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LYMPHOCYTE TRANSFORMATION,
LEUCOCYTE MIGRATION, SPECIFIC IgE, IgG
AND IgM BEFORE, DURING, AND AFTER
PENICILLIN TREATMENT WITHOUT
ADVERSE REACTION

A Follow-Up Study

By

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Using intradermal tests and passive hemagglutination technique, Levine et al. (7), Redmond & Levine (13), have previously shown that numerous subjects have undergone an immune response to penicillin. These subjects were not always found to be allergic to this drug, and with some, a subsequent use of penicillin did not induce a reaction. The lymphocyte transformation test (LTT) seemed more sensitive than skin tests (21). In most of the studies, the LTT was found to be negative in controls (i.e. non allergic subjects) whereas only allergic subjects had a positive LTT (4). However, the modifications of the *in vitro* test response by a penicillin treatment were not investigated in these non allergic controls.

The aim of this work was thus to examine *in vitro* the immune response to penicillin of non allergic controls. In addition to LTT, three other tests were used: passive hemagglutination which detects anti-penicilloyl IgG or IgM; passive anaphylaxis on monkey lung tissue for detection of serum anti-penicillin IgE; and leucocyte migration test. Non allergic

controls were tested before, during and after a well-tolerated course of penicillin therapy.

MATERIALS AND METHODS

Subjects Studied

Before treatment by penicillin: Twenty four subjects (5 females, 19 males; mean 32 years, range 17-48) of which six had a personal or family history of atopy. Further subdivision was as follows: 14 healthy volunteers who had received penicillin more than 1 year previously without incident; 10 patients with primary (two), secondary (six) and serologic (two) syphilis. None of them had received penicillin less than 1 year previously. None had history suggestive of allergy to penicillin.

Without treatment by penicillin: Five out of the 14 healthy volunteers were tested three times at intervals of 20 days thus serving as control group of normal variability.

During and after penicillin: The 10 syphilitic patients tested before treatment were tested again four times: the 8th and 15th day after treatment and 8 and 30 days after the end of therapy. This was scheduled as follows: daily intramuscular injection of sodium benzyl penicillin (400,000 IU) and benethamine penicillin (600,000 IU) for 15 days. Four of these patients could be studied again 90 days after the end of therapy, and one of them during and after a second course of penicillin.

In vitro Studies

For all tests sodium benzylpenicillinate (BP) was used as antigen.

Leucocyte migration test (LMT): The technique, similar to that of Søborg & Bendixen (20), has previously been described (15). Control migrations (six to ten) did not contain any antibiotic. Test migrations (3 to 5 for each dose) were performed with freshly prepared BP; 1,000, 5,000, and 10,000 IU/ml were used routinely but 10,000 IU/ml were found to be the most appropriate. Previous experiments (16) had shown that these concentrations did not induce a non specific inhibition of leucocyte migration. The interpretation that a penicillin-induced inhibition reflects a positive test has been discussed in detail elsewhere (16); it is recalled briefly here: a 0.80 migration index was considered to be the highest value for a positive test as it has been shown by numerous authors to be the limit for a significant inhibition in various antigenic systems; indeed intradermal tests with BP are not used routinely in this country and it was not possible to make a correlation between LMT and delayed cutaneous responses. However, using a micromethod, we found that the migration index was always above 0.80 in newborns when BP was used as antigen (unpublished results). Nevertheless, the significance of each inhibition was further assessed as follows: for each control migration serie the mean (M.C.) and the standard deviation

(s.d.) were calculated; in each experiment reported as positive in this study the mean of migration values with penicillin (M.P.) was: $M.P. \leq M.C. \pm 3 \text{ s.d.}$ (i.e. a significance at the 0.01 level). In a few cases a stimulation of migration was observed. As this phenomenon in the LMT is still under investigation (19), it was only noted here as such; it was outlined when $M.P. \geq M.C.$ (s.d. 3).

