

# Effects of gold salts and prednisolone on inflammatory cells

## I. Phagocytic activity of macrophages and polymorphs in inflammatory exudates studied by a 'skin-window' technique in rheumatoid and control patients

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Whatever the stimulus to inflammation, for example traumatic, bacterial or immunological damage to tissues, macrophages and neutrophil polymorphs usually accumulate in large numbers in the inflamed area, and there is abundant experimental evidence that these cells originate in the bone marrow (Volkman and Gowans, 1965a, b; Spector and Willoughby, 1968). Inflammation of synovial tissues is a cardinal feature of rheumatoid arthritis and variable numbers of macrophages and polymorphs are present within the synovium and synovial fluid in affected joints. Lysosomal enzymes of the macrophages and polymorphs present in rheumatoid pannus are actively concerned in the destruction of articular cartilage (Dingle, 1969; Chayen and Bitensky, 1971). In addition to their phagocytic and digestive role in inflammation, it is now widely acknowledged that macrophages are probably involved in the initial handling of antigens in some immune responses and are probably involved in various aspects of cell-mediated immune reactions. In this latter connection, in rheumatoid arthritis, a variety of autoantibodies directed against immunoglobulins and various organs are usually present, and cell-mediated immune reactivity to certain antigens has been demonstrated (Williams and Bruckner, 1971; Berry, Bacon, and Davis, 1972).

Since the activity of macrophages and polymorphs is fundamental to various aspects of rheumatoid arthritis, including the effects of anti-inflammatory therapy, it would seem likely that a method of measuring the activity of these cells could provide useful information. Of the known activities of macrophages and polymorphs, phagocytosis is easily assessed in the experimental animal. A variety of techniques have been used to assess phagocytosis in the human but they are time-consuming and are not suitable for routine use. We have adapted the 'skin-window' technique of Rebeck and Crowley (1955) to

investigate the phagocytic activity of inflammatory macrophages and polymorphs in rheumatoid and control subjects.

### Methods

#### ASSESSMENT OF PHAGOCYtic ACTIVITY BY 'SKIN-WINDOW' TECHNIQUE

An area of skin on the lateral aspect of either upper arm was swabbed with 'Repelcote' silicone solution (Hopkins and Williams) which was allowed to dry. A small area of the cleaned skin was then lightly scratched with a sterile needle, any oozing of blood being arrested by pressure, and an 8 mm. diameter glass coverslip (Chance Pilkington) which had been coated previously on one side with a dried layer of a suspension of colloidal carbon (Gunther Wagner, carbon code number C11/1431a) was applied to the abraded area. The coverslip was held in place by a circular plaster dressing (Band-Aid 7/8" Spots, Johnson and Johnson) having a 9 mm. diameter central pad. Three carbon-coated coverslips were applied in this way on each occasion. In the inflammatory response which followed the above procedure, macrophages and neutrophil polymorphs migrated to the inflamed area and adhered to the glass coverslip. 24 hours after application, the coverslips were removed, air-dried, fixed by immersion in absolute methanol for 2 minutes, stained with Giemsa 'R66' (George T. Gurr), and mounted with the cell-free surface uppermost. Examination of the stained coverslips revealed that the cell population was usually composed of about 75 per cent. macrophages and 25 per cent. neutrophil polymorphs, and a variable proportion of these phagocytic cells contained visible aggregates of phagocytosed carbon particles (Fig. 1, opposite). The percentages of macrophages and neutrophil polymorphs containing visible phagocytosed carbon aggregates were assessed by randomly selecting and examining a minimum of 300 cells of each type on each coverslip using an oil immersion objective (total magnification 1,000 ×).

