values when methimazole was administered.

The distribution of radiothyroxine at physiological levels of thyroxine between TBG and TBPA and the binding capacities of these carrier proteins were found to be unchanged by methimazole administration in 11 euthyroid subjects (Table II), nor was any change noted in the free-thyroxine values (percent or concentration). Methimazole was found to produce no significant change in the PBI.

Summary and conclusions. The effects of methimazole (60 mg/day) on peripheral thyroxine turnover and transport were investigated in 16 adult volunteers. In four subjects (two euthyroid individuals and two myxedematous patients in the euthyroid state on thyroxine replacement) using exogenously administered ^{131}I-labeled L-thyroxine, the radiothyroxine turnover was investigated. Simultaneously, the effects of methimazole administration (10 days) on thyroxine-binding serum protein carriers (11 subjects) and serum free-thyroxine levels (six subjects) were observed. Methimazole administration in man in these studies was shown to have no effect on peripheral thyroid indices: thyroxine binding to serum proteins, the serum free-thyroxine levels or the turnover and total quantitative degradation of thyroxine. This finding confirms a similar result on thyroxine turnover in one myxedematous patient reported earlier (2).

From the present data it would appear that methimazole does not alter the turnover, transport or quantitative degradation of thyroxine in man when compared to propylthiouracil.

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Effect of Phytohemagglutinin and Various Mycobacterial Antigens on Lymphocyte Cultures from Leprosy Patients (32698)

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Lymphocyte transformation in cultures from peripheral blood has been described as an *in vitro* reflection of immunological capacity. The phenomenon has been associated with the delayed type of hypersensitivity and with homograft histoincompatability. The morphological change is an *in vitro* transformation of small lymphocytes into large blast-like cells that are capable of mitosis; it can be brought about by a number of different stimulants (1, 2). Some stimulants, such as phytohemagglutinin (PHA) and streptolysin O, cause a profound response in

[1] Use of trade names is for identification only and does not constitute endorsement by the Public Health Service, or by the U. S. Department of Health, Education, and Welfare.

the lymphocytes of all normal persons; others, such as tuberculin, cause a limited response and only in persons sensitive to the antigen. Patients with lepromatous leprosy, the severe form of the disease, fail to respond to skin tests to lepromin, an autoclaved suspension containing standard concentrations of *Mycobacterium leprae* prepared from patients' tissues. In contrast, normal adults and patients with tuberculoid leprosy, the milder form of the disease, react with papule or nodule formation reaching a maximum at about 28 days (Mitsuda reading). We report here the lymphocyte response of patients with lepromatous and tuberculoid leprosy and of normal controls to antigens from *M. leprae* and *M. tuberculosis* and to phytohemagglutinin, an extract of the red kidney bean, *Phaseolus vulgaris*. A recent study of the lepromin and tuberculin reactions of the normal adults of Group I (below) includes a review of the relationships between the lepromin and tuberculin skin tests (3).

Materials and Methods. Lymphocytes were collected from (1) normal volunteers at the Atlanta Federal Prison, (2) patients with active and inactive lepromatous leprosy at the U.S. Public Health Service Hospital in Carville, Louisiana, and (3) normal volunteers and patients with active lepromatous and tuberculoid leprosy at the U.S. Public Health Service Hospital, San Francisco, California. A modification of the lymphocyte culture method described by Bach and Hirschorn was used (4). At the place of collection venous blood was mixed with heparin and allowed to sediment at 37°C (1–2 hours), the supernatant fluid of leukocyte-rich plasma was withdrawn and an equal volume of NCTC-109 medium was added. The neutrophils were removed by allowing them to adhere to glass, and relatively pure lymphocyte suspensions were obtained. These suspensions were then transported to the National Communicable Disease Center (NCDC), Atlanta, for further processing and culture. For culture the lymphocytes were diluted to 1×10^6 cells/ml and maintained in NCTC 109 (75%) and autologous plasma (25%) under an atmosphere of 3% CO_2. Two-ml aliquots of each cell suspension were placed into 15 × 150-mm screw-capped tubes and the following antigens added to single or duplicate cultures: PHA (PHA-P, Difco), 1 μl/ml; PPD (Merck, Sharpe, and Dome), 1×10^{-4} mg/ml; BCG, 1×10^{-2} ml/ml, extract prepared by disruption in a Mickle apparatus with fine glass beads of an autoclaved suspension containing 2.1×10^8 bacteria per milliter; *M. leprae*, 1×10^{-2} ml/ml, extract prepared similarly from an autoclaved suspension purified from a patient's spleen and containing 2.3×10^8 bacteria per milliter; and lepromin, 1×10^{-2} ml/ml, containing 2.5×10^7 bacteria per milliter disrupted similarly. Higher dilutions of all bacterial extracts were also included, but the lymphocyte response was lower in each case, so these results are not reported. After the cultures were incubated 5 days at 37°C, 0.02 ml 1×10^{-5} *M* colchicine was added per milliter of growth medium and the tubes incubated an additional 4 hours to arrest mitosis. The cells were washed in hypotonic solutions, fixed in ethanol and glacial acetic acid (3:1 v/v) with final suspension in 0.2 ml fixative. The cells were then pipetted onto three warm prewetted slides, allowed to dry, and stained with Wright's stain. Coverslips were mounted and 1500 cells scored from each culture (500 from each slide). The number of spontaneously transformed cells on the control slides was subtracted from the number transformed by the various antigens in each case.

