

## INTERMITTENT CORTICOTROPHIN THERAPY STUDY OF LYMPHOCYTE TRANSFORMATION *IN VITRO* IN RHEUMATOID ARTHRITIS

BY

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The assumption that the most effective way to use corticosteroids to suppress disease is to give these agents in divided doses two or three times daily was challenged by Reichling and Kligman (1961) and later by Harter, Reddy, and Thorn (1963). They suggested that alternate day administration could be equally satisfactory, with the advantage that less suppression of the hypothalamic-pituitary-adrenal axis was induced. Grant, Forsham, and DiRaimondo (1965) also found that one daily morning dose produced less adrenal suppression than divided doses. Intermittent treatment with corticotrophin based on a régime of injections of ACTH gel every 48 or 72 hours is also clinically effective (Nelson, Mackay, Sheridan, and Weaver, 1966). The nature of the therapeutic benefit induced by steroids or corticotrophin on cellular or enzymatic activity which persists during the interval between doses is not clear, but it does appear that many individuals with diseases such as rheumatoid arthritis or asthma are benefited for a period longer than the recognized metabolic effects of these drugs. We have administered corticotrophin gel to patients with rheumatoid arthritis and studied lymphocyte transformation *in vitro* of blood samples obtained at various times after administration of corticotrophin, in an attempt to determine whether changes in lymphocyte activity persist beyond the duration of the induced rise in plasma cortisol.

### Material and Methods

#### Patients

Fifteen patients with rheumatoid arthritis and four control volunteer subjects were studied.

In the first investigation eight patients had venous blood samples taken for lymphocyte culture at 10 a.m. on four consecutive mornings (Day 1-2-3-4) to act as a

control period. On the fourth morning (after the last control sample had been taken) the patients were given 40 units of corticotrophin gel\* intramuscularly and blood was again taken on four consecutive mornings (Day 5-6-7-8).

In the second investigation six patients had venous blood taken for lymphocyte culture at 10 a.m. and were then given 40 units of corticotrophin gel intramuscularly; in this instance a second sample of blood was taken for culture 4 hours later (2 p.m.). Similarly, the four control subjects had venous blood samples taken at 10 a.m. and 2 p.m. but without corticotrophin administration.

In a third study one patient had blood samples taken for lymphocyte culture on twelve consecutive days, 40 units of corticotrophin gel being administered intramuscularly on Days 3, 5, 7, and 9.

The plasma 11-hydroxycorticosteroids (subsequently referred to as plasma cortisol) in all these studies were estimated by a modified Mattingly (1962) technique.

#### Lymphocyte Cultures

Venous blood from the patients and volunteers was placed in preservative free, heparinized sterile containers (Evans Medical H.204). After settling at 37° C. for 90 minutes, the leucocyte-containing plasma was transferred to a sterile 30 ml. container and centrifuged for 10 minutes. The supernatant plasma was removed and replaced with culture medium (TC 199 with 20 per cent. autologous serum). The concentration of lymphocytes in the leucocyte suspension was  $1.5$  to  $2.0 \times 10^6$  per ml. No attempt was made to obtain a pure suspension of lymphocytes, and the cultures contained up to 25 per cent. of contaminating granulocytes.

2 ml. aliquots of each cell suspension were placed in four Kahn tubes for culture. Two cultures were unstimulated and two were stimulated by adding 0.05 ml. freshly prepared phytohaemagglutinin M (Difco) (PHA). After 48 hours' incubation the tubes were gently centrifuged (200 G. for 10 minutes). Most of the supernatant was removed and the cells were resuspended in the remaining culture medium by flicking the tube gently with the fingers. Films were made from the suspended cultures, stained by the May-Grunwald-Giemsa method, and examined microscopically. Damaged cells and cells showing the changes of necrobiosis were not counted.

\*Acthar gel (Armour Pharmaceuticals Ltd.)