#### RELIABILITY OF METHOD

Preliminary studies using one of us (BVR) as a healthy control subject showed that, in repeated estimates of

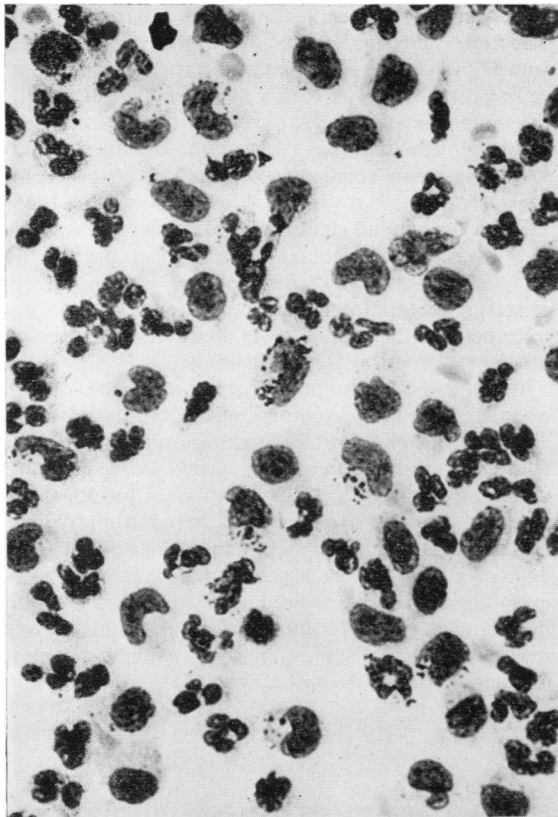


FIG. 1 *Macrophages and polymorphs adhering to glass 24 hrs after application of coverslip. The cytoplasm of many cells contains aggregates of carbon. Giemsa.  $\times 1,000$*

phagocytic activity carried out at short or prolonged intervals using the above technique, the variation in the numbers of macrophages and neutrophil polymorphs containing visible carbon was limited to about 10 per cent. (Fig. 2). Additional evidence for the reliability of the technique as a method of measuring phagocytic activity was provided by parallel studies in experimental animals given anti-inflammatory drugs, which showed a linear relationship between the percentage of cells containing visible carbon and the dose of sodium aurothiomalate or prednisolone (Vernon-Roberts, Jessop, and Doré, 1973).

SELECTION, GROUPING, AND CLINICAL ASSESSMENT OF SUBJECTS

Phagocytic activity was assessed in four groups of subjects who were matched for age and sex distribution insofar

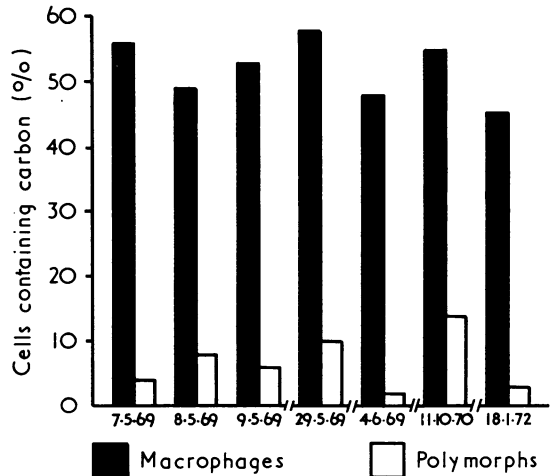


FIG. 2 *Percentage of macrophages and polymorphs containing carbon assessed at short and long intervals in a control subject*

as this was possible (Table I). The control group comprised fifty subjects who were either completely healthy or suffered from degenerative joint disease alone. The subjects in the other three groups were all suffering from classic or definite rheumatoid arthritis (Ropes, Bennett, Cobbs, Jacox, and Jessar, 1959), and comprised 61 patients (77 per cent. seropositive) not receiving gold salts or prednisolone, 51 patients (61 per cent. seropositive) receiving gold (sodium aurothiomalate—'Myocrisin', May and Baker), and 31 patients (71 per cent. seropositive) receiving prednisolone. At the time of presentation about 80 per cent. of the patients in each of the three groups of rheumatoid subjects were receiving drugs such as indomethacin, butazolidine, ibuprofen, paracetamol, or salicylates. In these cases, coverslip application was delayed until one week after withdrawal of these drugs, or, in the case of salicylates, until blood salicylate levels had fallen to zero. At the time of coverslip application, the rheumatoid patients were examined and the presence of active inflammation of the joints was scored on a 0 to 4 scale (0 = no inflammation; 4 = severe inflammation). The patients' notes were reviewed retrospectively and an assessment was made of the response to gold therapy based on an improvement in the severity of joint inflammation and the degree of disability. The tube latex test, haemoglobin, total and differential white cell count, and erythrocyte sedimentation rate was determined in each case. In the patients receiving gold therapy, serum gold