Lymphoblastic transformation test (LTT): Papiernick's technique (12) was used. Although usually performed in autologous serum, for the syphilitic group it was carried out in both autologous and homologous sera with similar results. This is in agreement with Muscher et al.'s work (10) on the effect of syphilitic sera on LTT. The cells were cultured for 4 days with 250, 500, 750 and 1,000 IU/ml of freshly prepared BP. Results were estimated cytologically by two observers counting 1,000 cells by slide. Cells were considered as blasts if they were greater than 30μ in diameter, hyperbasophilic with a reticulum pattern of the chromatin, and two or more nucleoli. According to Halpern (4) the LTT was considered positive if the percentage of blasts in the presence of penicillin was at least 4 per cent greater than those of cultures without penicillin.

Passive anaphylaxis on monkey lung tissue (PAM): This technique has been described by Ishizaka (5) for the detection of anti-ragweed serum IgE in man. The principle of PAM is passive binding of human serum IgE by monkey lung mast cells and evaluation of the release histamine by antigenic challenge. The following modifications were made in order to improve sensitivity and to reduce the occurrence of false negative results (1, 2). Serum was predialysed against isotonic glucose solution in order to decrease electrolytes and thus improve the lung tissue sensitization (8). Each tube contained 500 mg of lung tissue fragments. Five tubes were filled with glucose solution (4 ml) and EDTA only (non sensitized controls); three test tubes contained 0.5, 1 and 2 ml of the predialysed serum diluted in glucose solution (4 ml) and EDTA. After 2 h incubation at 37°C , the lung fragments were washed three times in Tyrode solution, and then challenged with BP (1,000 IU in 5 ml Tyrode solution) for 15 min at 37°C . After removing lung fragments, the released histamine was measured.

Histamine content was determined by biological assay on isolated guinea-pig ileum. Contractions due to the material released from lung tissue were inhibited by the addition of mepyramine confirming its identity with histamine. Histamine content was also determined by the automatic fluorometric method (18). Difference between these two assays was not found to be more than 5 per cent.

Previous studies (2) had shown that:

- (1) the PAM was only detecting IgE,
- (2) the technique could not be quantitative, even on the basis of the amount of histamine released; indeed this amount was found to be related both to the total histamine content of the lung and to the antibody levels. Thus the results are given only as "presence" or "absence" of anti-penicillin IgE.

PAM is reported to be positive when the histamine released after BP chal-

TABLE 1
Twenty-four Subjects At Least 1 Year after Penicillin Therapy.

No.	LMT	LTT	PAM	PH
1	0.86	0.0	—	—
2	0.83	0.9	—	—
3	0.83	2.6	—	—
4	0.61	0.6	—	—
5	0.65	ND	—	—
6	0.57	0.3	—	—
7	0.95	ND	+	—
8	0.98	1.2	+	—
9	0.68	0.1	+	—
10	0.58	7.1	+	—
11	0.95	0.3	—	—
12	1.05	1.2	+	—
13	1.03	0.2	—	—
14	0.85	4.1	—	—
15	0.98	1.1	—	—
16	0.88	2.1	—	—
17	1.55*	1.6	—	—
18	0.83	ND	—	ND
19	0.74	ND	—	ND
20	0.67	ND	—	ND
21	0.95	0.3	+	—
22	0.90	0.1	+	—
23	1.07	ND	—	—
24	1.25*	3.9	ND	ND
Total	24	18	23	20
% Positive	29 %	11 %	30 %	0 %

Lymphocyte transformation test (LTT) results are given as blasts per cent superior to the control culture. Leucocyte migration test (LMT) results are given as migration index.

Specific serum IgE by passive anaphylaxis on monkey lung tissue (PAM).
 Passive hemagglutination (PH).

ND: not done.

* Stimulation with a M.P. $\geq$ M.C. $\pm$ 3 s.d. (see Methods).

lence in one (or more) tube(s) containing sensitized lung was higher than the mean ($\pm$ 2 s.d.) histamine level in the five tubes containing non sensitized lung pieces.

Passive hemagglutination (PH): De Weck's technique (22) was used. Sera

were treated by mercaptoethanol (final concentration 0.1 M) to distinguish IgM from IgG.

