Further Studies on the Normal Lymphocyte Transfer Test in Man

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In a previous paper from the Nuffield Unit (Moorhead and Patel, 1964) it was recorded that differences of ABO blood group and of sex as between donor and recipient did not appear to influence the normal lymphocyte transfer test of Brent and Medawar (1963), a test which could be applied to the more effective choice of human homograft donors. A feature of the work of Moorhead and Patel was the high proportion of negative normal lymphocyte transfer reactions which occurred (24 out of 80 at 48 hours and 23 out of 80 at 11 days). Since negative normal lymphocyte transfer tests may imply *antigenic similarity* (and thus perhaps homograft acceptance), it was felt desirable to obtain additional experience of the test. We here describe a more precise technique which ensures accurate intradermal injections of the lymphocytes ; we have also studied further aspects of the test, as follows:

1. The normal lymphocyte transfer test has been carried out with dead lymphocytes so that a comparison can be made between the effects of live and dead cells.

2. The Mantoux test has been used to try to assess the contribution of non-specific skin hypersensitivity to the normal lymphocyte transfer test. This was done by comparing the area of erythema produced by positive reactors in both tests. Erythema (as opposed to induration) in the Mantoux test is generally regarded as non-specific (Irvine, 1954).

3. Reciprocal normal lymphocyte transfer tests have been carried out between the members of two small groups of volunteers (Experiments 1 and 2) in an attempt to analyse differences in histocompatibility antigens between a number of pairs of individuals. This was done as a result of a suggestion by Dr. A. J. Bateman, of the Paterson Laboratories, Christie Hospital and Holt Radium Institute, Manchester (see also Bateman, 1965).

4. *In vitro* lymphocyte cultures have been carried out in parallel with the normal lymphocyte transfer tests in Experiment 2, the number of transformed cells occurring in culture probably being a measure of difference of histocompatibility between individuals (Bain *et al.*, 1964 ; Bach and Hirschhorn, 1964).

5. The volunteers used by Moorhead and Patel were tested for leucocyte antigens and circulating leucoagglutinins in order to find out whether differences in leucocyte antigens influenced the results of the normal lymphocyte transfer test.

Methods

Normal Lymphocyte Transfer Test

1. *Separation of leucocytes* was accomplished essentially by the technique suggested by Brent and Medawar (1963), but we have found it convenient to obtain 50 ml. rather than 30 ml. of venous blood in order to obtain enough cells when the yield is low. Although variability in lymphocyte dosage has occurred in this work, there is some evidence that within the range 1–8 million cells this has relatively little effect (Hamburger *et al.*, 1965). All lymphocyte suspensions were stained with leucocyte-diluting fluid before counting, and tests for viability carried out by the dye-exclusion method. As has been said, a point of considerable importance lies in the exact technique used for intradermal injection. Variability in the depth of injection produces discrepant results, and negative reactions certainly follow inadvertent subcutaneous injections. The method recommended for Mantoux-testing (Irvine, 1954) has been adopted in the present work, and injections which do not immediately raise a bleb 6–8 mm. in diameter dimpled by hair follicles are at once repeated. All injections were given into the anterior surface of the upper forearm. In these experiments induration, more palpable than visible but corresponding to the "weal" of Moorhead and Patel (1964), has been measured as well as the erythema. The maximum induration usually occurred, as in previous experiments (Moorhead and Patel, 1964), on the second and eleventh days, and it is this and the erythema visible at these times which are recorded and subsequently referred to as the first and second reactions. No true weals—that is, transient urticaria-like reactions—were seen at 48 hours, though occasionally these occurred in the first 24 hours.

