between the patient groups were demonstrated. The results may influence the surgical management of these patients.

Discussion

PROF. V. WRIGHT (*Leeds*) I am interested to know how you determine when the wound is healed.

DR. MOWAT We did not find this in the medical notes but on looking carefully through the nursing notes we found enough information.

MR. S. J. BURROUGH (*Stoke Mandeville*) What significance do you attach to separation? Is a separated wound a delayed healing wound as far as you are concerned?

DR. MOWAT It is a delayed healing wound, but we have assessed independently those that were infected and those that were just simply separated with the wound-edge gaping.

MR. S. J. BURROUGH But there were rather more separated wounds in the rheumatoid group?

DR. MOWAT Yes, there were 15 compared with 5 in the control group ($P < 0.05$).

DR. D. A. H. YATES (*London*) You mentioned vasculitis in passing and the possible effects on wound healing. How many of this group had active vasculitis and was this relevant?

DR. MOWAT Something like four or five of our seropositive patients had an overt vasculitis. They did not appear to run into any problems. Overall, it is very difficult to say when a patient has vasculitis or not and indeed, even whether the seronegative patient may really have a vasculitis. Certainly we could not detect any obvious difference between our seropositive or seronegative patients, and similarly no apparent association with the severity of the disease.

DR. A. J. POPERT (*Droitwich*) Serum proteins have been amply demonstrated to be a significant factor in wound healing (Rhoads and Kasinskas, 1942). The severity and activity of the disease is also an important factor and it is closely bound up with related parameters such as haemoglobin concentration and rheumatoid factor titre. All these factors operate adversely in patients with the more severe kind of connective tissue disease and particularly when the disease is active. Such patients with active disease are often anaemic or have disturbed serum proteins with elevated globulin and low albumin or are underweight; they undoubtedly carry a high incidence of wound infection. Treatment with corticosteroids influences wound healing adversely only if it is given in a dose high enough to produce obvious hypercorticism. When the dosage is optimal, there is an improvement in the general condition of the patient and a favourable change in the anaemia and serum protein concentrations with hypercorticism; there will then result an improved wound healing rate and a reduced incidence of infection. Comparable patients not treated with steroids will heal less well. On the other hand, if the dose of steroids is enough to produce the clinical signs of Cushing's syndrome, then there will be a corresponding increase in the rate of wound dehiscence and infection.

DR. MOWAT With respect, sir, these are impressions that people have. They are largely undocumented impressions and we set out to investigate them. Our results suggest the very opposite of what many people think; haemoglobin values had nothing to do with wound healing and serum proteins seemed likely to produce little difference. We looked at the erythrocyte sedimentation rate and the activity of the disease and found no correlation.

DR. A. J. POPERT I was going to point out that yours is not the only paper which has been written on this subject. Peter Davis and I published a paper on this in 1958 (Popert and Davis, 1958) and I presented a paper on some eight cases to the Heberden Society in 1963 (Popert, 1963). There is a vast mass of other data (cited by Popert, 1962) on this subject, which you may not have fully culled.

DR. MOWAT I should be most interested to have those references. Most of the work is on animals and I was not aware that you had published in this field. Our results clearly disagree.

PROF. J. J. R. DUTHIE (*Edinburgh*) I should like to point out that the level of haemoglobin will not be significant until it has fallen to around about the 70 per cent mark. Your mean was 12.8g., which is 84 per cent so you did not have very anaemic patients for a start. The ESR was in the thirties, so that you did not have many active patients. You cannot say that anaemia and severity have anything to do with it when you did not have anaemic or active patients in your group.

MR. A. R. TAYLOR (*Stoke Mandeville*) I should like to make a final comment from the surgeon's point of view: there are very few of us who would operate with that degree of anaemia irrespective of whether the subjects were rheumatoid patients or not.

References

- Popert, A. J. (1962) M.D. Thesis, London
- (1963) *Ann. rheum. Dis.*, 22, 281 (abst.)
- and Davis, P. S. (1958) *Lancet*, 1, 21
- Rhoads, J. E., and Kasinskas, W. (1942) *Surgery*, 11, 38

Lymphocyte Sensitivity to Human Skeletal Muscle Antigen in Polymyositis. By M. M. ESIRI, B. L. HAZLEMAN, and I. C. M. MACLENNAN (*Radcliffe Infirmary, Oxford*) Evidence has been accumulating which suggests that polymyositis may be due to a breakdown of tolerance to skeletal muscle. If this is so, lymphocytes should be sensitive to muscle and express this sensitivity by undergoing transformation in the presence of muscle *in vitro*. This hypothesis has been tested using a quantitative method of estimating blast transformation.

