Original articles

The lymphocyte transformation test for diagnosis of drug-induced occupational allergy

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Twenty-five workers with clinically diagnosed or suspected occupational hypersensitivity caused by contact with bacampicillin, alprenolol, and/or quinidine were studied by the lymphocyte transformation test and by skin tests. Ten healthy exposed workers, 16 job applicants, and seven healthy nonexposed laboratory workers served as control subjects. Lymphocyte transformation was measured by ^{3}H-thymidine uptake into DNA and by counting of lymphoblasts on cell smears. Lymphocytes from workers with contact eczema or with eczema in combination with conjunctivitis and rhinitis responded to offending drugs in vitro as demonstrated by an increased ^{3}H-thymidine incorporation and by the presence of lymphoblasts in the cultures. In vitro proliferative responses were reproduced during a 4-year period. Drug-specific allergy was confirmed by positive patch test in most workers with eczema. In addition, bacampicillin-specific lymphocyte proliferation was also observed in workers with suspected bacampicillin hypersensitivity but with negative skin tests. They suffered mostly from eczema in combination with conjunctivitis and rhinitis or from conjunctivitis/rhinitis only. Lymphocytes from most control subjects did not respond in vitro to bacampicillin, alprenolol, or quinidine. Weak proliferative responses to bacampicillin were observed in two of the 16 job applicants. The exquisite specificity of drug-induced lymphocyte responses is demonstrated. Thus, lymphocytes from a quinidine-sensitive worker did not respond in vitro to the quinidine stereoisomer, quinine. Furthermore, lymphocytes from a bacampicillin-sensitive worker responded to some penicillins, such as pivampicillin and ampicillin, but not to others, such as benzylpenicillin or pivmecillinam. These data suggest the role of N-acylamido side chain in the sensitization of lymphocytes from this particular donor. In conclusion, lymphocyte transformation test can be used for the detection of offending agents in occupationally sensitized workers. Furthermore, lymphocytes from such individuals may serve as a model for study of specificity of cellular reactions underlying drug-induced hypersensitivity. (J ALLERGY CLIN IMMUNOL 77:411-26, 1986.)

Many drugs stimulate cell-mediated and humoral immunity in man and in experimental animals.[1, 2] Immediate and delayed-type hypersensitivity reactions have been described in patients treated with drugs, in members of the medical and veterinary professions, and in personnel employed in the pharmaceutical industry.[3-5] Pharmaceutical employees are exposed to drugs by contamination of the skin and by inhalation of polluted air. The offending agent is sometimes difficult to identify, since the employee often works with several pharmaceutical agents simultaneously. Contact dermatitis has been described in workers occupationally exposed to penicillins,[5, 6] alprenolol,[7] quinidine,[8, 9] and quinine.[10] Respiratory dyspnea and rhinitis have been experienced by some workers exposed to penicillin-containing dust.[11, 12] Positive patch tests were rare in workers in penicillin factories who suffered from chronic eczematous dermatitis, urticaria, and asthma.[3, 5, 11] Similar findings have been reported in workers with quinine-induced dermatitis; testing of

Abbreviations used

LTT:	Lymphocyte transformation test
PPD:	Purified protein derivative
PWM:	Pokeweed mitogen
RPMI 1640:	Roswell Park Memorial Institute culture medium
SI:	Stimulation index

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Received for publication April 12, 1984.

Accepted for publication July 18, 1985.

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12 workers elicited only one positive patch test.[10] Fourteen of 32 workers with suspected alprenolol-induced dermatitis demonstrated positive patch test reaction to alprenolol.[7] Furthermore, no IgE antibodies with penicillin specificity have been found in penicillin factory workers who experienced conjunctivitis, rhinitis, and asthma.[12]

Lymphocytes from man and experimental animals sensitized to various agents undergo morphological transformation on exposure to a sensitizing antigen in culture.[13-15] Peripheral blood lymphocytes from patients with hypersensitivity reactions after penicillin and quinidine treatment proliferate after the addition of these drugs in vitro, whereas lymphocytes from similarly treated but nonallergic patients are not stimulated.[16-18]

We have used the LTT and skin tests as a diagnostic tool in workers with suspected occupational allergy induced by bacampicillin, alprenolol, and/or quinidine. The possible use of this model for the study of specificity of cellular reactions in drug hypersensitivity is also indicated.

MATERIAL AND METHODS

Allergic workers and control subjects

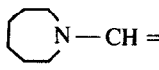
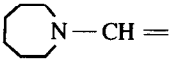
The present study has both a retrospective and prospective design because some of the LTTs were performed on individuals who already had been investigated by conventional clinical dermatology techniques. Twenty-five workers suffering from various hypersensitivity symptoms in connection with their work were studied. Eighteen workers employed at a bacampicillin plant experienced skin or mucosa reactions while they were working with bacampicillin, a prodrug to ampicillin. Nine of the workers exhibited eczema localized on the face, arms, hands, and neck; six had eczema in combination with rhinitis and conjuctivitis, whereas three suffered from conjunctivitis/rhinitis only. Five workers from another plant developed eczema localized to various parts of the body, and two workers had eczema and urticaria. These workers handled various pharmaceutical products, including alprenolol and quinidine. The control group consisted of 10 healthy workers who had worked at the bacampicillin or alprenolol and quinidine factory together with the affected workers for several years. Thus, they had the same exposure history as the study group. Sixteen job applicants and seven healthy laboratory employees served as additional control subjects.

LTT

Two to four 15 ml plain vacutainer tubes (Venoject; Mediplast AB, Stockholm, Sweden) were used for blood collection. Blood was immediately transferred into a 250 ml E flask with approximately 25 ml of 5 mm glass beads and defibrinated by shaking for at least 10 minutes. Lymphocytes and monocytes were obtained by Ficoll-Paque (Pharmacia Fine Chemicals, Uppsala, Sweden) gradient separation.[19] The cells were washed twice and diluted in RPMI 1640 medium with 10 mmol of *N'*-2-hydroxyethylpiperazine-*N'*-2-ethanesulfonic acid and 1 gm of $NaHCO_3$ per liter (Labassco AB, Gothenburg, Sweden). The medium was supplemented with gentamycin (Garamycina; Schering Corp., Kenilworth, N.J.) and glutamine. Medium older than 3 months from the day of preparation was not used for cultivation. One milliliter of lymphocyte suspension (1×10^6 cells per milliliter) was preincubated in 10 ml Falcon (Falcon Labware, Becton, Dickinson & Co., Oxnard, Calif.) tubes with 1 ml of various drug concentrations for 30 minutes at 37° C. The following concentrations were used in the LTT: alprenolol hydrochloride: (MW 285.9) 100, 20, 5, 1, and 0.2 μg/ml; 6-aminopenicillanic acid (6-APA): (MW 216.3) 250, 100, and 20 μg/ml; amoxycillin sodium: (MW 387.4) 250, 100, and 20 μg/ml; ampicillin sodium: (MW 371.4) 100, 20, and 2 μg/ml; azidocillin sodium: (MW 397.4) 250, 100, and 20 μg/ml; bacampicillin hydrochloride: (MW 502.1) 200, 100, 50, 20, and 4 μg/ml; bacmecillinam hydrochloride: (MW 462.1) 100, 20, and 2 μg/ml; benzylpenicillin sodium: (MW 357.4) 250, 100, and 20 μg/ml; metoprolol tartrate: (MW 417.5) 20, 5, 1, and 0.2 μg/ml; pivampicillin hydrochloride: (MW 500) 100, 20, and 2 μg/ml; pivmecillinam hydrochloride: (MW 448) 100, 20, and 2 μg/ml; and quinidine bisulphate: (MW 422.5) 50, 25, 5, and 1 μg/ml. Structural forms of penicillins are presented in Table I.