The apparently viable cells were classified in five types:

- (1) Small lymphocytes,
- (2) Transformed cells ("blasts"),
- (3) Cells showing some, but not all the characteristics of transformed cells ("intermediate cells"),
- (4) Eosinophil granulocytes,
- (5) Large cells with copious vacuolated cytoplasm, frequently containing azurophilic granules, and a mature, usually kidney-shaped nucleus having coarsely clumped chromatin and plentiful parachromatin ("macrophages").

200 cells from each tube were identified and the average differential count of duplicate cultures was calculated. Only those cells showing all the characteristics of transformed cells (Category 2 above) were counted as blasts, and were used as an index of the ability of the lymphocytes to respond to stimulation.

**Results**

Fig. 1 shows the effect of a single injection of 40 units of corticotrophin gel on lymphocyte transformation *in vitro* of blood samples taken from eight patients. There is no apparent change in the number of cells transforming into blast cells in the 4 days following administration of corticotrophin compared with the 4-day control period.

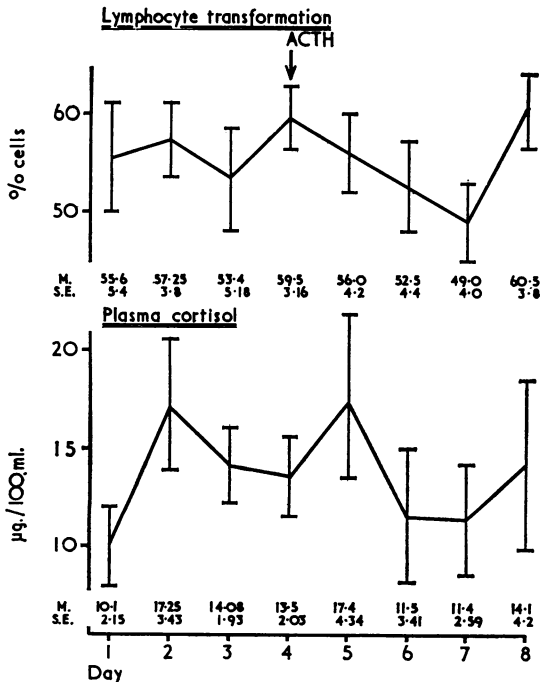


Fig. 1.—Daily levels (10 a.m.) of lymphocyte transformation *in vitro* in eight patients with rheumatoid arthritis. Corticotrophin gel 40 units intramuscularly was given on Day 4 at 10.15 a.m. (approx.).

In Fig. 2 the six patients who had 40 units of corticotrophin gel intramuscularly at 10 a.m. are contrasted with four control subjects. The mean plasma cortisol for the six treated patients were 13.75 µg./100 ml. (10 a.m.) and 50.25 µg./100 ml. (2 p.m.). At 4 hours after the corticotrophin injection all six treated subjects showed a decrease in the number of lymphocytes transforming to stimulation with PHA, but this trend was not seen in the untreated group of four subjects. The mean difference in lymphocyte transformation after corticotrophin therapy was a fall of 26 per cent. (using the paired "t" test;  $t=5.28$ ;  $0.01 > P > 0.001$ ); the difference in control subjects was 2.8 per cent. ( $t=1.79$ ;  $0.2 > P > 0.1$ ).

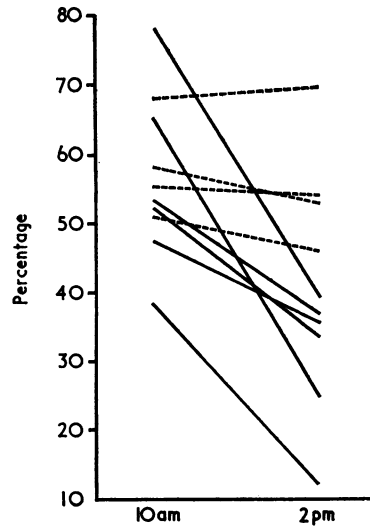


Fig. 2.—Lymphocyte transformation *in vitro* at 10 a.m. and 2 p.m.—Rheumatoid arthritis patients given 40 units corticotrophin gel intramuscularly at 10 a.m.—Control subjects (no treatment).

