Passive Transfer of Tuberculin Reactivity in Vitro

Abstract. *A factor capable of effecting passive transfer in vivo of delayed hypersensitivity to tuberculin to recipients that are tuberculin negative was isolated from the dialyzate of disrupted leukocytes of tuberculin-positive individuals. After this factor was incubated with cultures of peripheral leukocytes from tuberculin-negative individuals, the addition of purified protein derivative of tubercle bacilli resulted in leukocyte stimulation similar to that observed after addition of purified protein derivative to leukocytes from tuberculin-positive individuals.*

The addition of tuberculin antigens to tissue cultures of human peripheral leukocytes obtained from tuberculin-positive donors induces increased mitotic activity and morphologic alteration of small lymphocytes, which become larger lymphoid cells (*1*). Lawrence (*2*) and others (*3*) have shown that delayed hypersensitivity to tuberculin can be passively transferred to a previously tuberculin-negative individual with cell-free extracts of leukocytes from sensitive donors. We now report that an extract of disrupted leukocytes from highly sensitive tuberculin-positive individuals was capable of transferring reactivity to tuberculin in vitro; the maximum molecular weight of the extracted material is less than 4000, and the material contains nucleic acid. When leukocytes from tuberculin-negative individuals (*4*) were cultured in the presence of this low-molecular-weight transfer factor, the addition of tuberculin resulted in an increased transformation into large lymphoid cells and increased mitoses.

The leukocyte extract was prepared by a modification of the method of Lawrence *et al.* (*5*). Leukocytes were separated from heparinized whole blood from a highly sensitive tuberculin-positive (purified protein derivative, PPD) individual, by sedimentation in 2 percent dextran. The leukocytes, 1×10^9 in number, were mechanically disrupted by agitation with glass beads and then dialyzed against distilled water. After concentration by lyophilization, the dialyzate was fractionated on a column of Sephadex G-25, and a fraction was isolated which was capable of transferring delayed hypersensitivity. The fraction containing transfer factor was further purified on Sephadex G-10; comparison of its elution pattern with the elution of substances of known molecular weight indicated a molecular weight of 700 to 4000. By the same method of separation, a similar fraction was isolated from the dialyzate of the disrupted leukocytes from a tuberculin-negative individual, and passive transfer tests revealed that none of the fractions from such leukocytes were capable of transferring delayed hypersensitivity to tuberculin in man. Human peripheral leukocytes from tuberculin-negative and tuberculin-positive individuals were isolated and cultured by a modification of the method of Hirschhorn (*6*). One million leukocytes were cultured in 5 ml of Eagle's modified minimum essential medium (Spinner) containing 1 percent of 200 m*M* L-glutamine and 20 percent fetal calf serum. Leukocyte cultures were incubated at 37°C for 6 days after the addition of 0.05 mg of the PPD of tubercle bacilli, or 3 days after the addition of 0.1 ml of phytohemagglutinin. After an additional incubation period with 0.01 percent colchicine at 37°C for 2 hours, the cells were collected by gentle centrifugation at 1000 rev/min and washed twice in the culture medium. The cells were then fixed in a mixture of alcohol and glacial acetic acid (3 : 1) and stained with 1 percent acetic orcein. The percentages of small lymphoid cells, large lymphoid cells, and mitoses were determined by counting at least 1000 cells from randomly chosen fields. The sum of large lymphoid cells and mitotic figures was the total number of stimulated cells.

The response of cultures of leukocytes from PPD-negative individuals, from PPD-positive individuals, and from passively sensitized PPD-positive individuals is shown in Table 1. The addition of PPD to leukocyte cultures from PPD-positive donors resulted in a significant increase in the number of stimulated cells (20.5 ± 1.9 percent), when compared to the response of cultures from PPD-negative donors (2.7 ± 1.1 percent). Cultures of leukocytes from individuals who had been sensitized passively to PPD by means of an injection of low-molecular-weight transfer factor 1 to 4 weeks earlier also demonstrated an increase in the number of cells (8.5 ± 2.7 percent) stimulated by the addition of PPD. The cellular response to PPD in leukocyte cultures was considerably less in cultures from passively sensitized PPD-positive individuals than in cultures from individuals with naturally acquired delayed hypersensitivity to PPD. The response of cultures of leukocytes from either PPD-negative or PPD-positive individuals when transfer factor alone was added was similar to the response of leukocyte cultures to which nothing had been added, although the amount of transfer factor used represented the amount isolated from 1×10^6 leukocytes of a highly sensitive PPD-positive individual and was capable of passive transfer of tuberculin sensitivity in vivo. However, the addition of of PPD to the leukocyte cultures from PPD-negative individuals to which transfer factor had been added 1 hour earlier resulted in a significant increase

Table 1. Stimulation of leukocytes in vitro. Numbers in parentheses indicate the numbers of individuals whose leukocytes were cultured. Cultures from each individual were done in duplicate.

