ml. cultures were set up containing 1,500-3,000 cells/cu. mm. in Eagle's medium with 20% autologous plasma. The cultures were incubated at 37° C. for five days with three of the patients (one thymectomized, two nonthymectomized) and for seven days (with renewal of culture medium on the third day) with the other three (two thymectomized, one nonthymectomized). Tritiated thymidine 2 µCi was added to each culture 15 hours before harvesting. Identical cultures from normal control subjects were set up on each occasion.

*Harvesting.*—The cultures were shaken and 1-ml. portions removed from each and centrifuged at 100 g for 10 minutes; smears were made from the cell buttons and stained with Jenner-Giemsa. The remainder of each culture was cooled in ice and processed for total tritiated thymidine incorporation by the method of Dutton and Eady (1964). The slides were

analysed as "unknowns" by one of us (J.H.); at least 200 cells were counted on each slide and transformed lymphocytes differentiated from small lymphocytes and phagocytic macrophages.

### RESULTS

Both thymectomized and nonthymectomized myasthenics responded equally well to phytohaemagglutinin stimulation as measured by both deoxyribonucleic acid (D.N.A.) synthesis (50- to 100-fold increase over unstimulated cultures in patients and controls (see Tables II and III), and by blast formation (thymectomized patients, mean 48% with range of 13% to 70%, versus controls, mean 49% with range of 26% to 64% ;

TABLE II.—D.N.A. Synthesis and Blast Transformation in Thymectomized Patients and Control

Culture	D.N.A. Synthesis in c.p.m.		Per cent Blast Transformation		
	Mean	Range	Mean	Range	
Nil added	3,955	434-8,360 1,700-11,440	0.5	0-1.0	Patients Controls
	5,577 P > 0.5		0		
P.H.A.	210,260	158,000-285,000 155,000-256,000	48	13-70 26-64	Patients Controls
	193,000 P > 0.5		49 P > 0.5		
S.F.	77,860	22,800-124,500 9,190-22,580	13.5	0-30.5 7-12	Patients Controls
	18,060 0.5 > P > 0.2		9 P > 0.5		
S.L.O.	56,620	8,260-121,000 1,318-128,500	19	6-32 19-29.5	Patients Controls
	47,072 P > 0.5		22.8 P > 0.5		
P.P.D.	50,613	4,860-144,700 14,300-131,200	19	0-57 3-38	Patients Controls
	59,483 P > 0.5		19 P > 0.5		
N.T.H.	2,909	217-7,950 302-8,710	0.33	0-1.0 0-1.5	Patients Controls
	3,543 P > 0.5		0.5 P > 0.5		
M.T.H.	2,909	217-7,950 159-9,350	0	— 0-2.5	Patients Controls
	4,254 P > 0.5		1.3		
N.M.H.	4,475	1,075-8,070 998-8,620	0	— 0-2	Patients Controls
	3,520 P > 0.5		1.10		
T.C.S.*	7,940	—	0	—	Patients Controls
	8,620		1.5		

\* Only one patient and control.

TABLE III.—D.N.A. Synthesis and Blast Transformation in Non-thymectomized Patients and Controls

Culture	D.N.A. Synthesis in c.p.m.		Per cent Blast Transformation		
	Mean	Range	Mean	Range	
Nil added	3,015	106-7,400 530-3,578	0.17	0-0.5 0-1.5	Patients Controls
	3,067 P > 0.5		0.5 0.5 > P > 0.2		
P.H.A.	286,800	128,000-486,000 46,000-266,000	62	60-65 52.5-67.5	Patients Controls
	155,330 0.5 > P > 0.2		61.3 P > 0.5		
S.F.	84,844	1,033-173,600 3,720-12,700	23.5	25-42 4-7	Patients Controls
	8,533 0.2 > P > 0.1		5.3 0.2 > P > 0.1		
S.L.O.*	183,400	34,800-332,000 1,398-48,400	21.25	12.5-30 10.5-20	Patients Controls
	24,859 P > 0.5		15.25 P > 0.5		
P.P.D.	38,700	29,400-44,000 32,950-194,000	11.3	4-16 16-30	Patients Controls
	104,116 P > 0.5		24.5 P > 0.5		
N.T.H.	353	104-444 302-1,062	3.3	0-10 0-4	Patients Controls
	796 P > 0.5		1.6 P > 0.5		
M.T.H.	134	80-217 159-1,190	0.6	0-1 0-1	Patients Controls
	562 P > 0.5		0.5 P > 0.5		
N.M.H.	1,541	108-2,985 998-1,529	0.8	0.5-1 0-1	Patients Controls
	1,036 P > 0.5		0.5 P > 0.5		
W.L.M.†	998	—	1	—	Patients Controls
	4,410		—		

\* Only two patients and controls.