The longer study in one patient (Fig. 3) who received repeated corticotrophin injections at 48-

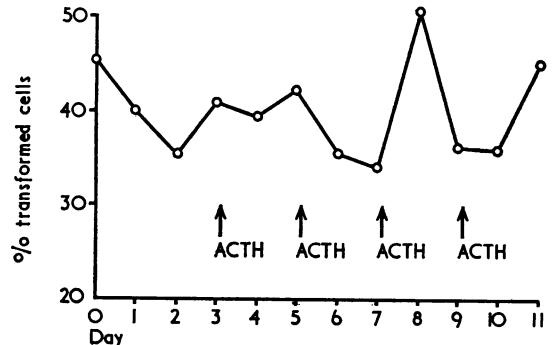


Fig. 3.—Lymphocyte transformation *in vitro* in a patient with rheumatoid arthritis. Corticotrophin gel 40 units was given intramuscularly as indicated by arrows on Days 3, 5, 7, and 9.

hour intervals showed no cumulative effect of the corticotrophin injections on lymphocyte transformation.

### Discussion

There is evidence that alterations in antibody mechanisms following steroid administration are due to a failure of production rather than increased antibody catabolism (Fischel, Stoerk, and Bjørneboe, 1951). The decreased antibody production probably correlates with the effect of adrenal steroids or corticotrophin in decreasing lymph node and thymus weight, associated with degeneration of lymphocytes in the germinal centres of the nodes and within the thymus (White and Dougherty, 1944; Weaver, 1955).

Although there is debate about the relevance of the phenomenon of lymphocyte transformation *in vitro* to immune responses *in vivo* (particularly delayed hypersensitivity), it is apparent that some relationship exists. Blast cell transformation can be induced in a proportion of lymphocytes from "immune" subjects cultured in the presence of the appropriate antigen. For example, when tuberculin is added to lymphocyte cultures from Mantoux positive subjects, transformation is observed, but this does not occur in cultures from those who are Mantoux negative. A high proportion of the peripheral blood lymphocytes in a culture can also be induced to undergo transformation into cells resembling lymphoblasts by a non-specific stimulus such as PHA. Such transformed lymphocytes have been shown to contain gammaglobulin (Elves, Roath, Taylor, and Israëls, 1963), and Bach and Hirschhorn (1963) concluded from their experiments that transformed lymphocytes can synthesize gamma globulin. It is of particular interest that Bartfeld and Juliar (1964), using a fluorescent antibody technique, were able to demonstrate the presence of rheumatoid factor in transformed lymphocytes from patients with rheumatoid arthritis.

When lymphocyte cultures are set up using cells from patients on "anti-inflammatory" drugs or in stressful situations, marked changes in transformation and ability to elaborate gamma globulin are seen. Serial measurements *in vitro* of culture of lymphocytes from patients on treatment with prednisone and cortisone show a fall in globulin synthesis after such therapy (Forbes and Henderson, 1966). Forbes and Smith (1967) also studied other drugs used in rheumatoid arthritis (phenylbutazone, salicylates, chloroquine) and showed that they all caused inhibition of protein synthesis by lymphocytes *in vitro* at concentrations within the

range of therapeutic blood levels. Altered ability of lymphocytes to transform in response to PHA stimulation has been found after treatment with immunosuppressive agents (Rubin, Stenzel, Hirschhorn, and Bach, 1964) and after surgical operations (Riddle and Berenbaum, 1967). The depression after surgery was considered as possibly mediated by the effect of stress acting through increased pituitary-adrenal stimulation.