Table I *Number, age, and sex of control, rheumatoid, gold-treated rheumatoid, and prednisolone-treated rheumatoid groups of subjects investigated by the 'skin-window' technique*

Group	No. in group	Sex		Age (yrs)	
		Male	Female	Mean	(Range)
Controls	50	18	32	46	(20-80)
RA	61	27	34	54	(30-82)
RA/Gold	51	21	30	52	(19-70)
RA/Pred	31	11	20	57	(31-84)

estimations were carried out using the method of Lorber, Cohen, Chang, and Anderson (1968).

## Results

### PHAGOCYTTIC ACTIVITY OF MACROPHAGES

Fig. 3 shows that the range of 'scores' for the percentage of macrophages containing visible carbon was in

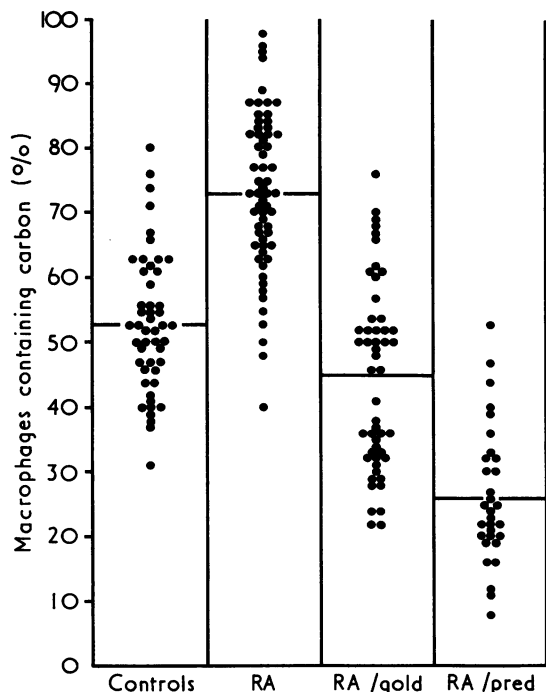


FIG. 3 Percentage of macrophages containing carbon in non-rheumatoid controls, rheumatoids (RA), and rheumatoids treated with gold (RA/GOLD) and prednisolone (RA/PRED)

**Table II** Comparison of means of percentage of macrophages containing carbon in non-rheumatoid, rheumatoid, gold-treated rheumatoid, and prednisolone-treated rheumatoid subjects

Groups under comparison				Probability of significance of difference between means
Group	Mean $\pm$ S.E.	Group	Mean $\pm$ S.E.	
Controls	53 $\pm$ 2	RA	73 $\pm$ 2	P < 0.001
Controls	53 $\pm$ 2	RA/Gold	45 $\pm$ 2	P < 0.01
Controls	53 $\pm$ 2	RA/Pred	26 $\pm$ 2	P < 0.001
RA	73 $\pm$ 2	RA/Gold	45 $\pm$ 2	P < 0.001
RA	73 $\pm$ 2	RA/Pred	26 $\pm$ 2	P < 0.001
RA/Gold	45 $\pm$ 2	RA/Pred	26 $\pm$ 2	P < 0.001

**Table III** Mean percentage of macrophages containing carbon in rheumatoid patients exhibiting a positive or negative beneficial response to treatment with sodium aurothiomalate

Beneficial response to chrysotherapy	No. of cases	Percentage of macrophages containing carbon (mean $\pm$ S.E.)	Probability of significance of difference between means
Definite response	33	46 $\pm$ 3	P < 0.05
Doubtful or absent	15	56 $\pm$ 3	

the order of 50 per cent. for each of the four groups. The mean score of the non-rheumatoid control group was 53 per cent.; the scores in the rheumatoid group were generally higher with a mean of 73 per cent.; in the gold-treated rheumatoids the mean score was 45 per cent.; and in the prednisolone-treated rheumatoids the mean score was reduced to 26 per cent. Analysis of these results (Table II) shows that these mean scores are all significantly different. Thus, relative to the non-rheumatoid control subjects, the phagocytic activity of macrophages is significantly raised in rheumatoid arthritis, is suppressed during treatment with gold, and is markedly suppressed during treatment with prednisolone.