2. Lymphocytes were killed by freezing as described below.

3. Mantoux reactions were obtained by the intradermal injection of 0.1 ml. 1/10,000 Old Tuberculin (Evans).

In-vitro Culture and Counting of the Lymphocyte Series

Culture Method.—Lymphocyte suspensions were prepared in essentially the same way as reported by McFarland and Heilman (1965). Venous blood containing 10 I.U. of preservative-free heparin per ml. was allowed to sediment for one to two hours at 37° C. The cell-rich plasma was removed, mixed with an equal volume of medium NCTC 109 (Difco) in 4-oz. (114-ml.) flat bottles, and incubated for 20 minutes at 37° C. to allow the polymorphonuclear leucocytes to become attached to the glass, after which the lymphocyte-rich suspension was decanted. Finally, the concentration of mononuclear cells was adjusted with NCTC 109 to give 1×10^6 cells/ml. in a plasma concentration of 25–40%. Dead lymphocyte suspensions were prepared by spinning down cells from the final culture preparation. The cells were washed twice with phosphate-buffered saline and resuspended in phosphate-buffered saline at a concentration of 1×10^6 in 0.1 ml. This suspension was frozen and thawed five times, acetone and CO_2-snow freezing mixture being used. Cultures were set up at the time of the normal lymphocyte transfer test and again 11 days later, cells of each of four donors (Experiment 2, Table I) being cultured with cells of each of the other three. Cells were set up alone and with phytohaemagglutinin, added at 0 days or at 4 days, as a control to test the capacity of the lymphocytes to undergo transformation. Preparations were also set up so that each donor's lymphocytes were cultured with dead lymphocytes from each of the other donors, 0.1 ml. of dead lymphocyte preparation being mixed with 1 ml. of live lymphocytes.

Method of Counting Cells.—Samples were taken from the various cultures at seven and eight days and in some experiments at four and five days. After spinning down the cells

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smears were made on coverslips and stained with Giemsa. Differential cell counts of small lymphocytes, eosinophils, macrophages, transformed cells, and cells showing mitotic figures were made on at least three samples, each of 1,000 cells, over the whole smear area. Cells which were large and had strongly basophilic cytoplasm, large nuclei with fine chromatin, and abundant nucleolar material were scored as "transformed." These cells and also those which showed mitosis were recorded and expressed as a percentage of the surviving lymphocyte population.

Leucocyte Typing

The donors and recipients were tested with the antisera that were available. These were anti-4a and 4b, supplied by Dr. J. J. van Rood, serum T.A. from a multitransfused patient, sera P.A. and C.A. derived from immunized mothers, and serum No. 254 from a normal blood donor. The antigens 4a and 4b belong to a reasonably well defined genetic system (van Rood and van Leeuwen, 1963). The other antisera used are under investigation, and it is not yet known whether they define antigens corresponding to any described by other workers, but they do not belong to group 4. It was arranged, so far as possible, to test first the people who had received the injections and then the donors of the lymphocytes—after the tests of the recipients were completed. One sample of blood was taken into ethylenediamine tetra-acetate and another into a dry bottle. The samples were flown from Liverpool to London and the tests made on the same day as the samples were taken. The method used for leucoagglutination was substantially that used by van Rood *et al.* (1964), except that acetic acid was not added before the tests were read. The only difficulty experienced was the development of plasma clots during incubation in two specimens. The sera were inactivated and stored. When the donors' lymphocytes were typed they were also tested against the sera of the recipients.

Results

Normal Lymphocyte Transfer Test Results

1. Dead lymphocytes in no case produced any appreciable first or second reaction, and, as before, no positive autologous reactions were ever obtained.

2. A positive correlation between the areas of erythema in the normal lymphocyte transfer test and Mantoux reaction is

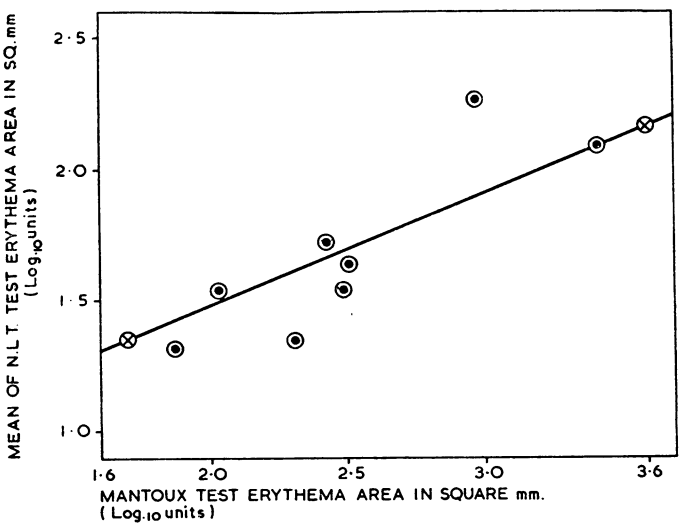

FIG. 1.—Relationship between the area of erythema (in square mm.) in individuals with *positive* normal lymphocyte transfer and *positive* Mantoux first reactions in square mm. with the fitted regression line ($r = +0.480$; $0.05 > P > 0.02$). No correlation is present between the areas of induration in the two tests.

