

From the Research Laboratory of AB Draco, Lund,
Tornblad Institute, University of Lund, and Department of Neurology,
University of Lund, Sweden.

ON THE REACTIVITY OF HUMAN LEUKOCYTES TO PPD IN CLAUSEN'S AGAROSE MIGRATION TECHNIQUE

By

HAKAN BERGSTRAND, BENGT KÄLLÉN & OLLE NILSSON

In animals, especially guinea pigs, the macrophage migration inhibition technique is a standard *in vitro* method, regarded as a test of delayed type hypersensitivity. Many efforts have been made to find an equivalent method suitable for study of human leukocytes isolated from peripheral blood. Søborg & Bendixen (24) introduced a method in which human buffy coat cells migrate from glass capillaries. They found results which correlated well with skin reactivity, but not with circulating antibody. The method has been tried extensively. Initially, the results were not very satisfying. As recently as 1971, Alzer et al. (1) reported no success with the method. A micro-method introduced by Federlin et al. (15) was said to give reproducible results with PPD as an antigen. During recent years, this or similar modifications have been extensively used and have also been applied in a number of studies on "cellular immunity" in various autoimmune diseases (e.g., Calder et al. 6). Recently, Maini et al. (18) described a standardized system and analysed in great detail the technical variations inherent in the method.

Similar, but technically different, is the agarose method introduced by Clausen (7). Peripheral blood leukocytes are allowed to migrate from holes punched in an agarose gel.

They move out between the agarose and the plastic vessel and form a circular area of migrated cells, mainly polynuclear leukocytes. The migration is inhibited if the cells are preincubated with the antigen in question or with a preincubate supernatant from an antigen-stimulated lymphocyte culture (Clausen 7, 9). An inhibiting factor is formed also in the supernatant of cultures where lymphoid cells are stimulated with allogeneic cells in a mixed lymphocyte reaction (Clausen 8). Weisbart et al. (26, 27) also demonstrated that an inhibiting factor was formed by human lymphocytes when cultured for seven days in the presence of PPD. This factor inhibited the migration of polynuclear cells in the agarose test. After serial dilution of the medium, activity disappeared but could be restored with antigen. Clausen (7, 11) compared the agarose method with the glass capillary technique of Søborg & Bendixen (24) and found the former more sensitive and less apt to an escape phenomenon. The agarose method has been successfully used also by other investigators. Astor et al. (2) obtained results that correlated well with the cutaneous delayed hypersensitivity reaction when three different antigens were studied: PPD, candida, and streptokinase-streptodornase, a good correlation was found; but when coccidioidin was used as an antigen, no correlation was found. The present authors (5) have used the method in a study of cells from healthy individuals, from patients with multiple sclerosis, and from patients with other neurological diseases, using basic encephalitogenic protein as antigen.

It is not clear which immunological parameter is evaluated with leukocytes migration inhibition methods. Maini et al. (18) stressed that the "clinical significance" of the method was "not proven and probably irrelevant". Senyk & Hadley (23) studied 12 normal adults and used three antigens: PPD, streptokinase-streptodornase (SK-SD), and candida (DO). They compared the reactions in a lymphocyte transformation test (LT) and in leukocyte migration inhibition tests (ILM), using both Federlin's modification of the glass capillary method and Clausen's agarose method. The results of the tests

were compared with the skin delayed type reactivity (DCH) of the cell donors. A good correlation between DCH and both LT and ILM was seen only with PPD. For SK-SD, DCH and LT gave a good correlation; but ILM appeared to be less sensitive, as positive results were obtained only in about 50 per cent of the tests performed with cells from individuals with a positive DCH. With the DO antigen, both DCH and LT were positive, but ILM was consistently negative. To explore this further, the authors studied the ability of DO to evoke MIF production, as tested on guinea-pig peritoneal macrophages. DO as well as PPD and SK-SD could stimulate to MIF production. The authors stress that the reaction of polynuclear leukocytes in the tests in question depends upon "the complex interplay of diverse influences and events, the release of MIF by lymphocytes, triggered specifically or otherwise, being but one of them".