In none of the groups was there a significant degree of correlation between individual macrophage scores and factors such as age, sedimentation rate, white cell count, duration of disease, or a crude scored estimate of disease activity. In the gold-treated group, there was no significant correlation between individual macrophage scores and serum gold levels on the total dose of gold which each patient had received. However, those patients who had exhibited a definite beneficial response to gold had a significantly lower mean macrophage score than those patients in whom a beneficial response was absent (Table III).

### PHAGOCYTTIC ACTIVITY OF NEUTROPHIL POLYMORPHS

Fig. 4 shows that the percentage of polymorphs containing visible carbon were generally appreciably lower in all four groups than was observed in the case of macrophages (Fig. 3). The range of scores for the percentages of polymorphs containing visible carbon also showed greater variation than in the case of

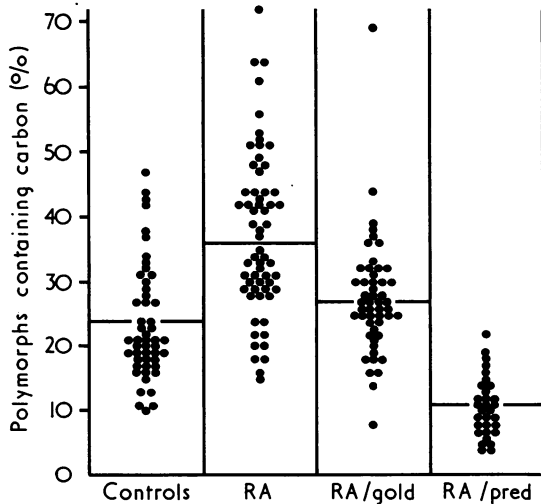


FIG. 4 Percentage of polymorphs containing carbon in non-rheumatoid controls, rheumatoids (RA), and rheumatoids treated with gold (RA/GOLD) and prednisolone (RA/PRED)

macrophages, and was least in the prednisolone-treated group (about 20 per cent.) and greatest in the gold-treated group (about 60 per cent.).

The mean score of the non-rheumatoid control group was 24 per cent.; the scores in the rheumatoid group were higher with a mean of 37 per cent.; in the gold-treated rheumatoids the mean score was 27 per cent.; and in the prednisolone-treated rheumatoids the mean score was reduced to 11 per cent. Analysis of these results (Table IV) shows that, relative to the non-rheumatoid control subjects, the phagocytic activity of neutrophil polymorphs is significantly raised in rheumatoid arthritis and is markedly suppressed during treatment with prednisolone; although the mean phagocytic score of the gold-treated rheumatoid group was not significantly different from the non-rheumatoid control group, it was significantly lower than the mean score of the rheumatoid group not receiving gold or prednisolone.

As in the case of macrophages, there was no significant correlation between individual polymorph scores

and such factors as age, sedimentation rate, white cell count, duration of disease, or a crude scoring estimate of disease activity. In the gold-treated group, there was no significant correlation between individual polymorph scores and serum gold levels or the total dose of gold which each patient had received.

#### PHAGOCYtic ACTIVITY OF MACROPHAGES AND POLYMORPHS DURING GOLD THERAPY

In sixteen of the rheumatoid patients receiving gold, phagocytic activity was assessed at various intervals on two or more (up to five) occasions.