TABLE I.—*Areas of Induration and Erythema (in Square mm.) which occurred in the First and Second Reactions of the Normal Lymphocyte Transfer Test between Pairs of Volunteers in Two Sets of Experiments (A and B). The Upper Figures Refer to Induration and the Lower to Erythema. Part of the Data—that is, the Reaction of Mrs. C. when a Recipient—are Taken from the Paper of Moorhead and Patel* (1964). *No correlation appears to Exist between the Areas of Induration and Erythema in the Reciprocal First Reactions in Pairs of Volunteers* ($P > 0.1$). *Similarly there is no Correlation between the First Reactions in One Volunteer and the Second Reactions in the Other Member of Any Pair when either Induration Alone is Considered or when Erythema is Included* ($P > 0.1$)

A. FIRST EXPERIMENT

Donor	Recipient: Mrs. C.	Miss L.	Mr. M.	Mr. W.	Miss S.
First Reaction (48 Hours)					
Mrs. C.	Nil	23 23	4 4	Nil	Nil
Miss L.	,,	Nil	40 40	12 12	8 45
Mr. M. . .	,,	16 16	Nil	Not done	Not done
Mr. W. . .	,,	23 23	17 17	Nil	Nil
Miss S. . .	,,	55 55	12 12	6 6	,,
Second Reaction (11 Days)					
Mrs. C.	Nil	15 75	80 80	Nil	90 90
Miss L.	,,	Nil	Nil	Nil	35 35
Mr. M. . .	,,	29 29	Nil	Not done	Not done
Mr. W. . .	,,	15 67	6 6	Nil	80 80
Miss S. . .		18 100	Nil	,,	Nil

Donor	No. of Lymphocytes Obtained per ml.	Volume Injected (ml.)	Approx. No. of Lymphocytes Given
Mrs. C. . .	5×10^6	0·1	$0{\cdot}5 \times 10^6$
Miss L. . .	$11{\cdot}6 \times 10^6$	0·1	$1{\cdot}1 \times 10^6$
Mr. M. . .	65×10^6	0·075	$4{\cdot}8 \times 10^6$
,, W. . .	31×10^6	0·15	$4{\cdot}6 \times 10^6$
Miss S. . .	60×10^6	0·075	$4{\cdot}5 \times 10^6$

B. SECOND EXPERIMENT

Donor	Recipient: Mr. Gn.	Mr. Gd.	Mr. Gy.	Mr. Gs.
First Reaction (48 Hours)				
Mr. Gn. . .	Nil	8 8	150 310	40 175
,, Gd. . .	21 21	Nil	Nil	20 20
,, Gy. . .	4 4	6 6	,,	22 22
,, Gs. . .	4 30	40 40	5 22	Nil
Second Reaction (11 Days)				
Mr. Gn. . .	Nil	Nil	Nil	50 50
,, Gd. . .	22 100	,,	40 40	28 28
,, Gy. . .	Nil	,,	Nil	25 25
,, Gs. . .	,,	5 65	15 15	Nil

Donor	No. of Lymphocytes Obtained per ml.	Volume Injected (ml.)	Approx. No. of Lymphocytes Given
Mr. Gn. . .	18×10^6	0·1	$1{\cdot}8 \times 10^6$
,, Gd. . .	4×10^6	0·2	$0{\cdot}8 \times 10^6$
,, Gy. . .	$6{\cdot}4 \times 10^6$	0·2	$1{\cdot}3 \times 10^6$
,, Gs. . .	$11{\cdot}9 \times 10^6$	0·1	$1{\cdot}2 \times 10^6$

shown in Fig. 1, and is statistically significant (0.05>P>0.02). There is no significant correlation when induration alone is measured. It must be pointed out that in these and subsequent analyses the number of observations is small, and it is thus uncertain how effective $\log_{10}$ conversion is in producing a normal distribution. The validity of the analyses is consequently subject to this limitation.

3. The results of reciprocal normal lymphocyte transfer tests are shown in Table I. No correlation seems to exist between the areas of induration and erythema in the reciprocal first reactions in pairs of volunteers (P>0.1). Similarly there is no correlation between the first reactions in one volunteer and the second reactions in the other member of any pair when either induration alone is considered or when erythema is included (P>0.1).

Results with Lymphocytes Cultured in vitro

1. *Cultures of Lymphocytes Made at Beginning of the Normal Lymphocyte Transfer Test.*—The percentages of transformations observed in the mixed live lymphocyte cultures of the different donors are summarized in Table II. No significant correlation (P>0.1) between the logs of the *in vitro* transformations and the mean of the logs of the areas of induration of the reciprocal first reactions of the normal lymphocyte transfer test is apparent, but if the total area—that is, induration plus erythema—is measured then there is a statistically significant correlation (0.05>P>0.02) (Fig. 2).