Results. Since the time between the initial processing at the place of blood collection and the final processing and initiation of cultures at NCDC was not the same, the responses in each group are given separately.

The response of lymphocytes collected from normal volunteers in Atlanta was similar to that seen in other studies (1,2). The average response to PHA was 78.1% (Table I, Fig. 1). Tuberculin positive subjects had an increased response to PPD and BCG (Table I). The *in vitro* response to BCG and especially PPD was correlated with the size of the skin reaction to tuberculin. The donors had been selected for another purpose on the basis of the lepromin reactions, and all had strong Mitsuda reactions with 14–22-mm induration and necrotic centers (3). There was little correlation between the size of the lepromin test and the *in vitro* transformation by extracts of *M. leprae* or lepromin.

TABLE I. Skin Tests and Lymphocyte Transformation of Normal Volunteers of Group I.

Subject	Skin tests: Tuberculin[a] (mm)	Skin tests: Lepromin[b] (mm)	Cells transformed (%): PPD	BCG	*M. leprae*	Lepromin	PHA-P
1	25	15	8.0	8.1	2.5	3.1	80.8
2	18	22	13.3	4.7	0.8	1.8	73.5
3	17	14	4.1	6.2	5.9	5.0	78.8
4	6	16	0.2	1.3	0.7	1.7	76.8
5	0	22	0.1	3.3	1.5	1.7	82.2
6	0	16	1.0	3.2	1.3	1.3	76.4

[a] Induration 48 hours after injection of 0.0001 mg PPD.
[b] Induration 28 days after injection of 0.1 ml standardized lepromin.

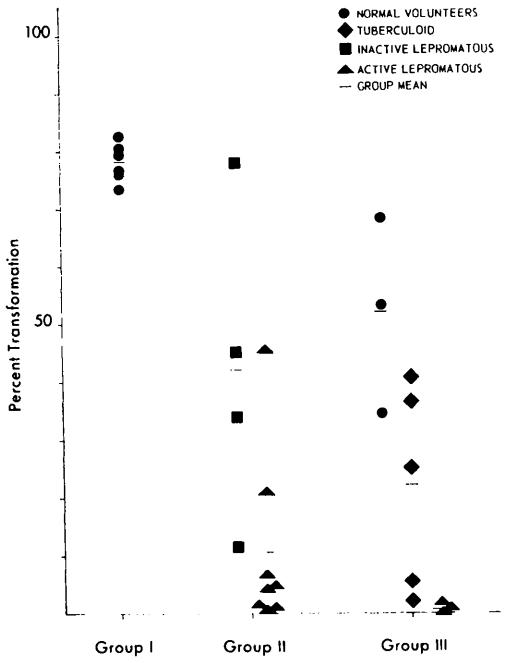

FIG. 1. Transformation by phytohemagglutinin of lymphocytes of normal persons and leprosy patients.

The second group was composed of leprosy patients at the Carville hospital. The eight active lepromatous patients had low responses to PHA (average 10.7%); the four inactive lepromatous patients averaged somewhat higher (42.2%) responses (Fig. 1, Table II). Four patients had erythema nodosum leprosum (ENL) at the time of collection; they were all among the active lepromatous group and they all had very low responses to PHA.

The third group consisted of active lepromatous and tuberculoid leprosy patients and normal volunteers in San Francisco. The three active lepromatous patients had very low responses to PHA; of the tuberculoid patients two also had low responses, and three had intermediate responses (Fig. 1, Table II).

Two of the three normal volunteeers had lower responses to PHA than the normal volunteers in Group I. These two lower responses to PHA might be connected with the greater time lapse that occurred between initial and final processing in Group III. However, the 68% response of the third volunteer compared favorably with earlier responses of the same individual (63–87%, average 76%, in six separate trials when the complete processing was carried out at NCDC).

The patients' skin test results with tuberculin and lepromin are given in Table II, where the results in the two grorups of patients are arranged in order of the PHA response *in vitro*. Several of the patients had strongly positive tuberculin skin tests even though they had very low responses *in vitro* to PHA and to PPD. One patient had recently been treated for active pulmonary tuberculosis; her tuberculin skin test measured 11 mm and her responses *in vitro* were very low. All the patients' responses *in vitro* to antigens of *M. leprae* were low and not correlated with the lepromin skin tests.

Discussion. The results with PHA revealed a depression in most of the leprosy patients. Of the 11 patients with active lepromatous disease, nine had severely depressed responses to a level below 7%. Of the five patients with tuberculoid disease, which is the milder form, two also had PHA responses in the same

TABLE II. Lymphocyte Transformation and Skin Tests of Leprosy Patients and Normal Volunteers of Groups II and III.