RESULTS

In vitro Evaluation of the Anti-Penicillin Immune State at least 1 Year after Therapeutic Use of Penicillin in 24 Subjects

Table 1 shows these results. Seven subjects were found to have a significant inhibition of leucocyte migration (29 per cent). Two showed a stimulation. Two subjects were found to have a lymphocyte transformation (11 per cent). Anti-peni-

TABLE 2
Spontaneous Variations in 5 Non Treated Subjects.

Patient no.	Test	Day		
		0	20	40
11	LMT	0.95	0.98	0.96
	LTT	0.3	ND	0.4
	PAM	—	—	—
	PH	—	—	—
13	LMT	1.03	1.00	0.83
	LTT	0.2	1.8	1.1
	PAM	—	—	—
	PH	—	—	—
14	LMT	0.85	0.82	0.94
	LTT	4.1	1.4	0.0
	PAM	—	—	—
	PH	—	—	—
15	LMT	0.98	0.95	0.88
	LTT	1.1	0.0	0.0
	PAM	—	—	—
	PH	—	—	—
16	LMT	0.88	0.82	0.88
	LTT	2.1	0.0	0.5
	PAM	—	—	—
	PH	—	—	—

For abbreviations, see legend Table 1.

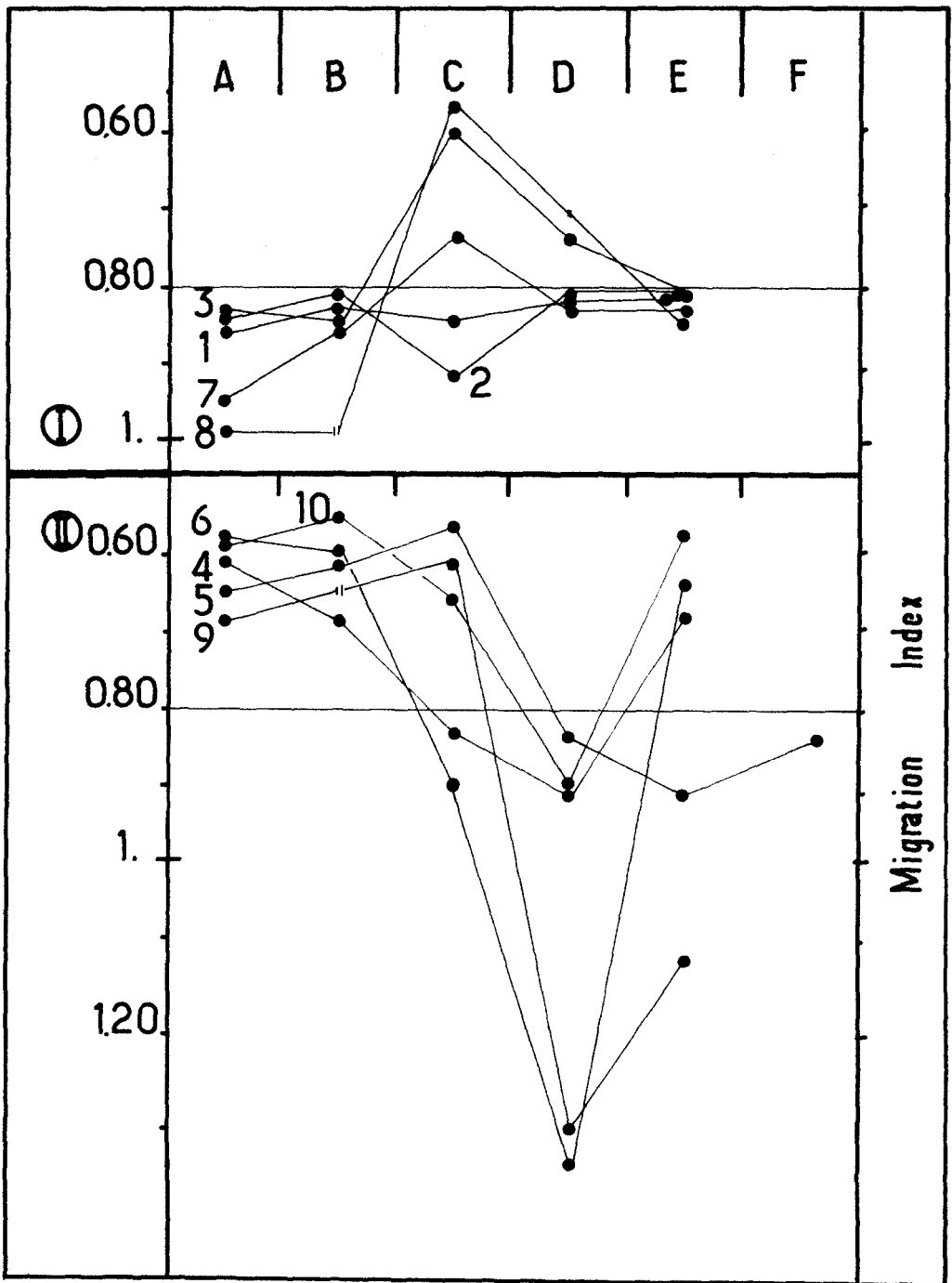


Fig. 1.

Follow-up study of leucocyte migration in 10 syphilitic subjects.

- (A) before treatment.
- (B) the 8th day of treatment with penicillin.
- (C) the 15th day of treatment.
- (D) 8 days after the end of treatment.

cillin IgE were present in the serum of seven subjects (30 per cent). No anti-benzyl penicilloyl IgM or IgG were detected. Two patients had more than one positive test: LMT and IgE (no. 9), LMT, IgE and LTT (no. 10). No significant difference was found between the healthy volunteer group and the syphilitic group before treatment.