2. *Results of Mixed Live Lymphocytes and Dead Lymphocyte Cultures.*—Here the highest transformation which occurred was only 0.22% either before or 11 days after the normal lymphocyte transfer test.

TABLE II.—*Percentage Transformation in Mixed Live Cultures of Lymphocytes from Volunteers in the Second Experiment Set Up at the Beginning of the Normal Lymphocyte Transfer Test and Examined Seven and Eight Days Later. The Areas of Induration of the Reciprocal Normal Lymphocyte Tests (1st Reaction) are Given for Comparison. There is a Trend Towards a Correlation, but this Reaches Significance only if Induration Plus Erythema is Considered*

Donor Mixture	7th Day *in vitro*	8th Day *in vitro*	N.L.T. Test Reciprocal Reading of 1st Reaction
Gn + Gd ..	3·5%	2·6%	(8 and 21)
Gn + Gy ..	1·9%	5·2%	(150 „ 4)
Gn + Gs ..	4·4%	7·95%	(40 „ 4)
Gd + Gy ..	0·6%	0·9%	(Nil„ 6)
Gd + Gs ..	2·0%	1·7%	(20 „ 40)
Gy + Gs	2·0%	1·7%	(22 „ 5)

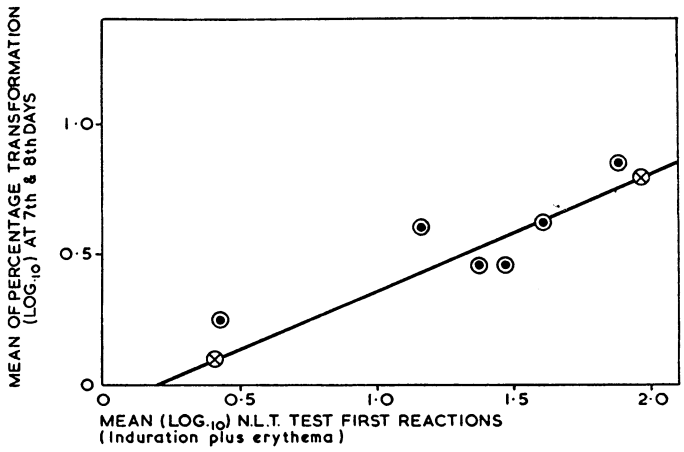

FIG. 2.—Relationship of the *in vitro* transformations with the mean of the areas of induration plus erythema in square mm. of the reciprocal first reactions of the normal lymphocyte transfer test with the fitted regression line (r = +0.853; 0.05>P>0.02). Although the correlation is statistically significant it is based on only small numbers. Furthermore, if induration only is considered, there is no significant correlation. Angular measure of percentage transformation instead of log conversion yields a similar result.

3. Cultures with phytohaemagglutinin added either at 0 days or at 4 days showed a very good response in terms of transformation (minimum about 50%).

4. Controls consisting of live lymphocytes cultured alone invariably showed a transformation of less than 1%.

5. Cultures of lymphocytes were also made at the time of the secondary reaction (11 days). Transformation of mixed live lymphocyte cultures read five and seven days later are shown in Table III. The proportion of transformed cells is not strictly comparable in the two experiments—that is, at the beginning and after the second reaction of the normal lymphocyte transfer test—but there was no increase in percentage transformation to suggest sensitization by the normal lymphocyte transfer test itself.

TABLE III.—*Percentage Transformation in Mixed Live Cultures of Lymphocytes from Volunteers in Second Experiment Set Up 11 Days After the Normal Lymphocyte Transfer Test and Examined Five and Seven Days Later. The Areas of Induration of the Reciprocal Normal Lymphocyte Tests (2nd Reaction) are Given for Comparison. No Trend Towards a Correlation is Apparent when either Induration or Induration Plus Erythema is Measured*

Donor Mixture	5th Day *in vitro*	7th Day *in vitro*	N.L.T. Test Reciprocal Reading of 2nd Reaction
Gn + Gd ..	0·36%	4·2%	(Nil and 22)
Gn + Gy ..	1·2%	1·9%	(Nil „ nil)
Gn + Gs ..	1·3%	4·2%	(50 „ „)
Gd + Gy ..	0·0%	0·48%	(40 „ „)
Gd + Gs ..	0·1%	0·19%	(28 „ 5)
Gy + Gs	0·0%	0·29%	(15 „ 15)

Leucocyte-typing Results

The results of the leucocyte antigen tests are given in Table IV, together with the areas of skin reactions of the 10 male and 10 female recipients reported in the paper of Moorhead and Patel (1964).