In the present paper, we report some experiences of Clausen's agarose method using PPD as an antigen, with the hope of further illuminating the processes involved.

MATERIAL AND METHODS

Venous blood was drawn from 21 healthy adults and from 10 normal newborn infants. Repeated samples were taken from eight of the former. Leukocytes were separated by gravity sedimentation after mixture of the heparinized blood with a fourth of its volume of 6 per cent dextran (Macrodex, Pharmacia, Uppsala, Sweden). The leukocytes were washed three times by centrifugation with Parker 199 (SBL, Stockholm, Sweden) and a cell suspension was prepared in Parker 199 with a cell count of 2.2×10^6 cells/ml. It was divided into 50 μ l lots to which were added 5 μ l of antigen (PPD) or saline (controls). Incubation took place at 37° C for 30 minutes. Agarose plates were prepared in disposable petri dishes (Millipore Ltd.) as described by Clausen (7) and holes were punched in the agarose in a specific pattern. Into each hole, 7 μ l of cell suspension was placed, usually 5-7 holes being filled with material from each incubation tube. Care was taken to randomize the preparation as described previously by us (5). The agarose plates were fixed, after appr. 20 hours' incubation, for a few hours in methanol. The agarose could then easily be removed, leaving the migrated cells in the petri dish. The size of the migrated area was determined planimetrically and was expressed in arbitrary units, one unit = 0.08 mm². The PPD used was obtained from Statens Seruminstitut (Copenhagen, Denmark) as a stock solution

of 1 mg/ml without chinosol. In some experiments, puromycin treatment was used. Crystallized puromycin hydrochloride was bought from Sigma Ltd. Fresh solutions were prepared in saline. For further methodological details, see Clausen (7) and Bergstrand et al. (5).

RESULTS

1. Sources of Technical Variation

Incubation of cells with antigen takes place in small test tubes. From each tube, a number of samples are tested, each in one hole in the agarose. There are thus two sources of technical variation in the material: one related to the incubation process; the other to differences between "identically" prepared agarose holes. Table 1 analyses the relative significance of these two sources of variation. The "between holes" variance is almost identical in incubations without PPD ("controls") and with PPD; and the common estimate of 421, based on 607 d.f., must be regarded as a fairly exact estimate of this variance. The effect of different incubation tubes could be tested in 30 experiments, where two "identically" prepared control incubations were performed. The variance between them amounts to 787, estimated from 30 d.f.; this can hardly be an estimate of the "between hole" variance— $F = 1.9^{**}$.

TABLE 1
Variance Analysis of the Significance of Technical Error Associated with Incubation of Cells with Antigen.

Source of variation	d.f.	variance	F
<i>"Between holes" from the same incubation tube</i>			
Controls	344	418.5	} 1.01 NS
PPD	263	423.0	
All	607	420.84	} 1.87**
<i>"Between identically prepared control incubation tubes"</i>	30	786.7	

NS = non-significant; ** $0.01 > P > 0.001$; F = variance ratio.

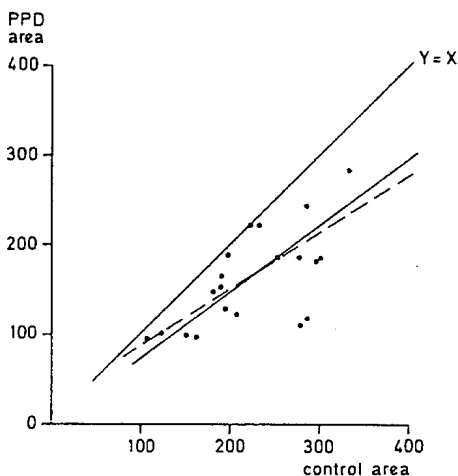


Fig. 1.