Variations in the Absence of Treatment

These variations were slight as can be seen in Table 2.

Effect of Penicillin Treatment in 10 Syphilitic Patients

In only one patient (no. 1) were all the tests negative throughout the study.

LMT results: The treatment induced three kinds of response (Fig. 1). In five patients with a negative LMT before penicillin, it was found to be transiently positive (nos. 3, 7, 8) or unmodified (nos. 1, 2). In five patients with positive LMT before the treatment, the tests became negative during and just after it; the desensitization was transient for three subjects (nos. 4, 6, 10) and longer for two others (nos. 5, 9).

LTT results: The treatment also induced three kinds of response (Fig. 2). Three patients (nos. 1, 2, 9) had a negative test during the study. In the six others (nos. 3, 4, 5, 6, 7, 8) the test became positive on the 15th day of the treatment; three (nos. 3, 5, 6) remained positive for 30 days and only one (no. 5) 90 days after its termination.

In the patient with positive LTT before treatment (no. 10) the test became negative in a transient manner. This phenom-

Fig. 1. (continued).

(E) 30 days after.

(F) 90 days after.

I. Five subjects with a negative LMT before treatment.

II. Five subjects with a positive LMT before treatment.

In the two cases of stimulation in "D" in patients 6 and 9 the M.P. was $> \text{M.C.} \pm 3 \text{ s.d.}$ (see Methods).