Patient	Lymphocyte transformation (%)			Skin tests		Clinical status[c]
	PHA	PPD	BCG	PPD[a]	Mitsuda[b]	
Group II						
1	77.9	1.0	3.3	0	0	L (I)
2	45.1	0.0	1.1	12.5	0	L (I)
3	45.1	0.0	0.0	0	0	L (A)
4	34.1	16.4	17.0	20	7	L (I)
5	21.3	0.8	0.0	0	0	L (A)
6	12.0	0.1	0.0	22.5	0	L (I)
7	6.9	1.7	0.6	0	0	L (A)
8	5.3	3.6	0.3	0	0	L (A)
9	4.7	0.6	0.3	19	0	L (A)
10	1.5	1.5	0.0	30	0	L (A)
11	0.9	0.0	0.0	0	0	L (A)
12	0.5	3.9	0.0	0	0	L (A)
Group III						
1	40.7	1.3	0.0			T
2	36.6	0.0	0.0			T
3	25.1	1.4	0.0	27.5	1	T
4	5.3	0.1	0.2			T
5	2.9	0.0	0.0	15	2	T
6	0.6	0.0	0.0	0	0	L (A)
7	0.3	0.0	0.0	11	3	L (A)
8	0.0	0.0	0.0	0	0	L (A)
9	68.4	0.9	0.0	3	8.5	Normal
10	34.9	3.3	0.8			Normal
11	52.7	1.8	1.0	0	4	Normal

[a] Induration 44 hours after injection of 0.0001 mg PPD.
[b] Induration 28 days after injection of 0.1 ml standardized lepromin.
[c] L = lepromatous, T = tuberculoid, I = inactive, A = active.

range. Patients with inactive disease and three of those with tuberculoid disease had intermediate responses.

The results of other workers show a depression in the ability to develop delayed hypersensitivity in leprosy patients. Bullock (5) found an impaired ability to develop sensitivity to picryl chloride in lepromatous and tuberculoid patients. Lepromatous patients with ENL were not significantly different from those without. Waldorf *et al.* (6) found an impaired ability to become sensitized to 2,4-dinitrochlorobenzene (DNCB) in lepromatous patients. The depression was more severe in lepromatous patients without ENL, and was less marked or absent in lepromatous patients with ENL, in inactive lepromatous patients, and in patients with borderline disease (a form with features of both lepromatous and tuberculoid leprosy). Job and Karat (personal communication) have recently observed that the rejection of skin homografts was delayed in four patients with lepromatous disease. Sheagren *et al.* (7) have reported recently in abstract that patients with lepromatous disease have impaired lymphocyte transformation to Streptolysin "O" but not to phytohemagglutinin. Perhaps a more complete analysis of their results will explain the apparent discrepancy with ours. Thus, although the accumulated evidence is not in complete agreement, there is considerable support for the view that there is a generalized

depression of immunity of the delayed type in leprosy.

Defective lymphocyte transformation has previously been noted in chronic lymphatic leukemia (8), Hodgkin's disease (9), primary acquired agammaglobulinemia (10) and ataxia telangiectasia (11). In these conditions depressed lymphocyte transformation has been associated with immunological deficiencies.

Olson *et al.* (12) have described depressed lymphocyte transformation to PHA in four infants with congenital rubella. The obvious parallelism between congenital rubella and lepromatous leprosy lies in continued production of their infectious agents. The infants with congenital rubella were able to develop and express delayed hypersensitivity in an apparently normal manner, thus indicating a dissociation between the PHA response and the functions of lymphoid cells in delayed hypersensitivity (12). As mentioned, patients with lepromatous leprosy are usually unable to develop delayed hypersensitivity to such antigens as picric acid (5) and DNCB (7). They are, however, frequently able to express tuberculin sensitivity, although probably with decreased frequency and intensity in comparison to controls (13). Some of our patients who were distinctly reactive in tuberculin skin tests had very low lymphocyte transformation to PHA and PPD as well.

Whether the depressed immunologic capacity of active lepromatous patients is a result of the infection or a genetic or other type of constitutional factor that determines the course of the infection is an important question in leprosy. Our limited data from inactive lepromatous patients suggest that the depression is a result of the infection. These patients had been treated for many years, and their skin smears converted to bacterial negativity; their PHA responses were normal to only moderately depressed.

The combined results thus demonstrate a generalized immunologic deficit in leprosy, one that is not specifically directed toward *M. leprae*. However, they do not explain the specific inability of the lepromatous patient to react to suspensions of *M. leprae* in the lepromin test. These patients react normally to suspensions of *M. tuberculosis* and many other mycobacteria and it is only to suspensions of *M. leprae* that they are nonreactive (14, 15).

Summary. Leprosy patients and normal controls were studied for lymphocyte transformation *in vitro* to phytohemagglutinin (PHA) and to antigens from *M. leprae* and *M. tuberculosis*. Most patients with active lepromatous leprosy, the severe form of the disease, had markedly depressed lymphocyte responses to PHA as well as to the mycobacterial antigens. The response to PHA was only moderately depressed in patients with tuberculoid leprosy, the milder form of the disease, and in patients whose lepromatous disease had been rendered inactive by long-term therapy.

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