A minimum of three concentrations of each drug was used per donor. PWM (Gibco, Grand Island, N. Y.) at a concentration of 1 μg/ml and/or PPD (Statens Serum Institut, Copenhagen, Denmark) at a concentration of 5 μg/ml was used as a positive control of lymphocyte proliferation. (Most of the adult population in Sweden has been vaccinated with bacille Calmette-Guérin vaccine and demonstrates Mantoux-positive skin test.) Control lymphocytes (one to three parallel cultures) were incubated without any antigen. After 30 minutes, 0.2 ml of heat-inactivated (60 minutes at 56° C) pooled human AB^+ serum was added to each tube. In some experiments 0.2 ml of heat-inactivated autologous instead of homologous serum was added to cultures before the antigen. The idea was to test if the addition of the donor's own serum can affect drug-induced lymphocyte proliferation. The cultures were then incubated at 37° C and 100% humidity in 6% CO_2 for 5 days. This is the optimal period for measurement of ^{3}H-thymidine incorporation in antigen-treated cultures as found in our preliminary experiments and by others.[20] During the 5-day culture, various cell-derived factors accumulate in the medium and may interfere with the measurements of ^{3}H-thymidine incorporation.[21, 22] Thus, after cultivation, 1 ml of the culture medium was removed, and 4 ml of RPMI 1640 containing 5% human serum was added to each tube. The cells were then spun down at 300 × g in a Beckman (Beckman Instruments, Inc., Spinco Div., Palo Alto, Calif.) (model TJ-6) centrifuge for 10 minutes. After washing, the viable cells were counted either manually (in Türk solution) or in the celloscope (cell counter 134, Analys Instrument AB, Stockholm), and cell concentration was adjusted to 5×10^5 cells per milliliter. In the early experiments all cultures were counted. Since we had observed that the lymphocyte survival in control and drug-treated cultures was similar, only control cultures were later counted. For 100 donors tested in our laboratory, the mean survival of control cultures was 61 ± 27% (range 19% to 140%).

TABLE I. Structure of penicillins used in lymphocyte transformation test

Generic names	Structure	
	R1	R2
6-Aminopenicillanic acid (6-APA)	NH_2-	$-H$
Ampicillin	$-CH(NH_2)-CONH-$	$-Na$
Amoxycillin	$HO-C_6H_4-CH(NH_2)-CONH-$	$-Na$
Azidocillin	$-CH(N_3)-CONH-$	$-Na$
Benzylpenicillin	$-CH_2-CONH-$	$-Na$
Bacampicillin	$-CH(NH_2)-CO-NH-$	$-CH(CH_3)-OCO_2C_2H_5$
Bacmecillinam	$HCl \cdot$ 	$-CH(CH_3)-OCO_2C_2H_5$
Pivampicillin	$-CH(NH_2)-CONH-$	$-CH_2-OCO-C(CH_3)_2-CH_3$
Pivmecillinam		$-CH_2-OCO-C(CH_3)_2-CH_3$

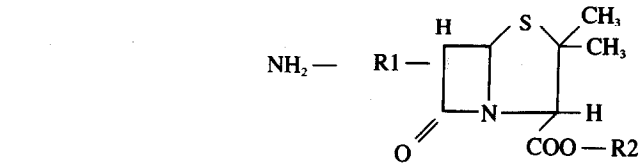


TABLE II. Skin test and lymphocyte proliferation in allergic workers employed in bacampicillin production

Subject			Symptoms and period of work before the onset (mo)	Patch test with bacampicillin	
Code	Sex	Age (yr)		Year	mg/ml*
2	M	41	Eczema: face, arms (1)	1979	6.25 + + 12.5 + +
24	M	43	Eczema: face, arms (4)	1981	3.12 + 6.25 + +
27	F	45	Eczema: hands (3)	1982	3.12 + + 6.25 + + +
62	F	30	Eczema: hands (12)	1978	12.5 + + 25.0 + +
65	M	22	Eczema: face, hands (0.5)	1983	3.12 + 6.25 + +
74	M	34	Eczema: face, hands (0.5)	1983	3.12 + 6.25 + + +
75	M	22	Eczema: face, arms (1)	1983	3.12 + + + 6.25 + + +
94	M	21	Eczema: face, neck (1)	1983	3.12 + 6.25 + +
4	M	40	Eczema: face Rhinitis, conjunctivitis (18)	1983§	3.12 + + + 6.25 + + +
28	M	23	Eczema: face, neck Rhinitis, conjunctivitis (1)	1982§	3.12 + + 6.25 + +
38	M	57	Rhinitis, conjunctivitis (36)	1983¶	12.5 neg 25.0 neg

ND = not done; neg = negative.

*Lowest concentrations of bacampicillin resulting in positive skin reaction. The plus marks indicate the degree of positivity of the patch test.

†Bacampicillin concentrations that induced maximal lymphocyte proliferation.

‡Spontaneous blastogenesis.

§This patient has also been prick tested with bacampicillin and found negative.

‖Eczema and rhinitis at the time of lymphocyte transformation test. After the transfer to other work, the patient became free from symptoms.

¶This patient has also been prick tested with bacampicillin and found positive. Patch test was negative.

Incorporation of radiolabeled thymidine into DNA

From each culture, 1×10^5 cells in triplicate were transferred to V-shaped microplates (Titertek; Flow Labs, Irvine, Scotland) and pulsed with 1 μCi of 5′-^{3}H-thymidine (TRK 328, Radiochemical Centre, Amersham, U. K., specific activity 5 to 14 Ci/mmol) for 4 hours to determine lymphocyte proliferation.[20] The cultures were harvested in a Skatron (Skatron, Lier, Norway) semiautomatic cell harvester, and the radioactivity was counted in a liquid scintillation counter. The results of ^{3}H-thymidine incorporation into DNA are expressed as mean counts per minute (cpm ± SD) for the triplicate cultures or as an SI

$$\frac{\text{cpm in culture cultivated with antigen}}{\text{cpm in control culture}}$$

SI ≥ 2 was regarded as a sign of a positive proliferative response. The values ≥10 have been rounded off to the nearest decimal.