Twelve of the sixteen patients receiving *weekly* injections of gold exhibited a progressive reduction in macrophage phagocytic scores during the period of observation (intervals of 1 day to 9 months), but the polymorph scores were variable and did not show a uniform pattern of response although a reduction was observed in the first few days after gold injection in many cases. It was generally observed that there was usually relatively little change detectable in macrophage scores assessed at daily intervals, and the greatest reduction in phagocytic activity was usually observed if at least 5 to 7 days were allowed to elapse between phagocytic assessments: this reduction was particularly marked about 5 to 7 days after each gold injection. The findings in two of the patients in this group are illustrated in Fig. 5 (overleaf). In both subjects the serum gold levels were elevated 24 hours after each gold injection, and fell to lower levels 1 week later. In contrast, there was a progressive fall in the percentage of macrophages containing visible carbon. In patient J.D. the polymorph scores were variable; but in patient V.J. the polymorph scores were reduced 24 hours after each gold injection, but had returned to the higher level at the time when the next injection was due.

In three of the sixteen patients, there was no observed change in macrophage scores. However, one of these, who was receiving *weekly* injections of gold, had only two phagocytic assessments which were carried out on successive days; and the other two patients, who were receiving *monthly* injections of gold, had three phagocytic assessments carried out at

Table IV Comparison of means of percentage of polymorphs containing carbon in non-rheumatoid, rheumatoid, gold-treated rheumatoid, and prednisolone-treated rheumatoid subjects

Groups under comparison				Probability of significance of difference between means
Group	Mean ± S.E.	Group	Mean ± S.E.	
Controls	24 ± 1	RA	37 ± 2	P < 0.001
Controls	24 ± 1	RA/Gold	27 ± 1	P > 0.05
Controls	24 ± 1	RA/Pred	11 ± 1	P < 0.001
RA	37 ± 2	RA/Gold	27 ± 1	P < 0.001
RA	37 ± 2	RA/Pred	11 ± 1	P < 0.001
RA/Gold	27 ± 1	RA/Pred	11 ± 1	P < 0.001

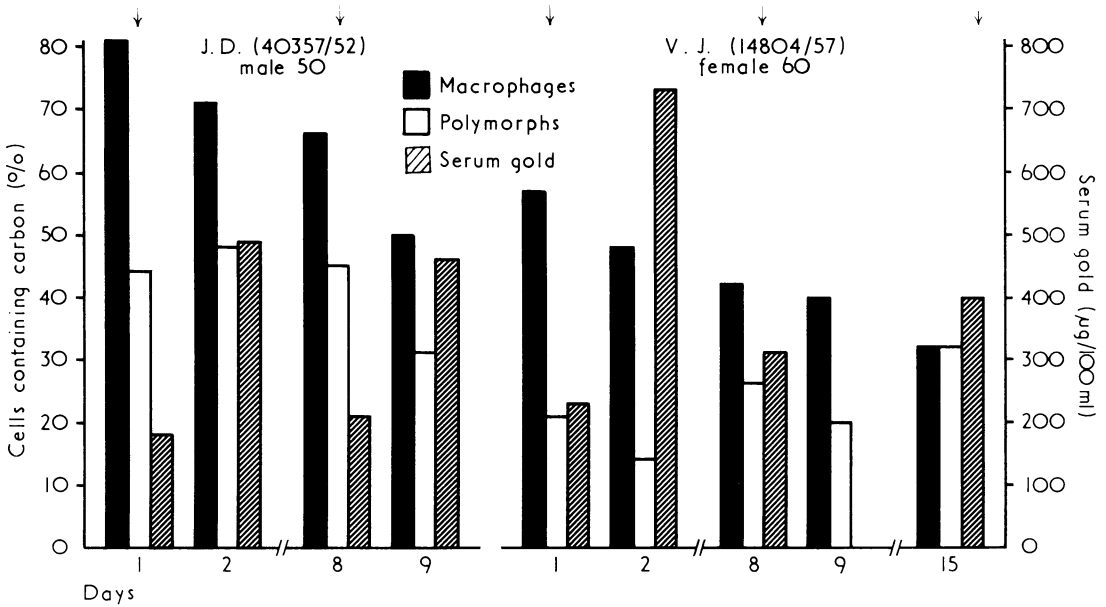


FIG. 5 Changes in serum gold and macrophage and polymorph phagocytic scores during treatment with weekly injections of 50 mg. sodium aurothiomalate (arrows) in two rheumatoid patients

monthly intervals in one case and 2-monthly intervals in the other.