Diagram showing the relation between mean area in control migrations (abscissa) and mean area in PPD migrations (200 $\mu\text{g}/\text{ml}$) (ordinate). Each dot represents the first test of cells from each of 21 normal Mantoux-positive individuals. Three lines are drawn: the line of equality ($Y = X$, no effect of PPD), the best-fitting line not necessarily passing through the origo (dashed line $Y = 19.7 + 0.65 X$), and the best-fitting line passing through the origo ($Y = 0.74 X$, corresponding to a MI of 74 per cent). Areas expressed in arbitrary units.

The variances agree well with those we observed in our earlier study (5), but the increase in the material now ensures that the difference reaches statistical significance. As was done in the earlier study, the mean value of the various determinations performed on one incubation are used as variates in the following analysis.

2. Methods of Evaluation of PPD Inhibition

Fig. 1 shows the relation between the mean areas of control tests and of PPD tests performed on 21 healthy adults (always first test performed on each individual). Each experiment is thus represented by a dot, whose co-ordinates show the mean control area (abscissa) and the mean PPD area (ordinate). If X represents the former and Y the latter, a regression line fitted with the least squares method is found to be: $Y = 19.7 + 0.65X$ (eq. 1)

The sum of residual squares around this line is 24,184 at 19 d.f., i.e. the variance of the variates around the line is 1,273. If, instead, a regression line passing through the origo is fitted, $Y = 0.74X$ (eq. 2)

The sum of residual squares is then 26,301 at 20 d.f. This alternative represents the graphical equivalent of the classical migration index; namely, Y/X .

Equation (1) thus eliminates a sum of residual squares of 2,117 (1 d.f.) which is not significant compared to the variance around the regression line, 1,273 ($F = 1.66$, N.S.). The line through the origo—the migration index line—will therefore be considered adequate to characterize the relation between X and Y . The variance around this line will then be 1,315; this is significantly higher than the variance between the means from two control incubations performed on each of 30 individuals: this variance is 133 at 30 d.f. The variance ratio is $F = 9.9$ at 20 and 30 d.f., $P < 0.001$. The migration inhibiting effect of PPD

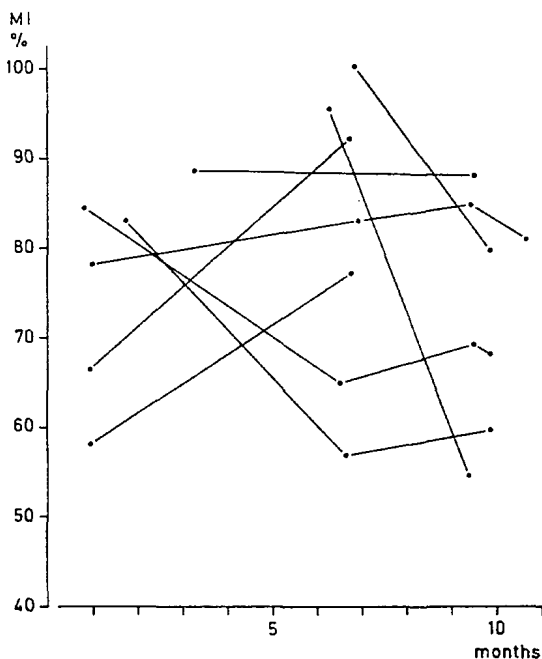


Fig. 2.

Diagram showing MI recorded with PPD at 200 $\mu\text{g}/\text{ml}$ using cells from eight Mantoux-positive individuals tested repeatedly over eleven months. Abscissa gives time axis.

thus differs in different experiments, but this does not depend on the migration rate of control incubations; if it did, the regression line would not have gone through the origo.