There seemed to be no simple correlation between the size of the reaction in the normal lymphocyte transfer test and differences in leucocyte antigens between donor and recipient in the systems which we tested. Thus, in the 4a and 4b system, injection of incompatible lymphocytes does not produce larger skin reactions than compatible ones. However, the data are being further analysed by Dr. Moorhead. They have also

TABLE IV.—*Leucocyte Antigens of Recipients and Donors and the Normal Lymphocyte Transfer Test Reactions Recorded by Moorhead and Patel* (1964)

Recipients	4a	4b	T.A.	P.A.	C.A.	254	N.L.T. Reactions in sq. mm. (Donors)							
							A♂		A♀		B♂		B♀	
							1	2	1	2	1	2	1	2
Males														
1	+	+	+	+	+	+	423	0	140	0	151	26	64	0
2	+	−	.	+	−	−	12	40	40	22	0	20	0	25
3	+	+	.	+	−	+	98	46	8	14	48	24	25	7
4	−	+	+	+	−	+	34	38	0	0	0	31	0	21
5	+	+	+	+	−	+	130	24	240	34	150	25	52	24
6	+	+	+	+	+	+	116	34	125	17	17	45	6	28
7	+	+	−	+	−	−	42	0	0	0	0	0	0	0
8	.	.	.	.	.	.	22	14	25	14	31	13	0	0
9	.	.	.	.	.	.	0	21	32	0	28	0	0	0
10	+	+	−	−	+	−	29	325	0	143	0	120	0	205
Females														
1	+	−	.	+	−	−	50	52	0	0	0	0	56	15
2	.	.	.	.	.	.	11	21	90	28	9	23	0	48
3	+	+	+	+	−	+	6	14	38	22	9	23	25	9
4	−	+	+	+	−	.	0	0	0	0	0	0	0	0
5	+	+	.	+	−	−	26	25	75	13	0	18	25	59
6	+	+	−	+	−	+	26	59	14	22	140	56	0	0
7	−	+	+	+	+	+	184	34	18	11	6	15	6	12
8	+	−	+	−	−	+	64	22	32	19	67	17	24	24
9	+	−	−	+	+	+	0	0	30	0	46	0	0	0
10	+	+	.	+	−	−	18	188	14	27	32	30	0	66
Donors														
A♂	+	+	−	+	−	−								
A♀	+	+	−	−	+	−								
B♂	+	+	+	+	+	.								
B♀	+	+	+	+	+	+								

been expanded by Dr. van Rood, and this will be the subject of a further paper.

All the tests for presence of leucoagglutinins in the sera of the recipients were negative except in the case of female 9 (Mrs. F.), in whose serum a leucoagglutinin of the specificity anti-4b was found. Although all four donors were (fortuitously) 4b+, the antibody was present at the same titre before and after the injections of the incompatible lymphocytes. It is probable, therefore, that this antibody had been formed as the result of the pregnancies Mrs. F. had had about 20 years previously. Typing of the family showed that Mrs. F. was incompatible with her husband on the 4b system. So it appears that leucoagglutinins may persist for many years after the pregnancies which stimulated their formation. No red-cell antibodies were detected in Mrs. F.'s serum.

Discussion

Interpretation of the Normal Lymphocyte Transfer Test

The fact that dead lymphocytes do not produce any appreciable first (48 hours) reaction supports the view that this is predominantly activated by a donor-*versus*-recipient reaction depending upon the presence of live lymphocytes, as suggested originally by Brent and Medawar (1963). On the other hand, Amos *et al.* (1965) state that host factors are also important in the first reaction, and they think that the test is less reliable as an indicator of histocompatibility in man than it is in the guinea-pig. The absence of second reactions with dead lymphocytes is not in accord with the second reactions, being a host-*versus*-graft response, but it is possible that antigens are liberated from the dead lymphocytes and are lost by diffusion before the second reaction can occur.