† Only one patient and control.

nonthymectomized patients, mean 62% with range of 60% to 65%, versus controls, mean 61%, with range of 52% to 67% (see Tables II and III). The difference between the thymectomized group and controls and the nonthymectomized group and controls was due to a difference in potency between two batches of phytohaemagglutinin used in these experiments.

No significant differences at the 10% level (*t* test) were observed between patients and controls in either D.N.A. synthesis or in blast transformation in response to staphylococcal filtrate, streptolysin O reagent, and purified protein derivative. In both groups D.N.A. synthesis increased up to 100-fold (mean 15-fold) compared with unstimulated cultures, and blast transformation ranged from 0 to 57% (mean 17%) (Tables II and III). No increase in D.N.A. synthesis or blast transformation in response to the muscle and thymus preparations or to thymus culture supernatant was observed either in patients (mean tritiated thymidine incorporation 2,000 c.p.m., with range 104-8,070 c.p.m., mean blast transformation 1.0%, with range 0-10%), or in controls (mean tritiated thymidine incorporation 2,200 c.p.m., with range 159-9,350 c.p.m., mean blast transformation 1.0%, with range 0-2.5%) (see Tables II and III). The 10% blast transformation count in one patient in response to normal muscle homogenate was not supported by evidence of increased D.N.A. synthesis.

### DISCUSSION

These results do not provide evidence for a lymphocyte-mediated immune response involving skeletal muscle and thymus in the pathogenesis of myasthenia gravis, but on the other hand they do not rule it out. It is possible that the thymus and muscle antigens are labile and were destroyed during the preparation and storage of the homogenates. However, contractile and sarcoplasmic muscle protein antigens should have been present in suitable form and quantity in the homogenates which were used in these experiments. Alternatively, the failure to produce lymphocyte transformation may have been due to the absence of detectable numbers of sensitized cells in the peripheral blood; it is conceivable that they are located at the target tissues and not free to circulate in sufficient numbers to be detected by this technique.

There are conflicting reports on depression of immunological competence in myasthenia either as a result of the disease *per se* or of thymectomy. Adner *et al.* (1964) reported normal antibody responses but diminished capacity to develop delayed hypersensitivity to dinitrochlorobenzene. On the other hand, Kornfeld *et al.* (1965) found diminished antibody responses in myasthenics, which were more pronounced in those who had been thymectomized (though they were uncertain whether this more pronounced depression was due to thymectomy or to more severe disease). Furthermore, they found that both thymectomized and nonthymectomized patients showed normal ability to develop delayed hypersensitivity to dinitrochlorobenzene. Studies of patients with lymphoproliferative diseases and sarcoidosis (Hirschhorn *et al.*, 1964) and ataxia telangiectasia (Oppenheim *et al.*, 1966) reveal a correlation between intact delayed hypersensitivity and adequate lymphocyte transformation. However, the lymphocytes of our patients, both thymectomized and nonthymectomized, responded normally in vitro to phytohaemagglutinin and antigenic stimulation, providing no evidence of loss of immunological competence.

### SUMMARY

Three thymectomized and three nonthymectomized myasthenia gravis patients were investigated for possible depression of immunological competence and for immune responses to skeletal muscle or thymus antigens by in-vitro cultures of peripheral blood leucocytes in the presence of phytohaemagglutinin, various antigens, and skeletal muscle and thymus preparations.

No depression of immunological competence or abnormal stimulation by muscle or thymus was found.

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## Medical Memoranda

### Spontaneous Recovery of Gastric Secretion

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The gastric acid secretion in any one person when "maximally" stimulated is thought to vary very little from day to day. Kay (1953) found that repeated tests on successive days on the same person by use of the augmented histamine method gave results within 5%. According to Sircus (1959), when tests were repeated on one or more occasions in 15 subjects over a two-year period the coefficient of variation was 9.7%.

The purpose of the present communication is to record a marked variation of the maximal gastric acid secretion in a normal person within a period of 30 months.

#### METHODS AND RESULTS

The subject was a normal man aged 32 weighing 82.5 kg. His weight was constant from January 1964 to June 1966, these being the dates on which two sets of secretion tests were performed. In 1964 there was no symptomatic evidence of any stomach disorder, a barium-meal examination showed no abnormality, and the haemoglobin was 12.3 g./100 ml. and M.C.H.C. 32%.

The collection of gastric secretion was as described by Makhlof *et al.* (1964); the stomach tube was placed, under x-ray control, in the most dependent portion of the stomach.

Subcutaneous histamine and gastrin II were used as gastric secretagogues. The doses given were histamine 3.3 mg. (40 µg./kg.) and gastrin II 165 µg. (2 µg./kg.). Mepyramine maleate 100 mg. was injected intramuscularly 20 minutes before a histamine test. The gastric acid secretion was measured as the number of milliequivalents secreted during the first hour after injection of either histamine or gastrin II. At least three days elapsed between each test of both series of investigations.