3. *Reproducibility of Inhibiting Effect of PPD*

A total of 21 repeated tests were performed with cells from eight different individuals (number of tests for each person: 4, 4, 3, 2, 2, 2, 2, 2). Large variations were seen in the degree of inhibition obtained with PPD at 200 $\mu\text{g/ml}$. In two instances, one test gave no significant inhibition ($\text{MI} = 100.5$ and 95.5 per cent, resp.) but the other gave a strong one ($\text{MI} = 79.7$ and 54.4 per cent, resp.). Also in the other repeated tests when inhibitions existed in all tests, large variations were seen between the degree of inhibition. Fig. 2 illustrates the variations in MI with time: the study extended over appr. one year.

In order to analyse this problem statistically, a co-variance analysis was performed on these data, as shown in Table 2. It is obvious that the various cell donors do not differ significantly from each other compared with the differences noted between repeated tests performed with cells from the same individual.

TABLE 2
Co-variance Analysis of PPD Effect on Inhibition of Cells from Eight Individuals, Studied in Repeated Tests.

Source of variation	d.f.	co-variance	F
"Between individuals"	7	1222.5	} 1.32 ^{NS}
"Between repeated tests on cells from same individual"	13	929.7	

NS = non significant; F = variance ratio.

4. *Response to PPD by Leukocytes from Umbilical Cord Blood of Newborn Infants*

Cells from ten normal newborn infants were studied for reactivity to PPD. Fig. 3 shows the results, where the migra-

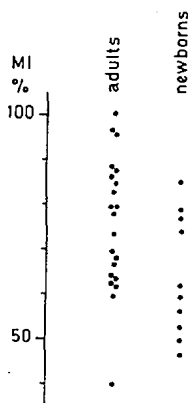


Fig. 3.

Comparison of MI with PPD at 200 $\mu\text{g}/\text{ml}$. 21 tests performed on cells from adult Mantoux-positive individuals, 10 tests performed on cells from the umbilical cord blood of normal newborn infants.

tion indices from these ten experiments are compared with those from the experiments with cells from the 21 adults (first tests only). The MI values lie within the same range, but if anything, the values for the newborn infants lie on average somewhat below those for the adults; but no statistically significant difference exists.

5. Effect of Various PPD Concentrations

In eleven experiments, the concentrations of 50 and 200 $\mu\text{g}/\text{ml}$ of PPD were tested simultaneously. The angle coefficients for the two concentrations, calculated as eq. (2) above, were 0.771 at 50 $\mu\text{g}/\text{ml}$ and 0.773 at 200 $\mu\text{g}/\text{ml}$, i.e. no difference was seen. When a multiple regression analysis, as described by Bergstrand & Källén (4), was performed ($X_1 =$ control, $X_2 = 200 \mu\text{g}/\text{ml}$, $Y = 50 \mu\text{g}/\text{ml}$), $a = 0.029 \pm 0.087$, thus confirming that no difference in effect between the two concentrations exists. Apparently, at 50 $\mu\text{g}/\text{ml}$, a level of inhibition has been reached which cannot be exceeded by increas-

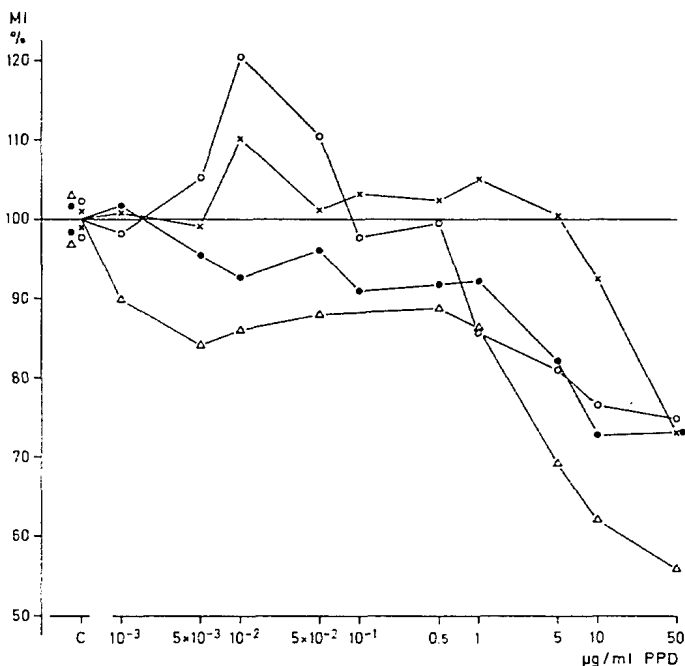


Fig. 4.