By use of reciprocal normal lymphocyte transfer tests it was hoped to be able to score for degrees of histocompatibility between individuals, but this has not proved to be the case. Thus no consistent trend has been found between the size of the first reaction of donor A into recipient B and the second reaction in the reciprocal experiment when B is the donor and A the recipient. Such concordance would be expected if both reactions were due to the same histocompatibility antigens present both on lymphoctyes and on tissue cells. The reason for the discordance may be that completely different antigenic systems are responsible for the first and second reactions, but it is perhaps more likely that the second reaction is so overlaid by non-specific effects as to prohibit simple analysis. Thus many factors—cellular, humoral, and non-specific—may impinge on the second reaction, making it a much less simple phenomenon than the first.

Correlation between the size of reciprocal first reactions might suggest multifactorial inheritance or a system of alleles controlling weak and strong antigens, but in fact no quantitative correlation can be detected between the first reactions of the reciprocal normal lymphocyte transfer test (Table I). Although Brent and Medawar (1964) have convincingly indicated that the normal lymphocyte transfer test is dependent upon genetic factors in animals, and in man identical twins give no reactions to each other's lymphocytes, yet no clear pattern of genetic control has become evident in this work. Various genetic models have been considered, including systems depending upon both major genes and polygenes (Simonsen, 1965), but none is convincing in fitting the available data sufficiently well to be acceptable (see Bateman, 1965).

Reading the Test

After intradermal injections of lymphocytes induration increases to its maximum at 48 hours, when it has been measured and recorded. It is evident that when either a weal with flare or erythema alone occurs, as commonly happens in the first 24 to 36 hours, it is a host reaction and is probably of the accelerated hypersensitivity type. In some individuals the area and the intensity of this erythema have begun to recede by the time the induration has reached its maximum. In addition we have noticed, in common with other workers (Amos *et al.*, 1965), that quite light rubbing will markedly increase the measurable erythema. These observations made it difficult to accept erythema as a reliable measure of histo-incompatibility, and common sense suggests that induration, since it is comparatively stable, is probably a more consistent guide.

This impression is strengthened by the statistically significant correlation that we have demonstrated between the erythema of the Mantoux and normal lymphocyte transfer tests. An individual may thus have a fairly constant ability to produce erythematous reactions after injection of any foreign material. This lack of specificity is recognized in Mantoux-testing, in which erythema is ignored and induration alone is measured.

Negative Reactions in Normal Lymphocyte Transfer Test

In the present work few negative first normal lymphocyte transfer test reactions have been encountered compared with the Moorhead and Patel experiment ; but they have still been found, and their occurrence and the more frequent weak reactions may indicate antigenic similarity. This is the practical application of the test, for among negative or weak reactors may be found the most suitable donors of homografts.

Nevertheless there are two other hypothetical explanations for negative reactions. First, injected lymphocytes may not react with foreign-tissue antigens unless the lymphocytes have had previous immunological contact with them. Such prior sensitization of the donor to prospective recipient antigens may be necessary before a first reaction can occur, and this might happen either by two-way exchange of leucocytes between mother and foetus, or, in post-natal life, by inhalation and ingestion of antigenic material. If this were the case it should be possible to convert a negative or weak normal lymphocyte transfer test into a positive one by repeated injections of lymphocytes or by skin-grafting.

Second, it seems highly probable that the reaction against foreign cells is not entirely due to immune mechanisms. For example, patients with advanced malignant disease or with uraemia often react weakly to the normal lymphocyte transfer test (Bridges *et al.*, 1964), and, as pointed out by Amos *et al.* (1965), it is precisely the uraemic subjects who are most in need of a test for histocompatibility. Thus in man the normal lymphocyte transfer test may be influenced by factors not directly related to genetic differences.

In-vitro Transformations of Mixed Lymphocytes

In this work the maximum *in vitro* transformation was 8%, but other workers have obtained larger proportions. A figure of over 70% in some cases was reported by Hirschhorn *et al.* (1963), and Elves and Israëls (1965) obtained a maximum transformation of just over 20%. It is probable that these differing results are attributable to differences in technique and scoring.

The comparison of the normal lymphocyte transfer test first reactions of two individuals and the percentage of the *in vitro* transformation when their lymphocytes are cultured together has been noted earlier. The very small percentage of transformations which occur in our hands when live lymphocytes are cultured with killed cells is interesting, but this is not the experience of everyone. Elves and Israëls (1965), using a mixture of live and killed lymphocytes, obtained a reduced

transformation which was relatively larger than ours, and it is likely that transformation can be induced by material from dead lymphocytes. That transformations of similar size were obtained in mixtures of lymphocytes taken before and after the normal lymphocyte transfer test is surprising, since it might be expected that the intradermal injection of lymphocytes would sensitize the recipient's lymphocytes. This would be comparable to the *in vitro* response of lymphocytes from tuberculin-positive individuals when these cells are cultured with added tuberculin (Pearmain *et al.*, 1963). Our failure to demonstrate increased transformation may have been because the rather small numbers of lymphocytes (about 10^6) given in this normal lymphocyte transfer test were insufficient in the experiment to produce sensitization. Skin-grafting experiments now in progress will allow both normal lymphocyte transfer tests and *in vitro* methods to be carried out before and after rejections. Evidence of conversion of the normal lymphocyte transfer or of accelerated *in vitro* transformation would then suggest sensitization.