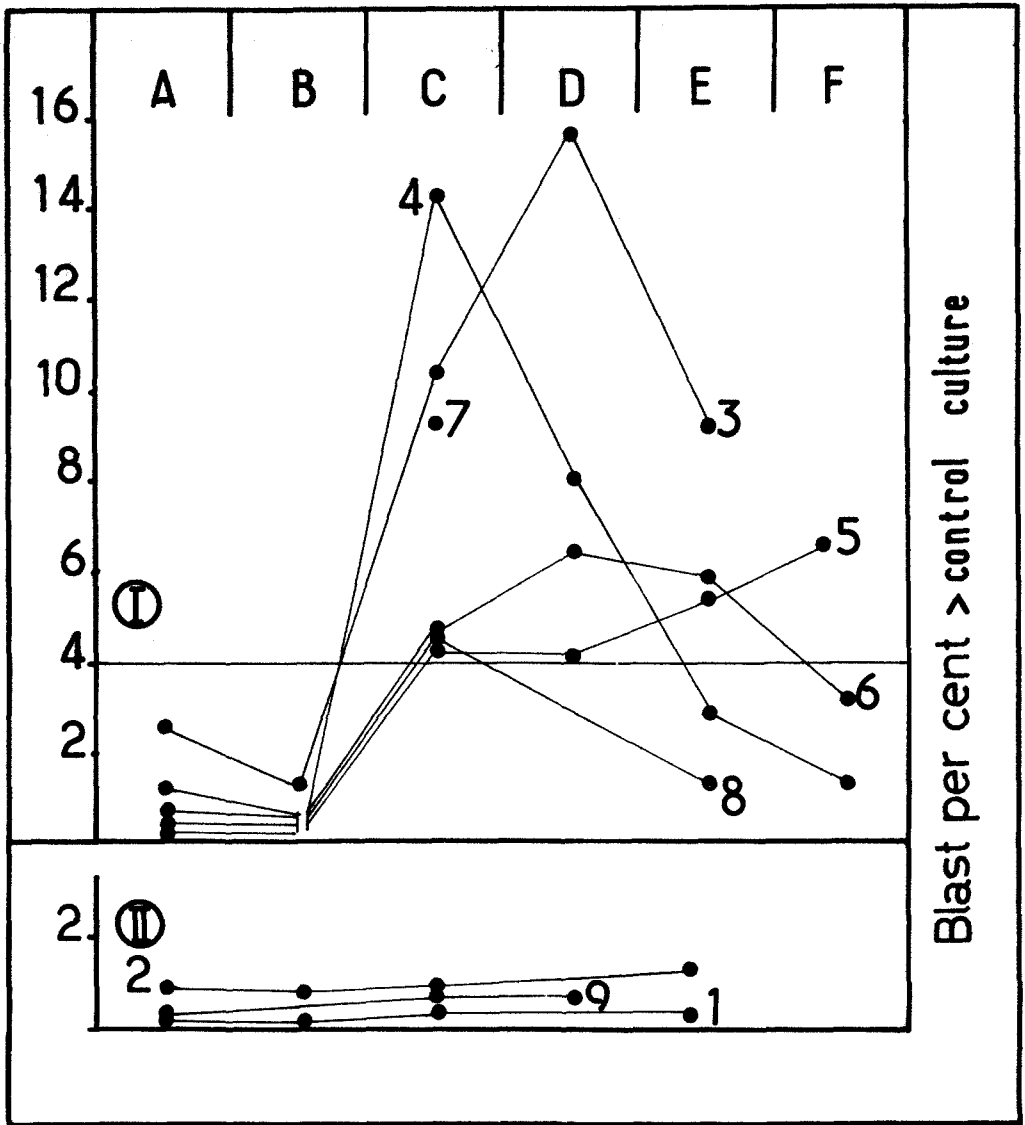


Fig. 2.

Lymphocyte transformation. Follow-up study in nine subjects with a negative test before the treatment.

I. Positive conversion in six subjects.

II. No variation in three others.

For the A.B.C.D.E.F., see legend Fig. 1.

enon was repeated during a second course of treatment (Fig. 3).

PAM results: In this test the treatment also induced three kinds of response (Table 3). Six patients (nos. 1–6) had no

TABLE 3
*Follow-up Study of Passive Anaphylaxis on Monkey Lung Tissue in
 10 Syphilitic Subjects.*

Patient no.	Before	Day during		Day after penicillin		
		8th	15th	8th	30th	90th
1	—	—	—	—	—	
2	—	—	+	—	—	
3	—	+	—	—	—	
4	—	—	—	—	—	—
5	—	—	—	—	+	+
6	—	—	—	—	—	+
7	+	—	—	—	—	
8	+	ND	+	ND	—	
9	+	ND	—	—	ND	
10	+	+	+	—	—	—

ND: not done.

detectable IgE before treatment. Two of them (nos. 1, 4) had no modification by treatment. The four others were found to have anti-penicillin IgE during and after the treatment. Four patients (nos. 7, 8, 9, 10) had these antibodies before the beginning of the treatment, but they were no longer detectable during and after treatment.

Passive hemagglutination: A slight IgM anti-penicillin production was found in three (nos. 2, 4, 6) of the patients (Table 4).