MI recorded at different concentrations of PPD with cells from four adult Mantoux-positive individuals.

ing PPD concentration. The effect of concentrations lower than 50 µg/ml was studied on cells from four individuals. Fig. 4 shows the MI values at different concentrations. For two individuals, a persisting inhibiting effect was seen down to very low concentrations. One of them was tested twice with a two-day interval between and with almost identical results. In the other two, a stimulating effect was seen at low concentrations, in one of them amounting to an increase of 20 per cent above controls. This test too was repeated, resulting in an equally large stimulation.

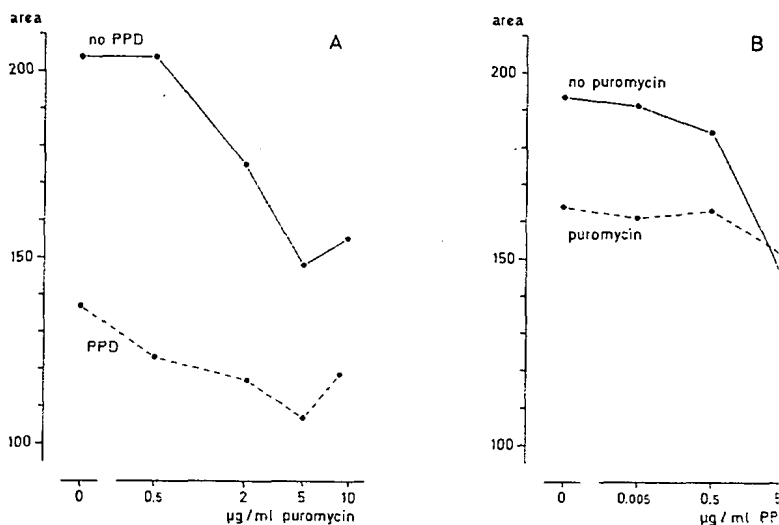


Fig. 5.

Size of mean migration area in tests performed with cells from two adult Mantoux-positive individuals. A. PPD 50 µg/ml, different puromycin concentrations.

B. puromycin 5 µg/ml, different PPD concentrations.

6. Effect of Puromycin on the Test

Experiments were performed with leukocytes from four individuals where the effect of puromycin at concentrations between 0.5 and 10 µg/ml was studied. Puromycin was present during the whole incubation period, both when incubation was made with saline (controls) and when made with PPD, usually at 50 µg/ml concentrations. Fig. 5 shows the results of such an experiment. It can be seen that puromycin acts as a powerful suppressor of cell migration in control cultures, and at 10 µg/ml of puromycin, the migration is blocked almost as much as with PPD 50 µg/ml only. The combination of puromycin and PPD gives no or only a slightly increased inhibition. If standard MI is formed from this material, the results will be: PPD 50 µg/ml (Fig. 5A): no puromycin, MI = 67 per

cent, puromycin 0.5 $\mu\text{g/ml}$: 61 per cent, puromycin 2 $\mu\text{g/ml}$: 67 per cent, puromycin 5 $\mu\text{g/ml}$: 73 per cent, puromycin 10 $\mu\text{g/ml}$: 76 per cent. In Fig. 5B, MI is 75 per cent at 50 $\mu\text{g/ml}$ of PPD without puromycin and 92 per cent with puromycin, 5 $\mu\text{g/ml}$.

DISCUSSION

The usual way to express and evaluate antigen-dependent inhibition of the migration of macrophages or polynuclear leukocytes is to use the migration index (MI); i.e. the ratio between the mean area in antigen-treated cultures and the mean area in control cultures. The absolute values of control migration areas vary considerably owing to inter-individual differences and variations in experimental conditions.