Morphological observations

The proliferative responses in 5-day cultures were evaluated by counting of lymphoblasts on cell smears; 50 μl of cell suspension (5×10^5 cells per milliliter) was spun down at 900 rpm for 5 minutes in a cytocentrifuge (Cytospin 2; Shandon, Great Britain). The smears were fixed with methanol and stained with May-Grünwald/Giemsa. The cell preparations were first screened under low power magnification to determine if cells were evenly distributed over the entire area. If no lymphoblasts were observed during the preliminary screening, no count was made, and the cultures were considered negative. If lymphoblasts were noted, a minimum of 300 cells for each culture was counted, and the percentage of lymphoblasts from the total number of

	LTT				
	Control	Bacampicillin			
Year	cpm ± SD	μg/ml†	cmp ± SD	SI	%Lymphoblasts
1981	2002 ± 192	20	12,290 ± 923	6.1	14
		200	11,203 ± 905	5.6	9
1984	915 ± 234	10	3571 ± 238	3.9	6
		20	4629 ± 63	5.1	ND
1982	1000 ± 52	20	14,706 ± 928	15.0	9
		100	18,895 ± 1528	19.0	13
1983	1003 ± 324	20	2215 ± 302	2.2	2
		100	2508 ± 403	2.5	1
1983	4886 ± 638‡	20	15,650 ± 549	3.2	14
		100	12,324 ± 602	2.5	6
1983	4334 ± 902‡	20	21,548 ± 946	5.0	9
		100	15,994 ± 1323	3.7	3
1983	1136 ± 238	20	2114 ± 142	1.9	3
		100	4140 ± 50	3.6	3
1983	2087 ± 495	100	7014 ± 459	3.4	5
		200	6937 ± 1237	3.3	2
1981	1214 ± 91	20	10,801 ± 1680	8.9	3
		200	10,272 ± 1815	8.5	3
1982‖	1352 ± 241	50	7220 ± 1192	5.3	2
		150	9203 ± 148	6.8	3
1983	1203 ± 294	20	4319 ± 419	3.6	3
		100	1651 ± 832	1.4	2

lymphocytes was calculated. The control cultures usually contained no or just a few lymphoblasts (<1%). However, lymphocytes from some donors proliferated spontaneously in vitro, and the number of lymphoblasts in such cultures was higher (1% to 5%). Antigen-specific lymphocyte transformation was expressed as the percentage of lymphoblasts in antigen-treated cultures minus the percentage of lymphoblasts in control cultures.

Evaluation of LTT

The results of LTT were considered positive only if antigen-pulsed cultures exhibited increased ^{3}H-thymidine incorporation and contained greater numbers of lymphoblasts than the control cultures.

Experiments difficult to evaluate were those with weak, borderline responses. In such experiments, positive lymphocyte proliferation was recorded with one drug concentration only and was generally low (SI around 2 to 3 and specific blastogenesis around 1%).

Skin tests

All workers with eczema were patch tested. The subjects with conjunctivitis, rhinitis, and asthma were also prick tested. The patch test was applied to the back by use of the method of Pirilä.[23] The test patches were removed after 48 hours, and the reactions were evaluated after a further 24 hours. (Control tests with bacampicillin, alprenolol, and quinidine were made on 30 outpatients treated in the dermatology department, Södersjukhuset; all proved negative.) The reactions were ranked from 1+ (erythema) to 3+ (vesiculation). The concentrations of drugs in aqueous solution used for testing were as follows: bacampicillin hydrochloride: 25, 12.5, 6.25, and 3.13 mg/ml; alprenolol hydrochloride: 10, 5, 2.5, and 1.25 mg/ml; quinidine sulphate: 5, 2.5, 1.25, and 0.5 mg/ml; metoprolol tartrate: 10, 5, and 2.5 mg/ml; and benzylpenicillin sodium: 25, 12.5, 6.25, and 3.13 mg/ml.

Prick test[24] was performed with bacampicillin simultaneously with and in the same concentrations as for the patch test. Each worker was skin tested with several drug concentrations on the same occasion.

RESULTS

Tables II and III illustrate the age and sex distribution in the group of 18 individuals experiencing clinical symptoms in connection with their work. The 16 men had been employed at the bacampicillin plant; most worked as process operators. One woman (No. 27) developed symptoms while she was handling bacampicillin during the toxicology studies performed

TABLE III. Lymphocyte proliferation in skin test negative allergic workers employed in bacampicillin production

Subject			Symptoms and period of work before the onset (mo)	Skin test with bacampicillin	
Code	Sex	Age (yr)		Year	Type
3	M	27	Eczema: face, hands (2)	1980	Patch
23	M	32	Eczema: face, hands; conjunctivitis (3)	1979	Patch Prick
60	M	24	Eczema: face, hands; conjunctivitis (13)	1982	Patch
66	M	23	Eczema: hands; rhinitis (2)	1983	Patch Prick
29	M	23	Eczema: neck; conjunctivitis; rhinitis (3)	1983	Patch Prick
69	M	31	Conjunctivitis (24)	1983	Patch Prick
34	M	25	Conjunctivitis; rhinitis; Asthma (3)	1983	Patch Prick

ND = not done.
*Bacampicillin concentrations that induced maximal lymphocyte proliferation.
†Symptoms at the time of lymphocyte transformation test. After the transfer to other work, the subject became free from symptoms.
‡Spontaneous blastogenesis.

in 1975, and one woman (No. 62) developed symptoms at laboratory work. The latent period preceding the onset of clinical symptoms varied between 2 weeks and 3 years. Most eczema subjects (eight of nine workers) reacted with a positive patch test. However, only two of six subjects with eczema, rhinitis, and conjunctivitis were patch test positive, and only one of three subjects with conjunctivitis/rhinitis reacted to bacampicillin in the prick test. Lymphocytes from all 18 workers with suspected bacampicillin hypersensitivity proliferated on addition of bacampicillin in vitro (Tables II and III; Fig. 1A, 1B, and 1C.) The bacampicillin sensitivity of eight workers, as demonstrated by the positive LTT, was later confirmed by skin tests. This excludes the patch test as a possible cause of sensitization in these workers. The clinical symptoms of LTT–positive workers resolved after transfer to a different process in another part of the factory.