Correlation of each type of response in each subject (Fig. 4): It was not possible to bring about only one type of response. On the basis of the immune state found before the treatment, four types could be noted: (I) no detectable sensitization (subjects 1, 2, 3); (II) cellular immunity only (LMT, subjects 4, 5, 6); (III) IgE only (PAM subjects 7, 8); (IV) co-existence of cellular immunity with IgE (subjects 9, 10). The subsequent evolution of each group during and after the treatment, however, did not show a very distinctive pattern.

TABLE 4
Follow-up Study of Anti-Penicillin IgM in 10 Syphilitic Subjects.

Patient no.	Before	Day during		Day after penicillin		
		8th	15th	8th	30th	90th
1	—	—	—	—	—	
2	—	—	4	4	—	
3	—	—	—	—	—	
4	—	—	4	8	—	—
5	—	—	—	—	—	—
6	—	—	—	4	4	—
7	—	—	—	—	—	—
8	—	ND	—	ND	—	
9	—	ND	—	—	ND	
10	—	—	—	—	—	—

ND: not done

Numbers indicate the title of a positive hemagglutination.

DISCUSSION

Our study gives information on two points:

a) the sensitization to penicillin in non allergic subjects as assayed by the association of four *in vitro* tests: lymphocyte transformation; leucocyte migration; passive anaphylaxis on monkey lung tissue for anti-penicillin IgE; passive hemagglutination for anti-penicillin IgG or IgM.

b) the chronological evolution of each *in vitro* parameter during and after a well-tolerated treatment by penicillin.

To our knowledge such a study has not been reported previously.

The Sensitization State before Treatment

LTT was positive in 11 per cent of cases. This is in agreement with Vickers & Assem (21) who found one positive LTT in a group of 10 control subjects and others (5, 7, 13). This challenges the initial findings of Halpern who had no positive results in his control group (4).

LMT was positive in 29 per cent of cases. This percentage

is slightly greater than that of positive delayed skin tests to penicillin found by Redmond & Levine (13) with no recent penicillin therapy. This challenges Ortiz et al. (11) who found no positive LMT to benzyl penicilloylated human gamma globulin (BPO HGG) in their control group. This discrepancy can be explained by the difference in the selection of the subjects rather than by the antigen used. Indeed, we found no difference between LMT with penicillin and with BPO HGG (17).

Anti-penicillin serum IgE was found in 30 per cent of cases. Levine et al. (7) had found a lower incidence (5 per cent) of BPO skin sensitizing antibodies in a similar group but they used intra-dermal skin tests which cannot be strictly correlated with the circulating IgE detected by the PAM.

Passive hemagglutination was negative in all the subjects; this is not in agreement with Levine et al. (7) but can be explained by the less sensitive technique used by us (23).

We did not find a significant difference between the 14 non syphilitic subjects and the 10 syphilitic patients. This seems to permit interpretation of the results obtained in the syphilitic group during and after the treatment.

Effect of the Treatment

With daily evaluation it was seen that all the patients tolerated well the course of penicillin. Two kinds of modifications in the *in vitro* test responses were induced by the treatment. (All the modifications we have observed could be reasonably attributed to the administration of penicillin, as in five non treated control subjects repeatedly tested, such modifications were not seen).

Positive conversion of previously negative tests: This conversion occurred mainly after 15 days of daily therapy. This was obvious for LMT, LTT and IgM, and less clear for PAM. It seems important to note that this was briefly transient and that most of the tests were again negative 90 days after the end of the treatment.