If it is possible to assess the degree of histocompatibility by the *in vitro* culture of leucocytes it would constitute a considerable advance, since one disadvantage of the normal lymphocyte transfer test is that prospective donors could become sensitized to the patient's lymphocytes. In addition an *in vitro* test obviously does not carry the same risk of serum hepatitis as an intradermal test. However, at present the *in vitro* test is not yet a substitute for the normal lymphocyte transfer test, because we do not yet know enough about the relationship between the two, and it is not yet easily possible to distinguish the contribution of each donor to the total lymphocytic transformation. The use of appropriate mixtures of live and dead lymphocytes or of *x*-irradiated cells will probably allow this to be done eventually.

Although the number of tests is too small for certainty, analysis of the data comparing *in vitro* transformation and reciprocal normal lymphocyte transfer test first reactions reaches significance only when erythema is included in the area recorded and fails to do so with induration alone, though the trend here is also towards a positive correlation. Perhaps when more data are available induration will be shown to be correlated with peak *in vitro* transformations. If this proves to be the case there will then be little doubt that the normal lymphocyte transfer and *in vitro* tests are measuring similar histocompatibility components.

In the meantime some reservations must remain about the comparability of the *in vitro* and intradermal methods, and the question of erythema specificity must remain open. For this reason both induration and erythema are recorded in Table I.

Circulating Antibody and the Normal Lymphocyte Transfer Test

As recorded by Moorhead and Patel (1964), it did not appear that the ABO groups were playing a major part in the normal lymphocyte transfer test results. However, a factor which has to be considered when the donor and recipient are incompatible on the ABO system is the effect of circulating antibodies (anti-A and anti-B) on the injected lymphocytes, which may carry the ABH antigens (Berroche *et al.*, 1955). Because of this it is likely that incompatible lymphocytes will become coated with circulating antibody. Though these ABO antibodies are not usually cytotoxic (they do not, for example, inhibit the division of stimulated lymphocytes *in vitro*) yet the coating with gamma-globulin might interfere with antigen-antibody reactions. In fact there is a suggestion in the data of Moorhead and Patel (1964) that ABO incompatible injections do produce less reaction than compatible ones, but the differences are not statistically significant. Another point is that the injection of incompatible lymphocytes, together with the inevitable red-cell contaminant, did not produce a significant change in the titre of anti-B in any of the recipients.

A leucoagglutinin was detected in the serum of only one individual (female) and was of the type anti-4b, and this antibody was present before the normal lymphocyte transfer tests were performed. Here again, as with ABO, the effect of circulating antibody on the injected lymphocytes has to be considered. There was no systemic reaction when the normal lymphocyte transfer test injections were given to this female, and there was a complete absence of reactions at 11 days. It is possible that the lymphocytes were coated with antibody and removed from the site of injection, and therefore there was no visible hypersensitivity reaction.

Future Work

We are now directly comparing the normal lymphocyte transfer test results with the fate of skin grafts in volunteers in an attempt to evaluate the test in predicting suitable donors of homografts. The normal lymphocyte transfer test will be repeated when the skin graft is rejected, since it is assumed that sensitization of the recipients will then have occurred. Positive conversion of a previously negative first reaction, if it occurs, will suggest that prior sensitization of lymphocyte donors is necessary before a positive reaction can arise.

In addition, work is proceeding on the genetic control of the normal lymphocyte transfer reaction in man by using a standard donor of lymphocytes in a survey of a population sample. By these means it may be possible to obtain an approximation of the frequency of positive and negative reactions to this standard donor. Mendelian segregation is being tested for by reciprocal normal lymphocyte transfer tests in family studies, and these will be accompanied by parallel *in vitro* cultures.

Summary

The importance of a consistent technique in the normal lymphocyte transfer test in order to avoid false-negative results is emphasized.