The remaining patient of the sixteen was also receiving *monthly* injections of gold and showed a reduction in macrophage and polymorph scores during the first 3 days after injection (Fig. 6). However, immediately before the next gold injection 1 month later, it was observed that the macrophage and polymorph scores had returned to the previous high levels.

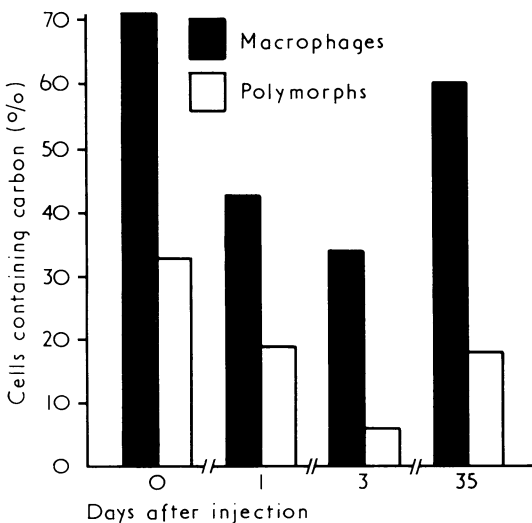


FIG. 6 Changes in macrophage and polymorph phagocytic scores in a rheumatoid patient during the month following a single 50 mg. injection of sodium aurothiomalate

These repeated assessments of phagocytic activity in rheumatoid patients receiving gold therapy revealed that macrophage phagocytic activity becomes progressively suppressed during treatment with weekly injections of gold, the greatest incremental reduction being observed 5 to 7 days after each gold injection. During treatment with monthly injections of gold, an initial fall in macrophage phagocytic activity is followed 1 month later by a return to the higher pre-injection level. The phagocytic behaviour of polymorphs during gold therapy is variable, but in many cases a reduction in polymorph scores was observed during the 3 to 4 days after each gold injection.

### Discussion

Using a modified skin-window technique to assess phagocytic activity, we have found that the phagocytic activity of macrophages and neutrophil polymorphs at inflammatory sites artificially induced in the skin is elevated in rheumatoid arthritis and suppressed during treatment with gold salts and prednisolone. This finding of a hyperphagocytic state of the inflammatory cells at extra-articular sites in rheumatoid arthritis indicates that, in rheumatoid arthritis, there must be present a factor or factors stimulating phagocytosis generally and these factors are not confined to the environment of inflamed joints.

There is morphological evidence of increased phagocytic and pinocytotic activity in the synovial

lining cells in rheumatoid joints, indicated by an increase in the number and length of filopodia and a striking increase in the number of lysosomes (Ghadially and Roy, 1969). There is similar morphological evidence of increased phagocytic and pinocytotic activity by macrophages and polymorphs in joint effusions in rheumatoid arthritis (Zucker-Franklin, 1966). The question obviously arises as to the nature of the stimuli to increased phagocytic activity by phagocytes within rheumatoid joints and whether such stimuli could also be the cause of the increased phagocytic activity which we have observed in extra-articular inflammatory sites in patients with rheumatoid arthritis.