Bergstrand & Källén (4) studied the effect of migration strength in control cultures on the antigen-induced inhibition, using guinea pig peritoneal macrophages and basic encephalitogenic protein as an antigen. They found that MI was high when control migrations were large, and vice versa. They thought that the phenomenon could be explained by the escape phenomenon known to occur in this type of test. But in this system, MI is not quite representative of the reactivity of a cell sample, as its value is influenced by the migration strength in control cultures.

Clausen (11) found that the escape phenomenon is less pronounced in the agarose technique than in the glass capillary technique, but an escape phenomenon could be demonstrated by comparing inhibition at 4 and at 20 hours incubation (10). We found that, within the interval studied, inhibition caused by PPD was not significantly influenced by the strength of the control migration. The MI can therefore be used as a relatively unbiased estimate of migration inhibiting effect of PPD.

The absolute values of migration areas enter the discussion also in another way. The technical error variance is of the same magnitude irrespective of the size of the migrations studied; as Table 1 shows, it is very similar in control cultures and

in PPD cultures, and yet the average size of the latter is only 75 per cent of the former. The relative size of the error will thus be larger in studies of small migration areas and also the MI determined will have a larger error than a MI determined from large migrations. Therefore we have preferred to use, in statistical considerations, the linear regression representing MI, and have analysed differences between groups etc. by co-variance analysis around such regression lines (cf. Bergstrand et al. 5).

Clausen (7) studied the reproducibility of the agarose method by twice testing cells from 22 individuals. The repeated test was performed within 2-11 days. He found a good reproducibility and reported that the 95 per cent confidence interval for a MI is represented by ± 13 per cent. This agrees with our experiences. However, when reactivity for PPD is studied over longer periods, marked variability can occur, as Fig. 3 illustrates. This could be due to systematic changes in the technical performance of the experiments, e.g., use of new sera etc.; but of the four tests performed during the first month of study, two showed reduced MI and two showed increased MI at re-test six months later.

This definitely argues against technical factors being the cause of the variation, but indicates that actual changes have taken place in the reactivity of the cells over some months. In an earlier study, we (5) noted relatively rapid changes in the reaction to basic encephalitogenic protein when studying cells from patients with MS.

Perhaps the most unexpected finding in the present study is that cells from newborn infants show a full responsiveness to PPD. Astor et al. (2) studied cells from four newborn infants and found a clear-cut reactivity to PPD in two of them. But they showed no clear reactivity to SK-SD, candida, or coccidioidin. This finding definitely argues against a simple correlation between cell-bound immunity and the leukocyte migration inhibition test.

Somewhat similar findings were made with the macrophage electrophoretic mobility test by Field & Caspary (16). They

claimed that lymphocyte sensitization is present in newborn infants when the mothers showed sensitivity; in five such infants, strong reactivity to PPD was seen, but reactions with other antigens were also described. These authors suggest a number of alternative explanations: transfer of maternal lymphocytes, influence from passively transferred antibodies or informative molecules, passive transfer of antigen.

We found that PPD concentrations above 50 $\mu\text{g/ml}$ did not inhibit any further migration. This agrees with the statement by Clausen (12) that on average he found the same MI with 50 $\mu\text{g/ml}$ and higher concentrations. In single instances, however, the higher concentration could result in an increased inhibition. Usually, doses between 50 and 100 $\mu\text{g/ml}$ have been used in order to get maximum inhibition. The absence of further inhibition can have different explanations. Receptors for PPD may be saturated at 50 $\mu\text{g/ml}$, and further increase in antigenic concentration could not then result in further migration inhibition. It is also possible that a certain cell population cannot respond more than to a certain degree to inhibiting factors induced by antigens. Part of the migrating cells might be unresponsive and will then give a migration area uninfluenced by the action of antigen. Reeves et al. (22) demonstrated a similar phenomenon for the standard macrophage migration system, using guinea pig peritoneal cells. They thought the migration inhibition reaction was a two-stage process. In the first stage, a uniform migration of cells takes place which cannot be influenced by MIF or related "immunological" factors. In the second stage, a further migration occurs under the control of MIF.