The lymphocytes of subject No. 27 repeatedly demonstrated strong proliferation in the presence of bacampicillin and pivampicillin in vitro (Fig. 1A, 1B, and 1C). Furthermore, her lymphocytes responded weakly to other penicillins, such as amoxycillin and azidocillin. Surprisingly, they did not respond to benzylpenicillin, pivmecillinam, and bacmecillinam in vitro. She consented to undergo skin testing and provocation with bacampicillin and benzylpenicillin to evaluate the biologic significance of the in vitro findings. Prick test with bacampicillin was negative at 15 minutes, but after 4 hours the test arm became red and swollen. Patch test was also positive (Table II). However, both prick and patch tests were negative with benzylpenicillin. The study proceeded with peroral provocation with 800 mg of benzylpenicillin. No reaction occurred. Last, she was administered 100 mg of bacampicillin perorally. After 4 hours she experienced itching and eczema starting on her hands. During the following hours, she developed pain in the stomach and diarrhea, and 24 hours later eczema was noted on the back at the site of former patch testing.

Table IV presents the results of patch tests and of LTT in the study of seven workers handling alprenolol and quinidine in another plant. Six of the workers reacted to alprenolol by patch testing. Two of these workers (Nos. 9 and 86) also exhibited quinidine-specific reactions, and one (No. 22) reacted to quinidine only. Lymphocytes from all patch test positive workers proliferated in vitro in the presence of the respective drug (Table IV, Fig. 2, *A* and *B*). The remarkably strong proliferation of V. J.'s lymphocytes to alprenolol requires a special comment. She became sensitized to alprenolol during the toxicology study of alprenolol in 1974 while she was working as an animal

	LTT				
	Control	Bacampicillin			
Year	cpm ± SD	μg/ml*	cpm ± SD	SI	%Lymphoblasts
1981†	3415 ± 97‡	20	7212 ± 443	2.1	2
		100	8376 ± 275	2.5	3
1982†	429 ± 55	20	1888 ± 118	4.4	3
		100	2354 ± 176	5.5	3
1982	587 ± 92	20	1823 ± 102	3.1	ND
		100	1265 ± 175	2.2	2
1983	6024 ± 747‡	20	14,430 ± 1269	2.4	6
		100	17,619 ± 3052	2.9	9
1983†	1323 ± 53	20	2221 ± 207	1.7	3
		100	5517 ± 889	4.2	4
1982	1362 ± 263	20	4360 ± 1227	3.2	2
		100	2693 ± 63	2.2	3
1982†	700 ± 360	20	4024 ± 564	5.7	4
		100	1295 ± 272	1.9	2

attendant. She had complaints of hand eczema during the work and was transferred to another job. In 1978 patch testing was performed that resulted in a strong local reaction. Since then she has not been in contact with alprenolol. We tested her lymphocytes in seven consecutive experiments during the period 1981 to 1984 and were repeatedly amazed by the strong, mitogen-like alprenolol-specific response. Her lymphocytes did not respond to other drugs such as bacampicillin or metoprolol in culture, although she had been similarly exposed to them. Several other workers suspected that metoprolol was an agent causing their symptoms (rhinitis and conjunctivitis). However, skin tests and LTTs with metoprolol were always negative.

Subject No. 22 began to work at the factory as a granulator and process operator in late 1981. He came in contact with several substances such as alprenolol, quinidine, metoprolol, and bacampicillin. After 6 weeks of employment, he had complaints of itching and hand eczema and was referred for patch testing. He was also transferred to another job. Patch testing in March 1982 with drugs suspected by the subject, bacampicillin, metoprolol, and alprenolol, proved negative. LTT, performed in June 1982, revealed a pronounced proliferative lymphocyte response to quinidine but was negative to alprenolol and metoprolol. Quinidine-specific sensitization was later confirmed by a positive patch test in September 1982. Quinidine-specific memory cells are still present in blood of this subject as demonstrated by positive LTT performed 2 years later (Table IV).

The remarkable specificity of drug recognition by lymphocytes is demonstrated in Fig. 2, *A* and *B*. Subject No. 86 worked as a granulator in quinidine production. Quinidine-specific dermatitis was diagnosed in 1979 by patch testing, and the subject was transferred to alprenolol production. After 6 months his eczema reappeared, and he was again transferred, this time to an administrative job. Two separate LTTs were performed, one in August (Fig. 2, *A*) and another in September 1983 (Fig. 2, *B*). The results confirmed the quinidine-specific sensitization. In addition, a strong proliferative response to alprenolol was noted, and alprenolol sensitivity was later confirmed by a positive patch test. Quinidine-specific lymphocytes did not react in vitro to the quinidine stereoisomer, quinine.

Lymphocytes from several other workers who exhibited positive drug-specific proliferative responses were examined in a series of consecutive experiments. Such lymphocyte responses were reproduced over a 4-year period (data not presented).

In vitro proliferative responses of 10 healthy subjects handling bacampicillin, alprenolol, and/or quinidine for several years are presented in Table V. Generally, the drug-specific LTT was negative, as demonstrated by an SI ≤ 2 and by the absence of specific blastogenesis (not presented). In two workers (Nos. 56 and 21) the maximal SI exceeded the value of 2, but no lymphoblasts were found by morphological evaluation of the cell smears. The experiment was therefore repeated within a 3-month period, and this

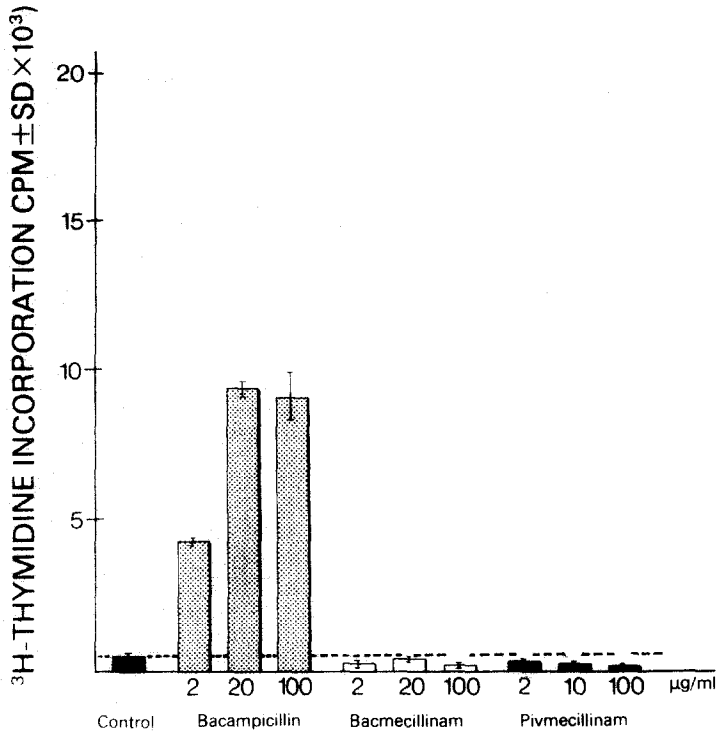


FIG. 1A. Lymphocyte proliferation induced by various penicillins in vitro (subject No. 27 noted in Table II). Experiment performed in May 1982.

time no increased thymidine incorporation was noted after the addition of bacampicillin and alprenolol to the respective cultures.