#### Maximal Gastric Acid Secretion in Milliequivalents in One Subject at an Interval of 30 Months

Gastric Stimulant	Dose	Acid Secretion (mEq)	
		Jan.-Feb. 1964	May-June 1965
Histamine .. ..	3.3 mg.	0.6, 1.5, 1.1*	20.5
Gastrin .. ..	165 µg.	2.0*	26.6

\* Reported in detail by Makhlof *et al.* (1964, Table II, Subject 15).

#### REFERENCES

- Adner, M. M., Sherman, J. D., Isé, C., Schwab, R. S., and Dameshek, W. (1964). *New Engl. J. Med.*, 271, 1327.  
Downes, J. M., Greenwood, B. M., and Wray, S. H. (1966). *Quart. J. Med.*, 35, 85.  
Dutton, R. W., and Eady, J. D. (1964). *Immunology*, 7, 40.  
Hirschhorn, K., Schreiber, R. R., Bach, F. H., and Siltzbach, L. E. (1964). *Lancet*, 2, 842.  
Kornfeld, P., Siegal, S., Weiner, L. B., and Osserman, K. E. (1965). *Ann. intern. Med.*, 63, 416.  
Nastuk, W. L., Plescia, O. J., and Osserman, K. E. (1960). *Proc. Soc. exp. Biol. (N.Y.)*, 105, 177.  
Oppenheim, J. J., Barlow, M., Waldmann, T. A., and Block, J. B. (1966). *Brit. med. J.*, 2, 330.  
Osserman, K. E., and Weiner, L. B. (1965). *Ann. N.Y. Acad. Sci.*, 124, 730.  
Russell, D. S. (1953). *J. Path. Bact.*, 65, 279.  
Simpson, J. A. (1960). *Scot. med. J.*, 5, 419.  
Strauss, A. J. L., van der Geld, H. W. R., Kemp, P. G., jun., Exum, E. D., and Goodman, H. C. (1965). *Ann. N.Y. Acad. Sci.*, 124, 744.  
— Smith, C. W., Cage, G. W., van der Geld, H. W. R., McFarlin, D. E., and Barlow, M. (1966). *Ibid.*, 135, 557.  
Sturgill, B. C., Carpenter, R. R., Strauss, A. J. L., and Goodman, H. C. (1964). *Proc. Soc. exp. Biol. (N.Y.)*, 115, 246.  
White, R. G., and Marshall, A. H. E. (1962). *Lancet*, 2, 120.

In January and February 1964 four secretion tests were performed, three after histamine and one after gastrin II; the latter and one of the histamine tests have been reported in detail elsewhere (Makhlof *et al.*, 1964, Table II, Subject 15). In June 1966 histamine and gastrin II secretion tests were repeated.

The results are given in the accompanying Table. They show an eighteenfold increase in the maximal gastric acid secretion.

#### DISCUSSION

The lower limit of normal of the maximal histamine test in a man in his early thirties is probably greater than 5 mEq/l. of acid (Baron, 1963). In the subject reported here the gastric secretion has changed from an abnormally low level to a normal value within a period of 30 months. The cause of the low level in 1964 is not apparent. Low levels of gastric secretion may be associated with severe iron deficiency (Badenoch *et al.*, 1957), and when the deficiency is corrected the maximum acid secretion may rise to normal levels (Shearman *et al.*, 1966; Jacobs *et al.*, 1966). Similarly, variations in maximal gastric secretion may occur in association with peptic ulceration (Card and Sircus, 1958) and after gastric irradiation. The above investigation showed no evidence of peptic ulceration on barium-meal x-ray examination and the blood picture was normal. The man did not receive an excessive dose of radiation and had not taken corticosteroids. It is conceivable either that he had a transient asymptomatic gastritis which temporarily depressed the gastric secretion or that the results represent a marked physiological variation.

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#### REFERENCES

- Badenoch, J., Evans, J. R., and Richards, W. C. D. (1957). *Brit. J. Haemat.*, 3, 175.  
Baron, J. H. (1963). *Gut*, 4, 136.  
Card, W. I., and Sircus, W. (1958). In *Modern Trends in Gastroenterology*, edited by F. A. Jones, 2nd series, p. 186. London.  
Jacobs, A., Lawrie, J. H., Entwistle, C. C., and Campbell, H. (1966). *Lancet*, 2, 190.  
Kay, A. W. (1953). *Brit. med. J.*, 2, 77.  
Makhlof, G. M., McManus, J. P. A., and Card, W. I. (1964). *Lancet*, 2, 485.  
Shearman, D. J. C., Delamore, I. W., and Gardner, D. L. (1966). *Ibid.*, 1, 845.  
Sircus, W. (1959). *J. roy. Coll. Surg. Edinb.*, 4, 153.