Low doses of PPD have not been tested so extensively as have high doses. In the original Clausen (7) paper, the lowest concentration tested was 12.5 $\mu\text{g/ml}$. In a later study (12), concentrations down to 1.6 $\mu\text{g/ml}$ were tested, and Mitchell et al. (19) used 1 $\mu\text{g/ml}$ as the lowest dose with the glass-capillary method. In only one of eight experiments with the use of that concentration was significant inhibition seen. We have studied concentrations down to 10^{-3} $\mu\text{g/ml}$. Experiments with

cells from four individuals, all showing a marked response to PPD at 50 $\mu\text{g}/\text{ml}$, were significantly influenced also in a low-dose region. These experiments were performed with three different antigenic dilution series to avoid accidental errors in the dilution series. In two of the experiments, a more or less marked stimulation was seen: in one series, to a MI of 120; in the other, to 110. The former value was further supported by two adjacent concentrations which gave MI of 105 and 110, resp. In the other two experiments, inhibition corresponding to $\text{MI} = 85$ to 90 was seen in low-dose regions. Maximum stimulations and inhibitions were seen at 10^{-2} or 5×10^{-3} $\mu\text{g}/\text{ml}$. As far as we know, no similar observations have been made with PPD as antigen.

Mitchell et al. (19) explicitly state that stimulations were never seen with PPD even at the lowest dose tested, 1 $\mu\text{g}/\text{ml}$. However, stimulation of migration has been described with guinea pig macrophage migration tests (cf. Bergstrand & Källén 3) and with human polynuclear cells studied in the capillary technique (e.g., Calder et al. 6, thyroid antigens) and in the agarose technique (Bergstrand et al. 5, basic encephalitogenic protein). It is often regarded as a low-dose phenomenon, due either to a low degree of sensitization of the cells under study or to a low concentration of antigen used, or to a combination of both. However, we have presented some arguments favouring that, at least under certain circumstances, stimulation might represent a reaction which is qualitatively different from reactions giving migration inhibition. This alternative obtains further support from the observation by Stevenson (25) that mononuclear leukocytes when cultured together with hydrocortisone produce a factor which stimulates the migration of polynuclear leukocytes in a capillary technique—the direct effect of hydrocortisone on polynuclear cell migration is inhibiting.

The usual way to demonstrate that the effective component in a migration inhibition test is a factor produced by lymphocytes after antigenic interaction is to block the reaction with puromycin or some other suitable inhibitor of protein synthe-

sis. In this way, an active synthesis of the migration inhibiting factor (MIF) reactive on guinea pig peritoneal macrophages can be demonstrated (David 13). Mitchell et al. (19) and Maini et al. (18) used puromycin to block the human granulocyte inhibition reaction. Puromycin was added to the medium during the exposure of leukocyte to antigen, and a marked increase of MI up to 100 per cent (no inhibition) was registered. We have verified this finding but, as Fig. 5 shows, the resulting migration area is the same irrespective of whether the cells are treated with PPD at high concentration, with or without puromycin. Mitchell et al. (19) also noticed that puromycin affected control migration: at 15 $\mu\text{g/ml}$ of puromycin, the mean migrated area was reduced to 50 per cent; at 5 or 7.5 $\mu\text{g/ml}$, the reduction amounted to 20–30 per cent. This is of the same magnitude as the reduction we noted. The relations between actually measured areas might therefore agree well with those presented in Fig. 5. Lockshin et al. (17), in a short paper, also report that puromycin at a concentration of 0.55 $\mu\text{g/ml}$ ($= 10^{-6}$ M) had no effect on the control migration of leukocytes from capillaries, but reduced or abolished the inhibiting effect of high doses of PPD (100–600 $\mu\text{g/ml}$). We cannot verify this in the present system.