The lymphocyte responses of 16 healthy applicants for work at the factory are illustrated in Table VIA and VIB. Fourteen of 16 subjects tested in culture with bacampicillin proved negative in the LTT. Subject No. 51 responded weakly to one of the bacampicillin concentrations, and subject No. 49 responded to two bacampicillin concentrations in both autologous and homologous serum. Four of 16 work applicants were also tested with alprenolol and quinidine in vitro with no response (Table VIB).

Seven healthy subjects working at the factory but not exposed to pharmaceutical agents served as an additional control group. The lymphocytes from these subjects were not transformed when they were cultivated with bacampicillin, alprenolol, or quinidine for 5 days. However, the same lymphocytes did transform and incorporate thymidine when they were cultivated with PWM or PPD (data not presented).

DISCUSSION

Antigen-specific lymphocyte proliferation in vitro represents an anamnestic response to a given antigen and indicates the presence of memory cells in the donor's blood.[14, 15, 25] To our knowledge, this is the first study that has used LTT for evaluation of drug-specific cellular responses in occupationally exposed personnel. The results indicate that drug-specific memory cells are present in the blood of workers who had experienced eczema and/or conjunctivitis and rhinitis during exposure to bacampicillin, alprenolol, or quinidine dust. LTT was negative in symptom-free workers, handling these drugs for several years. Similar results with lymphocytes from patients with adverse reactions after drug therapy have been published previously.[16-18] Hypersensitivity reactions in patients treated with penicillin are well-known,[26] and penicillin allergy can be diagnosed by the LTT.[16-18, 27] Recently, transformation of lymphocytes by quinidine in vitro has been reported in patients demonstrating hypersensitivity symptoms after quinidine treatment but not in the patients taking this drug without side effects.[18, 28] We have found that quinidine, but not quinine, induced in vitro proliferation of lymphocytes from a quinidine-sensitive worker. These results are in agreement with the stereoisomer-specific skin responses observed by Wahlberg and Boman[9] with the patch test technique. Thus, the workers sensitive to quinidine did not react when they were tested with quinine.

The pilot study of the specificity of lymphocyte reactivity in subject No. 27 indicated that occupational

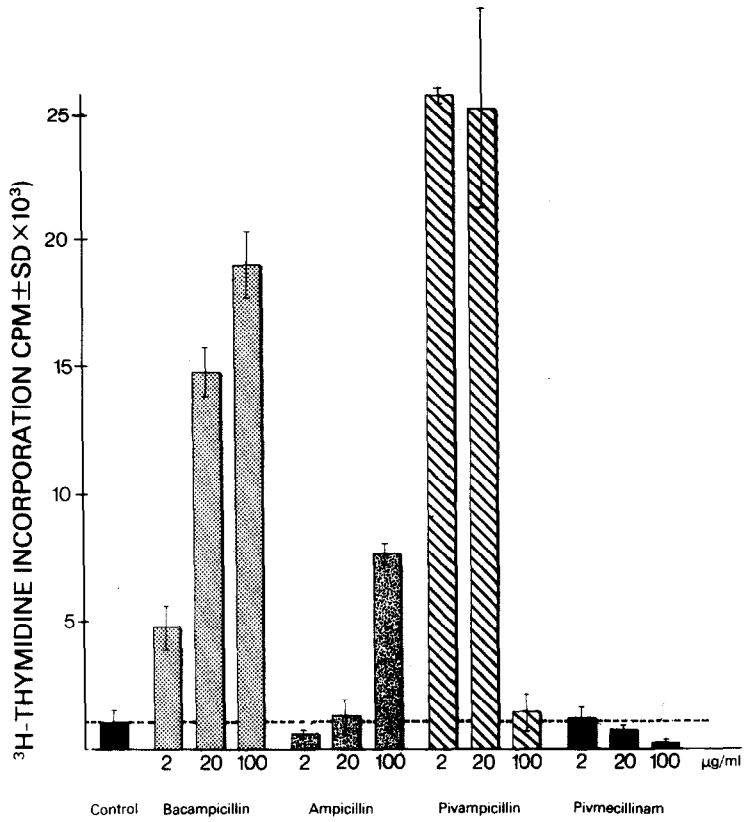


FIG. 1B. Lymphocyte proliferation induced by various penicillins in vitro (subject No. 27 noted in Table II). Experiment performed in October 1982.

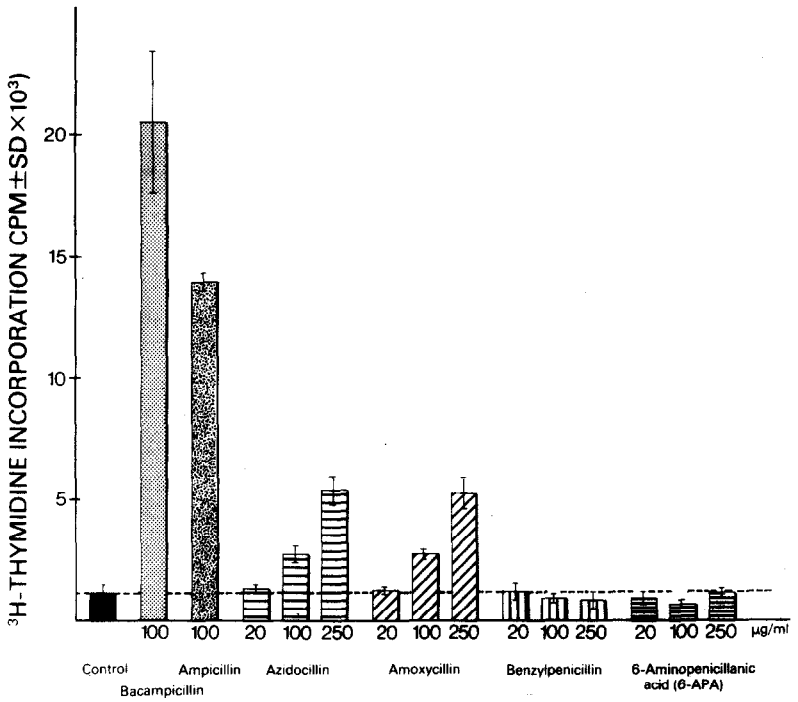


FIG. 1C. Lymphocyte proliferation induced by various penicillins in vitro (subject No. 27 noted in Table II). Experiment performed in February 1983.