Peripheral blood lymphocytes were separated from venous blood, suspended in tissue culture fluid, and incubated with varying concentrations of an homogenate of normal human muscle. Tritiated thymidine was added before taking the cultures down on the fifth day. The DNA was extracted and sampled in a liquid scintillation counter for the amount of incorporated tritium present. The amount of tritium incorporated by lymphocytes in the presence of muscle was compared with that incorporated in lymphocytes grown alone, the differences between these figures giving a measure of stimulation or inhibition produced by the muscle antigen. The results are seen in the

accompanying Table. Lymphocytes from patients with polymyositis and polymyalgia rheumatica showed stimulation by muscle, whereas those from normal healthy controls and patients with unrelated disease showed inhibition or insignificant stimulation. A lesser, but nevertheless significant, degree of stimulation was seen in patients with muscle wasting and rheumatoid arthritis. In patients with polymyositis and polymyalgia rheumatica the magnitude of the response was directly related to the degree of activity of the disease at the time of testing.

Response (counts per minute)	Poly- myositis	Poly- myalgia	Rheumatoid arthritis	Muscle wasting	Healthy and unrelated disease
Positive >400 <400	12 8	5 11	1 11	2 9	— 14
Negative	2	2	6	4	21
Total	22	18	18	15	35

Discussion

DR. H. L. F. CURREY (*London*) Did you test any other tissue extracts?

DR. ESIRI No.

DR. H. L. F. CURREY So it is really a guess that this antigen is specific to muscle?

DR. ESIRI Yes, absolutely.

PROF. J. J. R. DUTHIE (*Edinburgh*) I was going to make the same point. If you cut a piece of muscle out you are not just sampling muscle antigen. There will be antigens from every tissue and from any exogenous viral agent present. You are not proving anything by demonstrating antibodies to a homogenate of muscle.

DR. ESIRI No.

DR. M. I. V. JAYSON (*Bristol and Bath*) Was there any difference when healthy muscle or polymyositic muscle was used as the antigen?

DR. ESIRI No, we did not find any difference. We found comparable responses in patients tested with their own muscle and other people's muscle antigen.

DR. D. N. GOLDING (*Harlow*) Could some of the patients with polymyalgia in fact really have had polymyositis? Did you do serum muscle enzymes?

DR. ESIRI Yes, the serum muscle enzymes were done and were normal. Two polymyalgia patients had muscle biopsies and these also were normal.

DR. R. N. MAINI (*London*) The technique used was of tritiated thymidine incorporation. There are many problems about the use of this technique and one to which attention should be paid is how to express the magnitude of response at the sort of level of increased transformation rate that you are describing. I would guess, although you do not show us, that your PHA response was probably 1,000- or 10,000-fold higher than the sort of response that you showed us in your patient groups.

DR. ESIRI Quite so.

DR. R. N. MAINI Now I would submit that the degrees of increment that you are showing us have to be examined very carefully indeed and in a statistical way. How many cultures were you using and what level of confidence is there in saying this increment of 100 counts is significant?

DR. ESIRI Each culture was set up in triplicate and all I can say is that these results have been treated statistically.

DR. R. N. MAINI I know that your groups have been treated statistically, but I am questioning whether each individual assay has been treated individually. Unless you have some very special techniques the variation can be quite large at these low levels of counts.

DR. ESIRI We have found the results to be consistent and the differences between triplicate cultures were very slight.

DR. R. N. MAINI At what levels? What is your figure?

DR. ESIRI The differences were about 50 to 150. In some positive cases responses of several thousand were found.

DR. R. M. BENNETT (*London*) The increase in blast transformation occurred with a low dose of antigen and decreased with increasing doses of antigen. Can this be extrapolated to the clinical situation when the properties of antigen and lymphocytes are considerably different?

DR. ESIRI I should not like to try to do that. We do not know enough about the situation *in vivo*. There are obviously a lot of factors in this antigen and some are undoubtedly inhibitory in high concentrations and can more than counterbalance the positive effects of muscle antigen.

DR. C. J. GOODWILL (*London*) Have you any results on patients with muscular dystrophy?

DR. ESIRI There were ten patients with either Duchenne or facio-scapulo-humeral dystrophy included in the muscle-wasting group.

DR. D. A. H. YATES (*London*) Something like 50 per cent of rheumatoid patients show nodular myositis on muscle biopsy. Was there any correlation between those with an increased rate and the presence or absence of nodular myositis?

DR. ESIRI There was no correlation at all.

DR. D. N. GOLDING I don't think everyone would agree that there is invariably a good response to steroid therapy in polymyositis. It has been suggested that immunosuppressive drugs can be useful in such patients (Sokoloff, Goldberg, and Pearson, 1971) which would tie in with your immunological findings.

DR. ESIRI Yes, I agree; some of the patients had received steroids and had not responded.

DR. M. K. JASANI (*Horsham*) Since it is becoming increasingly important to know whether the clinically observed immunological abnormalities are the result or the cause of the disease under study, I wonder if you or others have any further data to support the suggestion that, in polymyositis, the abnormalities of lymphocyte function you describe might be the causative factors.

DR. ESIRI Results comparable with ours were obtained by Saunders, Knowles, and Currie (1969). Currie (1970) produced evidence for a cytotoxic effect of lymphocytes from patients with polymyositis on muscle cells grown in culture.