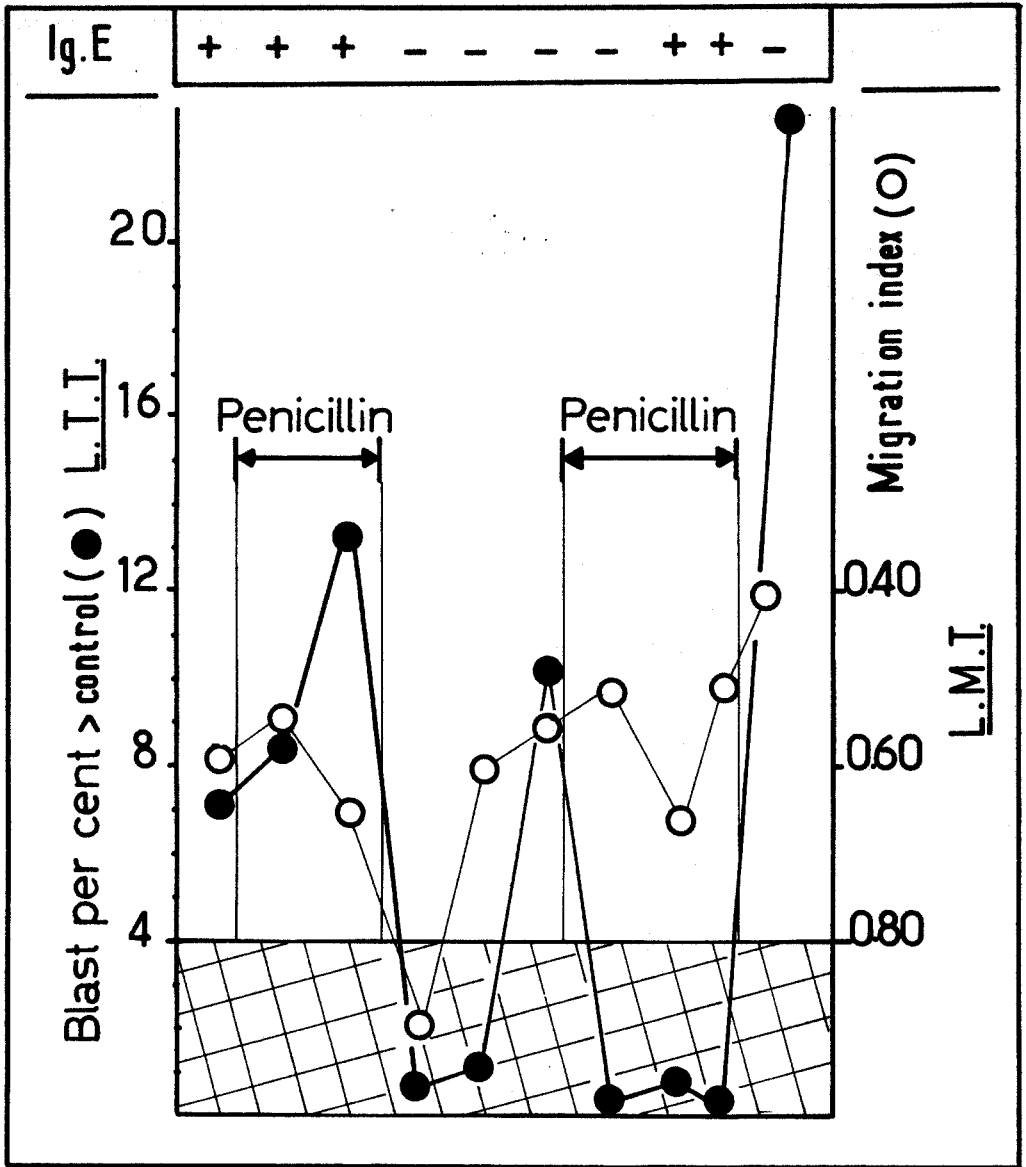


Fig. 3.

Follow-up study of the lymphocyte transformation test (LTT), leucocyte migration test (LMT) and specific anti-penicillin IgE in patient No. 10. Note the negative conversion of the three tests induced by the first penicillin treatment (1 million units/24 h) and the negative conversion of the LTT during a subsequent treatment (1 million units biweekly).

Negative conversion of previously positive tests: This was noted for all the four PAM (IgE); it is difficult to say whether the phenomenon reflects a decrease in production of anti-penicillin IgE or a formation of penicillin IgE complexes,

the antibody then becoming undetectable by the PAM. The same "desensitization" phenomenon was also noted for previously positive LMT. This finding can be compared with Redmond & Levine's (13). These authors have found a lower incidence of detectable delayed hypersensitivity (skin test) to penicillin among patients recently treated by penicillin. A prospective study made by Fellner et al. (3) also using skin test, showed that this was not due to the development of a tolerance but represented transient desensitization. The return of LMT to previous values in our study supports this interpretation. The positive LTT prior to treatment was also found to be negative, as was LTT, with the treatment. This was again induced by a second therapeutic course. The LTT became negative again but LMT was unchanged, perhaps because the penicillin dose was lower (Fig. 3).