The technique employed in setting up the cultures and in assessing the results in the present experiments is not regarded by us as providing more than a rough estimate of the activity of lymphocytes in culture. In mixed lymphocyte cultures, where activation seems to be associated with cell to cell contact, the response is affected by such factors as the population density in the culture (Leventhal and Oppenheim, 1968), the number of contaminating granulocytes (Jones, 1966), and perhaps even the number of red cells in the culture.

Satisfactory quantitation of lymphocyte activity in a culture is most readily obtained by measurement of the uptake of radioactive ( $^3\text{H}$  or  $^{14}\text{C}$ ) thymidine (Bain and Lowenstein, 1965; Caron, Sarkany, Williams, Todd, and Gell, 1965), or failing this, it is undoubtedly desirable to carry out only differential counts but also total white cell counts on the cultures, so that the actual numbers of transformed cells may be assessed. We were unable satisfactorily to overcome counting difficulties because of agglutination in the cultures, and were forced to resort to the purely qualitative measurement of the differential count.

With these reservations in mind, conclusions from the results presented here should be limited. Nevertheless, our findings were that the action of an injection of corticotrophin gel which is known to raise plasma cortisol levels at first maximally for 2-6 hours but lasting for 12-16 hours (Nelson and others, 1966) was not found to be associated with any alteration in the lymphocyte activity *in vitro* of treated patients extending beyond this period. Also, in the one patient to whom we gave repeated injections of corticotrophin every 48 hours, no evidence of cumulative effect was found. Since the experiments (Fig. 2) in which lymphocyte response was measured at the height of the response of cortisol to corticotrophin clearly showed a positive reaction, we conclude that, as regards lymphocyte transformation *in vitro*, increments in plasma cortisol exert influences corresponding only to the period of the increase in plasma cortisol. Thus, this study provides no clue to the prolonged clinical effects of steroids or corticotrophin administered in intermittent fashion yet having apparent therapeutic res-

ponses longer than could be expected from their known duration of action.

One explanation of these findings is that either the lymphocytes or the lymphocytic activity we have studied are not involved in the pathological processes of rheumatoid arthritis. Alternatively, only a small percentage of lymphocytes may be actively stimulated by the immunological reactions in rheumatoid arthritis and an experimental system which would allow isolation of these specific lymphocytes and their antigen might reveal an effect.

### Summary

Studies of lymphocyte transformation *in vitro* to phytohaemagglutinin were carried out in fifteen patients with rheumatoid arthritis at various times during treatment with corticotrophin gel. Inhibition of lymphocyte transformation correlated with the period of increment in the plasma cortisol level. The beneficial effects of intermittent steroid or corticotrophin regimes were not explained by these studies of lymphocyte activity.

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**La thérapie intermittente à la corticotrophine. L'étude de la transformation *in vitro* des lymphocytes dans la polyarthrite rhumatoïde**

**RÉSUMÉ**

Les études de la transformation *in vitro* des lymphocytes en phytohéماغلوتينine ont été faites chez quinze malades atteints de polyarthrite rhumatoïde à différents moments du traitement avec de la corticotrophine gel. L'inhibition de la transformation des lymphocytes correspondait à la période d'augmentation du niveau du cortisol dans le plasma. Les bons effets des différents traitements intermittents avec les stéroïdes ou la corticotrophine n'ont pas été expliqués par ces études de l'activité des lymphocytes.

**Terapia intermitente con corticotropina. Estudio de transformación de linfocitos *in vitro* en la poliartritis reumatoïde**

**SUMARIO**

Se realizaron estudios *in vitro* de transformación de linfocitos en fitohemaglutinina, en quince pacientes con poliartritis reumatoïde en diferentes etapas durante el tratamiento con gel de corticotropina. La inhibición de transformación de linfocitos tenía correlación con el período de incremento en el nivel de cortisol del plasma. Los efectos benéficos de regimenes intermitentes de esteroides o corticotropina no fueron explicados por estos estudios de la actividad de los linfocitos.