References

- Currie, S. (1970) *Acta neuropath. (Berl.)*, 15, 11. (Destruction of muscle cultures by lymphocytes from cases of polymyositis)
 Sokoloff, M. C., Goldberg, L. S., and Pearson, C. M. (1971) *Lancet*, 1, 14 (Treatment of corticosteroid-resistant polymyositis with methotrexate)
 Saunders, M., Knowles, M., and Currie, S. (1969) *J. Neurol. Neurosurg. Psychiat.*, 32, 569 (Lymphocyte stimulation with muscle homogenate in polymyositis and other muscle wasting disorders)

Antibody-producing Capacity in Rheumatoid Arthritis. By B. L. HAZLEMAN and H. L. F. CURREY (*The London Hospital*)

Tests for rheumatoid factor sharply separate patients with classical rheumatoid arthritis from those suffering from conditions such as ankylosing spondylitis and psoriatic arthritis. One possible explanation might be that these two populations differ in their potential humoral antibody response pattern. This was investigated by challenging 98 subjects (none of whom had suffered from typhoid or been injected with 'TAB') with a single, intradermal injection of 0.1 ml. monovalent typhoid vaccine. Blood samples taken immediately before, and 7 and 14 days after, injection were tested for typhoid agglutinins using the 'microtitre' technique. Total and 2-mercaptoethanol-resistant (MER) titres were determined. The method proved highly sensitive and the 'O' antigen titres were used for analysis as these were clearer and more reproducible than the 'H' titres. There was reasonably good correlation with the standard Vidal test. Appropriate experiments established that the presence of rheumatoid factor did not modify the 'O' titres, and that the MER titres reflected IgG activity. The opportunity was taken also to study the influence of certain drugs on this primary antibody response.

The conclusions were:

- (1) Patients with seropositive rheumatoid arthritis produced antibody responses indistinguishable from controls.
- (2) Patients with ankylosing spondylitis, psoriatic arthritis, and colitic arthritis produced responses (both total and MER) at least as great as those seen in seropositive rheumatoid arthritis.
- (3) Mean antibody responses in patients receiving azathioprine and/or prednisolone showed no evidence of suppression.
- (4) Unexpectedly, antibody responses (both total and MER) in patients receiving gold injections were significantly lower than in other rheumatoid subjects ($P < 0.01$).

Discussion

PROF. J. J. R. DUTHIE (*Edinburgh*) It seems quite clear that people using immunosuppressive drugs are not, in fact, getting any immunosuppressant effect. The drugs must be producing an anti-inflammatory effect and so why do you use a toxic drug when aspirin is just as good?

DR. CURREY Two groups of French workers (Bontoux, Kahan, Brouilhet, Amor, Delbarre, Jouanneau, Gnizdowska, 1971; Kahn and de Sèze, 1971) have reported that patients treated with chlorambucil (we do not use very

much in this country) show evidence of immune suppression, and that the degree of suppression correlates with the clinical response. I think that this does not apply to azathioprine.

DR. P. J. L. HOLT (*London*) The response to gold was interesting. Does it correspond to the clinical response? I ask this because gold is known to attach to immunoglobulins, particularly when complexed, and a low immunoglobulin response might thus be specific or non-specific (Lorber, Bovy, and Chang, 1972). We have now found that, of the non-steroidal anti-inflammatory agents so far tried, gold is the only one that suppresses complement conversion when it is accompanied by clinical improvement. It is quite dramatic in doing this and, of course, the rheumatoid factor titre also tends to fall (Versey and Holt, unpublished).

DR. CURREY We failed to show any correlation in the gold-treated groups between the reduction in response and clinical activity. Our groups were probably not large enough to show this.

DR. J. BALL (*Manchester*) As some of the antibodies produced were of the 7S class, I am surprised that you were not able to demonstrate some influence of rheumatoid factor in your tests. What did you think about that? Also, what was the distribution of rheumatoid factor titre in the gold treated group?

DR. CURREY The results were absolutely clear in showing no influence due to rheumatoid factor. The gold-treated patients were almost all seropositive.

DR. R. N. MAINI (*London*) Soothill and Steward (1971), in experimental work on mice who developed lupus-like nephritis, suggested that the affinity of the antibodies is at fault rather than the total quantity. Have you had a chance to look at that?

DR. CURREY No, we have done this only very crudely up to now.

DR. M. K. JASANI (*Horsham*) I should like to draw the attention of the Society to a possible relationship between your findings and those described by Dr. Esiri and her colleagues in the previous paper. Since, in experimental studies, steroids have been found to suppress more effectively the induction of a primary immunological response than either the established state, that is antibody production, or the secondary immunological response, I wonder whether the abnormality of lymphocyte function in polymyositis might not be incidental, whereas in RA it might not only be central but well established. Maybe some such explanation could help account for the observed fact that in polymyositis a good clinical response can be obtained readily using a high dose of prednisolone and maintained thereafter with a low dose, whereas in RA it is not uncommon to encounter difficulty in maintaining a good response even using a high dose of the steroid.

DR. CURREY Another way of looking at it is that polymyositis is a self-limiting disease and hence ideally treated with steroids, whereas rheumatoid arthritis is not.

DR. JASANI Although I agree that polymyositis is self-limiting, I wonder whether this is because in polymyositis