TABLE IV. Skin test and lymphocyte proliferation in allergic workers employed in alprenolol and quinidine production

Subject				Patch test			LTT	
					Alprenolol	Quinidine		Control
Code	Sex	Age (yr)	Symptoms and period of work before the onset (mo)	Year	mg/ml*	mg/ml*	Year	cpm ± SD
9	M	57	Eczema: face, neck; arms (2.5)	1979	5.00 + + + 1.25 + +	1.25 + + + 0.50 + + +	1981	1490 ± 1
86	M	31	Eczema: face, arms (2)	1979 1983	ND 2.50 + + + 1.25 + + +	Positive 1.25 + + + 0.50 + + +	1983	812 ± 193
71	M	40	Eczema: hands, face (1)	1983	2.50 + + + 1.25 + + +	ND	1983	3607 ± 729‡ 10,694 ± 1281‡,§
10	F	49	Eczema: face, neck (12)	1981	10.0 + + 2.50 +	Negative	1981	934 ± 153
V. J.	F	58	Eczema: hands, legs (0.5)	1976	2.50 + + + 1.25 + + +	ND	1981	455 ± 124 1373 ± 188§
70	M	40	Eczema: back; urticaria (18)	1983	5.00 + + 1.25 +	ND	1983	494 ± 101 1276 ± 263§
22	M	23	Eczema: arms; urticaria (1.5)	1983	Negative	2.50 + + 1.25 + +	1982 1984	326 ± 79 1252 ± 238

ND = not done.
*Lowest drug concentrations resulting in positive skin reactions; the plus marks indicate the degree of positivity of the patch test.
†Drug concentrations that induced maximal lymphocyte proliferation.
‡Spontaneous blastogenesis.
§Autologous serum in the cultures.

sensitization to bacampicillin resulted in memory cells with specificity directed against the *N*-acylamido side chain and the ester group of the bacampicillin molecule. Thus, the subject's lymphocytes did not react to benzylpenicillin or 6-aminopenicillanic acid, but they did cross-react with pivampicillin and ampicillin. Pivmecillinam and bacmecillinam, having the same ester group as bacampicillin but with the *N*-acylamido side chain replaced by an amidino side chain, were not recognized by bacampicillin-specific lymphocyte receptors. Skin and provocation tests were positive with bacampicillin but were negative with benzylpenicillin. The lack of cross-reactivity between ampicillin and benzylpenicillin has previously been observed by Schulz et al.[6] during the skin testing of workers occupationally sensitized with ampicillin. Thus, only five of 24 workers with ampicillin-positive patch test responded to benzylpenicillin. None of the 24 allergic workers responded to 6-aminopenicillanic acid. Thus, LTT could be used as a tool for the study of reactivity to various penicillins in occupationally and possibly therapeutically sensitized patients. Taken together, these results indicate that the recognition of a drug by immunologically committed cells involves the participation of a remarkably specific receptor system that can discriminate between closely related antigens.

To our knowledge there has been no published report concerning the allergenicity of alprenolol used therapeutically. Historically, however, there have been a number of workers exposed to alprenolol dust in the manufacturing process who exhibited skin manifestations and who gave alprenolol positive responses in the patch test. Two of the sensitized workers experienced flare-up of their eczema after ingestion of alprenolol tablets, thus proving the immunologic nature of sensitization.[7] In patch test positive subjects, the LTTs were also positive. Alprenolol-sensitive lymphocytes did not respond to alprenolol epoxide, which is an intermediate, synthesized during the manufacture of alprenolol (data not presented). This indicates that it is the final product, alprenolol, and not traces of an active contaminant that induces the lymphocytes.

All workers with eczema and with bacampicillin positive patch test had bacampicillin-specific lympho-

LTT							
Alprenolol				Quinidine			
μg/ml†	cpm ± SD	SI	%Lymphoblasts	μg/ml	cpm ± SD	SI	%Lymphoblasts
2	14,651 ± 667	9.8	12	20	28,697 ± 3029	19.0	15
20	16,619 ± 1372	11.0	13	100	14,714 ± 1349	9.8	8
2	6194 ± 318	7.6	4	5	8885 ± 1751	11	3
20	10,311 ± 739	13.0	5	25	9580 ± 699	12	5
2	9564 ± 240	2.7	6	1	4561 ± 29	1.3	0
2	45,710 ± 3122	4.3	9				
2	7818 ± 359	8.4	2	2	1444 ± 14	1.5	0
20	7495 ± 378	8.0	3	20	1462 ± 73	1.6	
2.5	14,373 ± 766	32					
12.5	23,973 ± 2901	53					
2.5	19,355 ± 2107	14					
12.5	22,032 ± 748	16					
2	1399 ± 71	2.8	3	1	414 ± 42	1.0	0
20	3407 ± 102	2.7	3	5	1677 ± 11	1.1	0
2	447 ± 58	1.5	0	2	8591 ± 1430	26.0	5
20	474 ± 106	1.5	0	20	8390 ± 1660	26.0	4
				4	4833 ± 18	3.9	3
				20	6327 ± 35	5.1	5

cytes in their blood. The same was true for workers who suspected bacampicillin as a causative agent of their symptoms but who had a negative patch test. Most workers exhibited eczema in combination with conjunctivitis and rhinitis or had conjunctivitis and/or rhinitis only. Generally, the clinical symptoms resolved after the worker had been transferred to another job outside the bacampicillin plant. However, drug-specific memory cells could be detected in the blood of such workers several years after the disappearance of clinical symptoms.

Negative drug-specific skin tests in subjects experiencing symptoms in connection with their work are usually attributable to the irritant effects of chemicals on the skin or mucous membranes.[10] The finding of drug-specific lymphocytes in such individuals strongly indicates the immunologic nature of the reaction. Positive lymphocyte proliferation but negative skin tests have been demonstrated in subjects occupationally exposed to tuberculin.[29] Serologic investigations have indicated that such individuals had recirculating PPD and/or PPD anti-PPD antibody complexes in their serum. These substances could block the PPD–specific T-lymphocytes in vivo. An alternative explanation for skin anergy in clinically normal individuals who, on being immunized with bacille Calmette-Guérin vaccine, failed to demonstrate a Mantoux-positive skin test has been presented by Muller et al.[30] The results of these authors suggest that lymphocytes from vaccinated, skin test negative individuals fail to demonstrate migration inhibiting–factor activity in response to PPD in vitro despite positive PPD–specific lymphocyte proliferation. This suggest that these subjects may have an immune deficiency at the level of lymphokine production. Last, LTT may merely be more sensitive than the skin test for demonstration of sensitization, since more cells with drug-specific receptors are available in cultures after clonal proliferation in vitro for 5 days.[25]

Lymphocytes from the 10 healthy workers who had been employed for many years on the drug production line did not respond to bacampicillin, alprenolol, or quinidine in vitro. However, lymphocytes from two of 16 applicants for work at the bacampicillin plant responded weakly to bacampicillin in LTT before employment. The presence of drug-specific lymphocytes in some apparently nonallergic subjects has been reported previously.[16, 27, 31] These persons could be latently sensitized by previous contacts with penicillins present in the environment (molds and food) or administered therapeutically. Asymptomatic subjects with positive drug-specific lymphocytes may constitute a risk population that could develop allergy if they were chronically exposed to sensitizing agents. This