The relative importance of induration and of erythema in the normal lymphocyte transfer test has been investigated by comparing it with the Mantoux reaction. It is concluded that induration is the more specific feature in the normal lymphocyte transfer test and that non-specific factors are partly responsible for the erythema. The relative importance of the specific component of erythema in any particular individual remains uncertain.

No progress has been made in determining the mechanism of genetic control of the normal lymphocyte transfer test.

Satisfactory *in vitro* lymphocyte cultures have been obtained, but no significant correlation has been demonstrated between the proportion of transformed cells and the area of induration in the normal lymphocyte transfer test, though a significant correlation is found when induration plus erythema is measured.

Differences in certain leucocyte antigens apparently do not influence reactions to the normal lymphocyte transfer test. The presence of circulating antibody may influence the results.

Indications for future work are discussed.

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The Epping Jaundice

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Early in February 1965 one of the medical students attached to St. Margaret's Hospital, Epping, reported sick with a history of severe upper abdominal pain of two days' duration followed by mild jaundice. At about the same time a woman anaesthetist married to a local general practitioner presented with similar symptoms. Soon after this several other cases appeared with jaundice, some with pain, others with discomfort, and it became apparent that these were not cases of biliary obstruction or of infectious hepatitis.

The general practitioners in the area were asked to report similar cases, and it was soon obvious that a local outbreak of jaundice was occurring which did not conform to any known clinical picture.

Clinical Presentation

Patients presented in three ways. The commonest form had an acute onset with severe intermittent pain in the upper abdomen and lower chest, mainly towards the right side. This could be severe and last from 24 to 36 hours, after which it was often noted that the motions were pale and the urine was dark. During the next four to five days these patients improved but still felt unwell, and then many of them became pyrexial with 'flu-like generalized aches and pains, rigors, and increasing jaundice. On examination the liver was enlarged and tender, occasionally reaching 3–4 in. (7.5–10 cm.) below the costal margin. The spleen was never palpable. This condition lasted a few days and was followed by general improvement, the liver becoming smaller and less tender, though the jaundice persisted in many cases for weeks, with pruritus nearly always present during the whole period. Despite being jaundiced the patients now felt better and had a good appetite but were not really well for some considerable time.

Other cases, often in the families of those described above, had only vague initial symptoms of upper abdominal discomfort, not severe enough to seek medical attention. About a week later, however, these too developed pyrexia, generalized aches, and increasing jaundice similar to the second phase in those previously described. On the whole jaundice persisted longer in these patients.

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The third and least common type of onset was in some elderly patients who, when first seen, had severe jaundice but gave a history of only minimal preceding symptoms. The liver in these patients was often greatly enlarged but was rarely tender on palpation.

Incidence and Clinical Findings

We have records of 84 persons who were affected by the disease. None of them had any relevant drug history prior to the illness. In 50 the onset was acute with fairly severe colicky pain of the type described, 29 had the more insidious onset, and only five presented with severe jaundice and minimal preceding symptoms. Fifty-seven of these patients were more fully investigated.

The degree of jaundice varied considerably. The highest serum bilirubin in the series was 29.3 mg./100 ml. The jaundice was usually much milder, however, and 35 patients had serum bilirubin levels under 5 mg./100 ml., 14 between 5 and 10 mg./100 ml., and only three had values of 20 mg./100 ml. or more. All the patients showed some rise in the serum alkaline phosphatase level, the highest being 84.5 K.A. units/100 ml., 22 having values between 20 and 30 K.A. units/100 ml., and 23 being below 20 K.A. units/100 ml. The thymol turbidity test was normal in all cases. The serum glutamic oxaloacetic transaminase level was invariably raised, in most instances to between 40 and 50 Karmen units, and took some time to settle to normal. Occasionally values of over 200 Karmen units were obtained early in the disease, while a few cases showed a secondary peak during recovery.

Needle biopsy of the liver was performed in four cases within two to three weeks of the onset of symptoms. All the biopsies showed cellular infiltration and cholestasis. There was evidence of damage both to the liver parenchyma and to the biliary tree. In two cases cholangitis was apparent. These findings were unique and differed from those produced by known infective, toxic, or therapeutic agents in man. A detailed description of the cases and biopsy findings will be published separately (Kopelman, Scheuer, and Williams, 1966).

At first an infective basis for the disease was suspected and investigations were instituted with this in mind. The Paul-Bunnell test was performed on 12 patients and was negative, as was the *Brucella abortus* agglutination test on 13. The results of investigations for the presence of enterovirus in stools