It has been suggested that the joint inflammation characteristic of rheumatoid arthritis could represent an immunopathological response to an exogenous or endogenous constituent present in the joint (Glynn, 1968). Immunoglobulins, rheumatoid factor (RF), and complement components have all been found in the intimal cells of the synovium (Rodman, William, Bilka, and Müller-Eberhard, 1967; Bonomo, Tursi, and Gillardi, 1968; Brandt, Cathcart, and Cohen, 1968) and within cytoplasmic inclusions of leucocytes of the synovial fluid (Zucker-Franklin, 1966; Vaughan, Barnett, Sobel, and Jacox, 1968) from patients with rheumatoid arthritis. The fact that we have been able to demonstrate the presence of IgM, IgG, and  $\beta_{1c}$  within macrophages and polymorphs adhering to skin-window coverslips in rheumatoid patients (Vernon-Roberts and Jessop, 1972) could suggest that similar immunological factors may influence the function of inflammatory cells at extra-articular sites. In the absence of acute inflammation, circulating immunoglobulin molecules do not easily diffuse into extravascular fluids (Mackness, 1970). However, the trauma and acute inflammatory response resulting from scratching the skin could feasibly allow the escape of circulating immunoglobulins or immune complexes into the inflammatory site, where they could stimulate phagocytic cells by direct action or indirectly by, for example, inducing the release of a phagocytosis-stimulating factor (Barnet, Pekárek, and Johanovský, 1968) from sensitized lymphocytes.

Prednisolone therapy is consistently effective in reducing inflammation in rheumatoid arthritis and we have found that it also induces a marked reduction in phagocytic activity below non-RA control level. This marked reduction in phagocytic activity must partly account for the increased susceptibility of prednisolone-treated patients to various infections. Gold salts were more variable in their effects and are significantly less potent than prednisolone in suppressing rheumatoid inflammation; and it is of interest that we have found that patients who exhibited a beneficial clinical response to gold therapy had significantly lower macrophage phagocytic scores

than patients in whom the beneficial response was absent.

While we could find no significant correlation between various clinical and laboratory findings and macrophage and polymorph phagocytic scores, serial observations in patients receiving weekly gold injections showed that there was a progressive decline in macrophage phagocytic scores as treatment progressed, indicating that gold salts have a progressive 'saturating' effect on these cells. The lowest levels of phagocytic activity were usually observed 5 to 7 days after each gold injection, and the results obtained in a single patient indicated that macrophage phagocytic activity were restored to pre-injection level within 1 month after the gold injection. While bearing in mind the small number of patients in whom serial observations were performed, these results are strikingly similar to our findings in rats (Vernon-Roberts and others, 1973).

The final and probably most important consideration is whether the coverslip test we have used could be of value in the assessment of rheumatoid disease activity or the response of RA patients to anti-inflammatory therapy. The changes in the percentages of carbon-containing cells on coverslips would be the result of changes in:

- (1) The number of monocytes and neutrophils produced in the bone marrow,
- (2) The number of cells released from the bone marrow pool,
- (3) The migration of cells from the blood into the extravascular inflammatory site,
- (4) The adherence of the cells to the glass of the coverslip,
- (5) Phagocytic activity.

However, there is no doubt that the end-result of the assessment—the phagocytic score—is significantly elevated in RA and reduced by anti-inflammatory therapy. These latter findings alone would appear to provide sufficient reason for the further investigation of the use of this simple technique as a means of assessing the efficacy of anti-inflammatory drugs and the response of individual patients to gold and prednisolone therapy. We plan to examine this aspect by the application of serial coverslips to individual RA patients before and during anti-inflammatory therapy and by correlating the findings with a documentation of clinical and laboratory parameters.

### Summary

The phagocytic activity of macrophages and neutrophil polymorphs in inflammatory exudates was studied using a 'skin-window' technique in rheumatoid and control subjects. The phagocytic activity of macrophages and polymorphs was increased in patients having rheumatoid arthritis when compared

to non-rheumatoid controls, was suppressed in rheumatoid patients receiving treatment with gold salts, and was markedly suppressed in rheumatoid patients receiving prednisolone. The mean macrophage phagocytic score was significantly lower in patients responding clinically to gold therapy than in those who failed to respond. A progressive reduction in macrophage phagocytic activity was observed during serial observations in some of the patients receiving weekly injections of gold. In one patient

receiving monthly injections of gold, an initial fall in macrophage phagocytic activity in the immediate post-injection period was followed by a return to the higher pre-injection levels 1 month later.

The authors are grateful to Dr. R. M. Mason, Dr. H. L. F. Currey, and Dr. C. G. Barnes of the Department of Rheumatology, The London Hospital, for permission to study their patients.

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