TABLE V. Lymphocyte proliferation in subjects handling drugs for several years

Subject				Control	Bacampicillin		
Code	Sex	Age (yr)	LTT (yr)	cpm ± SD	μg/ml*	cmp ± SD	SI
18	M	40	1982	1927 ± 290	2	2491 ± 81	1.3
					20	2197 ± 261	1.1
19	M	45	1982	611 ± 67	2	451 ± 44	0.7
					20	658 ± 140	1.0
20	M	52	1982	900 ± 82	2	788 ± 8	0.9
					20	798 ± 18	0.9
21	F	56	1982	1205 ± 104	20	2127 ± 102	1.8
			1983	653 ± 76	100	1223 ± 13	1.0
54	M	22	1983	2776 ± 373	2	2455 ± 578	0.9
					20	1526 ± 277	0.6
55	M	61	1983	6688 ± 623‡	2	7166 ± 220	1.1
					20	11,848 ± 986	1.8
			1984	985 ± 190	2	1156 ± 195	1.2
					20	1035 ± 103	1.0
56	M	34	1982	1301 ± 145	20	2134 ± 337	1.6
					100	3267 ± 155†	2.5
				2022 ± 25‡, §	20	2085 ± 95	1.0
					100	2396 ± 162	1.2
			1983	458 ± 109	20	323 ± 13	0.7
					100	361 ± 33	0.8
57	F	37	1983	8110 ± 397‡	2	5464 ± 215	0.7
					20	5218 ± 930	0.6
58	M	46	1983	8426 ± 980‡	2	7724 ± 314	0.9
					20	5671 ± 159	0.7
				10,361 ± 404‡,§	2	7262 ± 118	0.7
					20	5333 ± 225	0.5
59	M	41	1983	2653 ± 250	2	2670 ± 111	1.0
					100	3143 ± 437	1.2

*Two concentrations eliciting maximal prolification in vitro.
†No lymphoblasts in the culture.
‡Spontaneous blastogenesis.
§Autologous serum in the culture.

possibility is now under investigation in our laboratory. If this hypothesis can be verified, LTT could be used as a tool not only for the diagnosis of, but also for the introduction of, measures to avoid occupational drug allergy.

Many authors have tried the LTT for the diagnosis of hypersensitivity in patients with adverse drug reactions. The results have been variable. Some authors reported success,[16-18] whereas others were less successful.[32] The reasons for these discrepancies may be explained by variability in performance and evaluation of LTT in various laboratories. A detailed analysis of factors important for successful in vitro "cloning" of drug-specific memory lymphocytes is beyond the scope of this communication and will be presented in another article.* However, there are certain crucial factors that are relevant for a thorough evaluation of the results of LTT. This must be done both microscopically and by isotope incorporation. Morphological evaluation is the only way to establish whether transformed lymphocytes (lymphoblasts) are actually present in drug-pulsed cultures. Occasionally, we have observed increased thymidine incorporation in cultures containing only unstimulated lymphocytes and macrophages. The autoradiographic studies have demonstrated that in such cultures monocyte-derived macrophages incorporated ^{3}H-thymidine. Thus, one can get a false impression of lymphocyte proliferation

*Stejskal VDM, Olin R: In preparation.

Alprenolol			Quinidine			PPD	PWM
μg/ml	cpm ± SD	SI	μg/ml	cpm ± SD	SI	SI	SI
0.2	2033 ± 131	1.0	0.2	1645 ± 121	0.9	8	29
2	1740 ± 61	0.9	2.0	1347 ± 91	0.7		
0.2	429 ± 41	0.7	0.2	388 ± 45	0.6	7.1	48
20	390 ± 33	0.6	2.0	392 ± 99	0.6		
2	894 ± 78	1	0.2	1124 ± 158	1.2		57
20	750 ± 50	0.8	2.0	1466 ± 196	1.6		
2	2970 ± 255†	2.4	0.2	2093 ± 155	1.7	32	26
20	3345 ± 308†	2.8	2.0	1068 ± 17	0.8	109	
2	462 ± 153	0.7					
20	643 ± 164	1.0					
2	2455 ± 578	0.9	1	2265 ± 579	0.8	20	23
20	1234 ± 457	0.4	5	3068 ± 401	1.1		
						10	
						83	80
						100	
						71	
						195	
2	8188 ± 89	1.0	1	6962 ± 657	0.9	11	
20	2184 ± 160	0.3	5	3840 ± 386	0.5		
						9	
						9	
						6.4	

if the incorporating lymphoblasts cannot be verified microscopically. However, since morphological lymphoblasts evaluation is subjective, the method, if it is used alone, is open to criticism.[18]

Last but not least, the data in this study demonstrate that low-molecular-weight compounds can trigger human drug-specific memory cell proliferation in vitro. Since covalent binding of the drug or of drug metabolites to a protein carrier is considered to be a prerequisition for the antigenicity of low molecular chemicals,[1, 2] our data deserve further comments. The parenteral drugs alprenolol and quinidine do not bind covalently to the proteins. One could speculate that protein-reactive drug metabolites are formed in vitro as the result of an interaction between the drug and antigen-processing cells (monocytes and dendritic cells). The presence of cytochrome P_{450} in rabbit alveolar macrophages[33] and in mononuclear cells from human peripheral blood has been reported.[34, 35] In our culture system, one of the possible alprenolol metabolites, alprenolol epoxide, did not stimulate alprenolol-specific memory cells (data not presented). Since many epoxides bind covalently to protein, these preliminary observations appear to contradict rather than support the role of reactive metabolites in the stimulation of drug-sensitized lymphocytes. It should also be noted that some drugs, like paracetamol, do bind covalently to the proteins, but according to common experience, hypersensitivity to paracetamol is a rare event.

There may be an alternative explanation for the obvious efficiency of low molecular drugs to function as an antigen in vitro. Perhaps, once drug-specific receptors have been formed during the lymphocyte sensitization in vivo, the drug itself is able to switch on memory cell proliferation in vitro. It has been reported that low molecular *p*-aminobenzoate, which induces delayed hypersensitivity in vivo, does func-

TABLE VIA. Lymphocyte proliferation in work applicants

Subject			Control	Bacampicillin			PPD	PWM
Code	Sex	Age (yr)	cpm ± SD	μg/ml*	cmp ± SD	SI	SI	SI
32	M	22	1411 ± 183	2	2591 ± 169	1.8		20
				100	2197 ± 219	1.6		
44	M	44	920 ± 202	20	1212 ± 69	1.3		81
				100	969 ± 65	1.1		
45	M	36	528 ± 148	20	1092 ± 416†	2.1		26
				100	984 ± 544	1.9		
			473 ± 129‡	100	583 ± 75	1.2		27
				200	677 ± 89	1.4		
46	M	22	916 ± 193	20	955 ± 95	0.7		35
				100	517 ± 93	0.4		
47	M	26	1664 ± 151	20	2038 ± 52	1.2		74
				100	1508 ± 149	0.9		
48	M	22	1064 ± 259	20	1142 ± 383	1.1		16
				100	1138 ± 101	1.1		
49	M	39	2357 ± 160	20	7731 ± 1237	3.3		54
				100	6199 ± 559	2.6		
			1306 ± 398‡	20	2426 ± 305	1.9		11
				100	4864 ± 360	3.7		
50	M	29	1496 ± 218	4	2207 ± 418	1.5		86
				20	2621 ± 695	1.8		
51	M	22	1813 ± 372	20	2589 ± 533	1.4	19	53
				100	4983 ± 621	2.7		
52	M	22	4149 ± 1206§	4	3983 ± 288	1.0		19
				20	5054 ± 683	1.2		
53	M	26	2701 ± 497§	4	3627 ± 145	1.3	32	
				20	4648 ± 242	1.7		
67	M	52	1660 ± 347	20	1256 ± 223	0.8	12	
				100	1269 ± 122	0.8		