Additions to leukocyte cultures	Cultures of leukocytes from individuals who were:		
	PPD negative (8)	PPD positive (4)	PPD pass. positive (9)†
None	2.1 ± 0.9*	2.8 ± 1.2	1.9 ± 0.8
PPD	2.7 ± 1.1	20.5 ± 1.9	8.5 ± 2.7
Transfer factor	3.4 ± 1.1	3.0 ± 1.1	2.0 ± 1.3
Transfer factor + PPD	6.6 ± 1.2	26.1 ± 2.1	12.1 ± 1.5
Dialyzate from PPD-negative donor	3.0 ± 1.2	2.8 ± 1.3	3.1 ± 1.2
Dialyzate from PPD-negative donor + PPD	3.4 ± 1.0	3.0 ± 1.1	2.4 ± 0.9
Phytohemagglutinin	75.5 ± 4.7	81.5 ± 6.5	77.7 ± 3.9

* Mean percentage stimulated cells ± standard deviation. † Individuals sensitized passively in vivo with transfer factor.

in the number of stimulated cells when compared to the response of leukocyte cultures from the same individuals with PPD alone or with transfer factor alone ($P < .001$). There was no significant increase in the number of stimulated cells when PPD was added to cultures from PPD-negative individuals to which had been added the low-molecular-weight fraction prepared from leukocytes of a PPD-negative donor. The addition of PPD to leukocyte cultures from PPD-positive individuals to which transfer factor had been added 1 hour earlier increased the number of stimulated cells beyond that observed under the same conditions in the absence of transfer factor.

The data suggest that the response of leukocytes from PPD-negative and PPD-positive individuals to PPD was significantly increased by the addition of a low-molecular-weight dialyzate of leukocytes from PPD-positive donors which contained transfer factor. Whether this in vitro response of leukocytes is analogous to the in vivo passive transfer of delayed hypersensitivity to tuberculin with transfer factor remains to be seen.

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4. In this study individuals were PPD negative if they showed less than 5 mm of erythema and induration 24 to 48 hours after challenge with 5 μg of PPD. The PPD-positive (naturally acquired) individuals had at least 10 mm of erythema and induration 24 to 48 hours after challenge with 0.001 μg of PPD: the individuals passively sensitized with transfer factor had at least 10 mm of erythema and induration 24 to 48 hours after challenge with 5 μg of PPD.
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Interpopulation Variations in Calcium Metabolism in the Stream Limpet, Ferrissia rivularis (Say)

Abstract. *Significant differences between populations occur in calcium uptake during growth within one species of freshwater limpet. These are not related to environmental differences and may involve genetically determined physiological races. Such variation is significant in relation to aspects of evolution in freshwater animals and is important in assessing radionuclide contamination.*

Infraspecific physiological variation between populations of freshwater mollusks in aspects of growth, fecundity, and respiration has been reported (*1*, *2*), and its significance discussed (*1*, *3*). We now report on apparently extensive interpopulation variations in calcium uptake which occur during growth in the freshwater limpet *Ferrissia rivularis* (Say). The investigation was made in upstate New York with natural populations in freshwater creeks, whose mineral contents are very different.

If one considers only those fresh waters which can support mollusks with calcareous shells, the dissolved calcium content varies more than 100-fold. Within this range the distribution of many molluscan species is affected by calcium concentration. In temperate regions of the world, extremely soft waters (calcium concentrations < 3 mg/liter) can support only about 5 percent of the molluscan species of the region, moderately soft waters (Ca < 10 mg/liter) can support about 40 percent, intermediate waters (10 to 25 mg/liter) can support up to 55 percent, with hard waters (Ca > 25 mg/liter) being required for the rest (*3–5*). However, most of those species tolerant of low calcium could survive in, and are found in, harder waters (*3*).

The simplest possible case concerns those mollusks able to live in waters with a wide range of hardness and in whose shells and tissues the amount of calcium carbonate accumulated during growth varies directly with dissolved calcium content of the waters. Sampling of natural populations has shown that this is true in the extremely eurycecic snail *Lymnaea peregra* (*6*) where the thickness (and mass) of the shell varies according to the amount of calcium available. There are some earlier experimental data confirming this relation for some molluscan species (references in *4*, *7*), and culture experiments on *Biomphalaria pfeifferi* have demonstrated that there is an optimum concentration of environmental calcium for both fecundity and growth in this species (*8*).

The case of *Ferrissia rivularis* is apparently different. In upstate New York, this species lives in creeks containing from 10.4 mg to 67.6 mg of calcium per liter. In a survey of interpopulation variations in organic growth pattern, with carbon being assessed by a colorimetric method after "wet-oxidation" of the sample (*9*), it became clear that the calcium content of the shell of *Ferrissia* did not vary in a simple direct relation with the amount of environmental calcium available. Seven natural populations, in six creeks and on a section of the shore of Oneida Lake, were studied; all of these waters form part of the Oneida division of the Seneca-Clyde-Oneida system which flows by way of the Oswego River into Lake Ontario. The environmental concentrations of dissolved calcium and magnesium were analyzed by an EDTA (ethylenediaminetetraacetate) titration (Table 1). The total hardness was also determined chemically at the same time, and the average total hardness was determined from conductivity measurements of samples made on every visit throughout the year. Regular sampling of 50 to 100 *Ferrissia* at these localities on about 15 to 30 occasions over 18 months to 2 years has allowed growth cycles to be determined. For a range of shell lengths (ages), the total organic carbon content, weights of living specimens, weights of dried specimens, "ash-free" dry weights, and shell calcium carbonate were determined. If consideration of growth changes in the ratio of calcium to carbon is postponed, the mean figures (Table 2) for shell calcium (with the confidence limits of the mean at the 95 percent probability level, $\bar{X} \pm t_{0.05}\, s_{\bar{x}}$) for each population are from 82 ± 1.3 mg of calcium per gram live wet weight (Fish Creek) to 121 ± 1.0 mg of calcium per gram (Big Bay Creek). These data are plotted against environmental calcium (Fig. 1), and it is clear that there is no direct relation. Data from studies with radioactive tracers and microanalyses (*10*, *11*) show that there is direct active uptake of calcium from the medium by freshwater snails, and further that 80 percent of the calcium gained by grow-