One way to explain the increase in MI—that made by the authors quoted—is to suppose that puromycin blocks a process requiring protein synthesis and resulting in the production of a MIF-like substance which affects the migration inhibition. Another way to explain the findings is that, at a certain experiment, there is a minimum migration area below which migration cannot be reduced by active means, such as antigens or protein synthesis inhibitors (cf. the two-step migration suggested by Reeves et al. 22). This would fit well with the actual findings made. Further studies with various metabolic inhibitors are necessary to elucidate the role played by synthesis of soluble products from antigen-exposed lymphocytes. Incidentally, David (13), in his study on the effect of puromycin on guinea pig macrophage migration inhibition, noted an effect of puromycin on the control migration, but also demonstrated

a reduction of the PPD-induced inhibition regardless of the former effect. David (14) states that puromycin exerts its effect on the macrophage migration inhibition in two ways: the lymphocytes do not produce MIF and the macrophages do not react to MIF. We feel that the puromycin experiments performed so far fail to prove that the production of a MIF-like factor is the main active principle in the human polynuclear migration inhibition tests.

A comparison can be made with the results reached by Pritchard et al. (20) on the effect of puromycin on the macrophage electrophoretic mobility test. They showed that puromycin had no effect on the first stage of the reaction: the production by lymphocytes of soluble factors (MSF) after antigenic stimulation, but could abolish the reaction between MSF and the irradiated guinea pig macrophage, which is the target cell in this reaction. Also in the polynuclear migration inhibition test, soluble factors are undoubtedly effective, as is proved by the use of the indirect or two-step method (c.g., Clausen 8, Weisbart et al. 26, 27), but the identity or similarity between them and MIF is not well supported.

We also think that some facts definitely argue against the opinion that the agarose test is equivalent to the macrophage migration inhibition test and is therefore a correlate to delayed type hypersensitivity. The most powerful argument against this might be the strong reaction obtained with cells from newborn infants. The marked changes in reactivity noted in cells from the same adult in studies made 6 months apart perhaps also indicate that a phenomenon less stable than cellular immunity against tuberculin lies behind the reaction. A low dose phenomenon, as described in the present paper, is usually not met with when other parameters of cellular immunity are studied *in vitro*. The finding reported by Astor et al. (2) and Senyk & Hadley (23)—that the reaction in polynuclear migration inhibition tests correlate with delayed type skin reactivity only for some antigens and not for others—also argue in favour of the idea that the mechanism involved differs from that effective at delayed type hypersensitivity. The actual

parameter studied can correlate relatively well with delayed type reaction for some, but not for other, antigens. It should be pointed out, however, that Rauch & King (21) also compared the glass capillary leukocyte migration inhibition test with skin reactivity to PPD, candida, and coccidioidin antigens and found a good correlation with all three antigens. Perhaps variations in antigenic preparations or other technical differences can explain the discrepancies in the results of different workers.

SUMMARY

The Clausen agarose migration technique has been explored with PPD as an antigen. The response of leukocytes from normal, Mantoux-positive adult individuals shows marked changes during an observation time of 6 to 12 months. Leukocytes from newborn infants also show a marked response to PPD. Also at very low concentrations of PPD (about 10^{-2} $\mu\text{g/ml}$), inhibition or stimulation of leukocyte migration can be found. Puromycin reduces cell migration also in the absence of PPD, but little influences leukocyte migration when inhibited by PPD. The apparent prevention of PPD-dependent inhibition of migration seen with puromycin, therefore, does not necessarily mean that antigen-dependent migration inhibition is due to a factor produced by lymphocytes. These results and other observations from the literature suggest that the migration inhibition of human leukocytes in this test system is not based on completely the same immunological mechanism as that effective at delayed type hypersensitivity.

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Address of author:

Professor Bengt Källén,
Tornblad Institute,
Biskopsgatan 7,
S-223 62 Lund, Sweden.

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