*Two concentrations resulting in maximal proliferation in vitro.
†No lymphoblasts in culture.
‡Autologous serum in culture.
§Spontaneous blastogenesis.

TABLE VIB. Lymphocyte proliferation in work applicants

Subject			Control	Bacampicillin			Alprenolol			Quinidine			PPD	PWM
Code	Sex	Age (yr)	cpm ± SD	μg/ml*	cmp ± SD	SI	μg/ml	cpm ± SD	SI	μg/ml	cpm ± SD	SI	SI	SI
82	M	20	515 ± 113	20	664 ± 112	1.3	2	495 ± 206	1.0	1	537 ± 64	1.0	14	41
				100	647 ± 18	1.3	20	381 ± 84	0.7	5	661 ± 51	1.3		
83	M	21	516 ± 201	2	298 ± 89	0.6	2	219 ± 85	0.4	1	387 ± 1	0.8	27	
				20	589 ± 21	1.1	20	592 ± 311	1.2	5	295 ± 39	0.6		
84	M	21	494 ± 131	20	621 ± 246	1.3	2	222 ± 30	0.5	1	559 ± 63	1.1	48	
				100	622 ± 61	1.3	20	285 ± 77	0.6	5	258 ± 47	0.5		
85	M	20	476 ± 67	2	524 ± 246	1.1	2	362 ± 175	0.8	1	385 ± 15	0.8	73	
				200	517 ± 135	1.1	20	266 ± 27	0.6	5	403 ± 18	0.8		

*Two concentrations resulting in maximal proliferation in vitro.

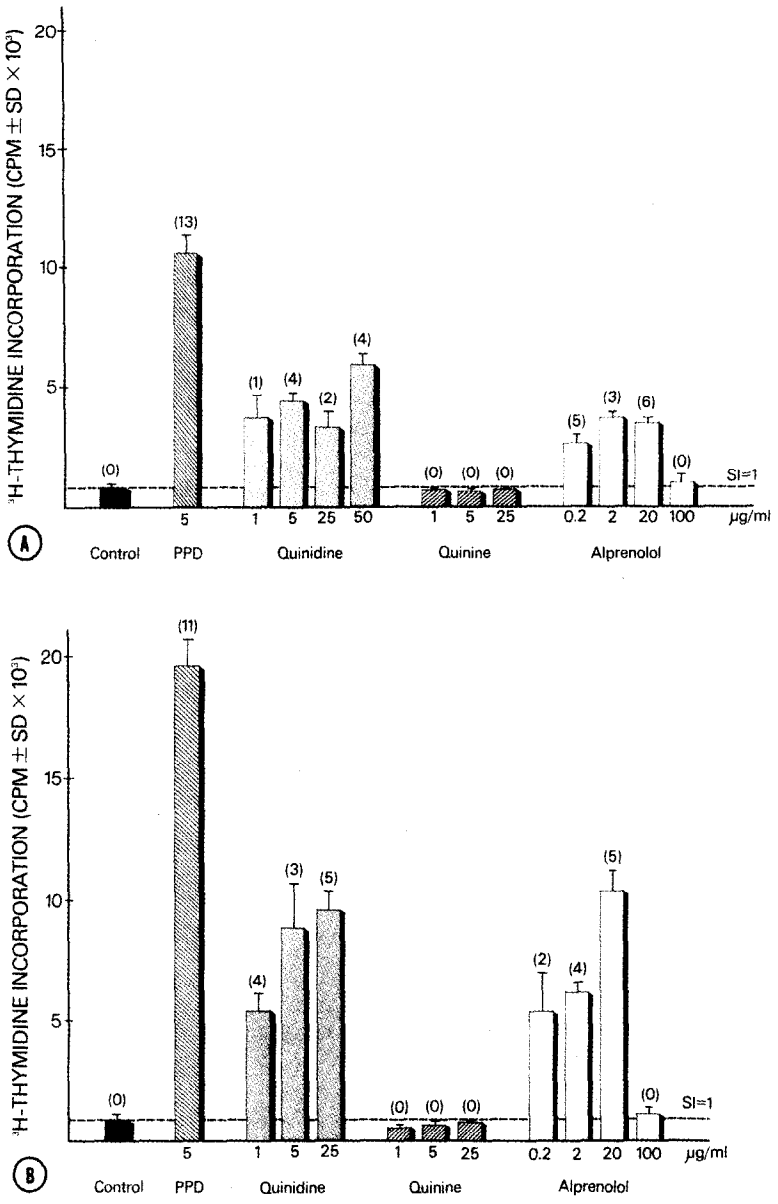


FIG. 2. Lymphocyte proliferation induced by alprenolol and quinidine in vitro (subject No. 86 noted in Table IV). **A-B,** Two independent experiments. Values in parentheses indicate the percentages of lymphoblasts in cultures.

tion as an antigen in vitro and induces the proliferation of para-aminobenzoate-specific memory cells.[36] Thus, covalent binding of the drugs to proteins may be just one of the ways that low molecular chemicals induce immune responses.

In conclusion, the results presented demonstrate that LTT can be used for the detection of offending agents in workers with suspected drug-induced occupational allergy. Furthermore, drug-specific lymphocyte proliferation may serve as a model for the study of lymphocyte receptor specificity and the cellular mechanisms underlying hypersensitivity reactions.

We thank Jan Larsson, M.Sc., president of Astra Pharmaceutical Production AB, for generous and enthusiastic support of this study, nurse Mia Westberg, Occupational Health Services, for her devotion in conducting the clinical survey. Dr. Ann Kristofferson and Dr. Brian Pring gave us valuable advice concerning the studies of penicillin specificity. Barbro Emanuelsson, Inga-Greta Johansson, Lab. Eng., Kerstin Sandelin, B.Sc., Birgitta Thorsin, and Birgit Sjöwall performed LTTs with remarkable skill. Last but not

least the assistance of Agneta Enström in preparing the manuscript and Jennifer de Paulis in revising English is gratefully acknowledged.

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