It was not possible to draw out significant interactions between these various parameters of the immune response to penicillin. Indeed, several types of responses can be postulated, as shown in Fig. 4. However, the number of subjects in this study is too low to allow a conclusion; the heterogenicity of the anti-penicillin immune response (7) probably accounts for this. Further studies will be necessary to bring about different kinds of responses, and to study their significance in this system.

The aim of this work has essentially been to evaluate the diagnosis value of four *in vitro* tests performed conjointly; and this prospective *in vitro* study of the immune response to penicillin in non allergic subjects has led to the confirmation of some already well-accepted ideas based on *in vivo* tests. These are:

- (1) the sensitization to penicillin of numerous subjects including their cellular and humoral IgE immune response.
- (2) the modification of the previous specific state by subsequent therapeutic use of penicillin; increase in the sensitization or transient desensitization.
- (3) the absence of allergic reaction in some sensitized subjects treated with penicillin.

		I				I				I				I				I			
		I	T	E	M	I	T	E	M	I	T	E	M	I	T	E	M	I	T	E	M
I	1	-	-	-	-	-	-	-	-	-	-	-	-	-	○	-	-	-	-	-	-
	2	-	-	-	-	-	-	-	-	-	-	E	M	-	○	-	M	-	-	-	-
	3	-	-	-	-	-	-	E	-	I	T	-	-	I	T	-	-	-	T	-	-
II	4	I	-	-	-	I	○	-	-	-	T	-	M	-	T	-	M	I	-	-	-
	5	I	○	-	-	I	○	-	-	I	T	-	-	-	T	-	-	-	T	E	-
	6	I	-	-	-	I	○	-	-	-	T	-	-	s	T	-	M	I	T	-	M
III	7	-	○	E	-	-	○	-	-	I	T	-	-	-	○	-	-	-	○	-	-
	8	-	-	E	-	○	○	○	○	I	T	E	-	○	○	○	○	-	-	-	-
IV	9	I	-	E	-	○	○	○	○	I	-	-	-	s	-	-	-	-	○	○	○
	10	I	T	E	-	I	T	E	-	I	T	E	-	-	-	-	-	I	-	-	-
		A				B				C				D				E			

Fig. 4.

Results of each test in each subject (1 to 10) all through the study; positive tests are noted as follows:

- I, (inhibition) for a positive leucocyte migration test.
 T, (transformation) for a positive lymphocyte transformation test.
 E, (IgE) for a positive passive anaphylaxis on monkey tissue.
 M, for anti-penicillin IgM.
 ○, not done.
 —, negative test.
 s, stimulation.

See legend Fig. 1 for abbreviations A.B.C.D.E.

These concepts had been established by cutaneous tests for immediate or delayed hypersensitivity and by passive hemagglutination. LTT (9), LMT, and circulating IgE have not been previously evaluated in such a prospective way. It seems important to keep these concepts in mind for the interpretation of *in vitro* tests used as a diagnostic tool after an allergic reaction to penicillin.

SUMMARY

This *in vitro* follow-up study of the penicillin-induced immune response by a well-tolerated regimen was conducted with an association of four tests: lymphocyte transformation test; leucocyte migration test; passive anaphylaxis on monkey lung tissue; and passive hemagglutination. It showed (1) that previously sensitized subjects (including IgE) tolerated the treatment by penicillin. (2) That the treatment induced either a transient positive conversion of previously negative tests or a negative conversion of previously positive tests. These *in vitro* findings are in accordance with previously established concepts based upon cutaneous tests and must be taken into consideration when interpreting *in vitro* diagnosis of penicillin allergy.

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Fig. 4. (continued).

Four groups were drawn up on the basis of the type of sensitization found before the treatment (see Methods): (I) no sensitization in subjects 1, 2, 3; (II) only cellular immunity (LMT) in subjects 4, 5, 6; (III) only IgE in subjects 7, 8; (IV) IgE and cellular immunity in subjects 9, 10. The subsequent evolution did not show a very distinctive pattern in each group.

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