A Correlation of in Vivo Delayed-Type Hypersensitivity with in Vitro Lymphocyte Transformation in Sarcoidosis*

O. P. Sharma, M.D.,** D. G. James, M.D.,† and R. A. Fox, M.D.‡

Induction of in vivo dinitrochlorobenzene (DNCB) sensitization has been correlated with in vitro cultured lymphocyte transformation in 21 patients with histologically confirmed sarcoidosis and 21 matched control subjects. Failure to sensitise with DNCB was noted in 71 percent of patients with sarcoidosis and in 14 percent of controls. Poor lymphocyte transformation occurred in 90 percent of patients with sarcoidosis and in only one of control subjects. The twin defects were a feature of 71 percent of patients with sarcoidosis, indicating that the in vivo cutaneous energy reflects the in vitro cellular hyporeactivity, probably due to immunologically incompetent lymphocytes.

The cardinal immunologic defect in sarcoidosis is depression of delayed-type hypersensitivity. This is readily demonstrated by skin tests using various antigens as tuberculin, Candida albicans, pertussis, mumps, trichophyton and even pine pollen.¹ Since lack of response to the intradermal injection of bacterial, viral, and fungal antigens is so evident, chemical contact allergens could also be expected to be met by a diminished response. Varied results have been reported according to whether the highly potent sensitiser pentadecyl catechol, found in poison ivy, was used or the moderately strong sensitizer 2:4 dinitrochlorobenzene (DNCB) or the least potent allergen, para-nitrosodimethyl aniline. Moreover response to DNCB varies depending upon whether sensitization is induced by the initial application to the skin of a 1 percent or 10 percent solution. Whichever method or antigen is used, the depression in delayed-type hypersensitivity is nonspecific. It is seen in the very young and very old, and can be brought about by immunosuppressive drugs, viral vaccines, irradiation and even by hypnosis.² Thus, any other parameter which may be correlated with this depression may go some way towards explaining the mechanism of the depression of delayed-type hypersensitivity. We have correlated the in vivo induction of DNCB sensitization with the in vitro response of cultured human lymphocytes to phytohemagglutinin.

**Patients and Methods**

The patients were attending the Royal Northern Hospital Sarcoidosis Clinic, London, England. A group of 21 patients with histologically-confirmed sarcoidosis and a group of 21 controls, matched for age and sex, were studied, with particular reference to their DNCB skin test response and the ability of their cultured lymphocytes to react in vitro.

**DNCB Skin Test**

The method used was similar to that described by Aisemberg³ (1962). The synthetic chemical contact sensitizer, 2:4 dinitro-1-chlorobenzene (DNCB) was used to investigate the incidence of delayed-type hypersensitivity. Initially 0.1 ml of the 10 percent solution in acetone was applied to forearm, followed one month later by the application of 0.1 ml of an 0.1 percent solution to the skin of the other forearm. A positive reaction comprised induration and vesiculation, in addition to erythema, at 48 to 96 hours.

**Lymphocyte Culture**

Thirty to 50 milliliters of venous blood was drawn into plastic disposable syringes containing 500 units of heparin. The needle was changed and the blood was allowed to

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*Presented before the Eleventh International Congress on Disease of the Chest, Lausanne, Switzerland, August 3-7, 1970.

**Present at University of Southern California, School of Medicine, Los Angeles, California.

†Royal Northern Hospital, London.

‡Royal Free Hospital, London.
sediment at room temperature for 30 to 45 minutes. The leukocyte-rich plasma was then discharged into a sterile container. Total and differential white blood cell counts were performed and calculations made so that the culture tubes contained one million lymphocytes. The final culture volume was 3 ml, which consisted of leukocyte-rich plasma in appropriate volume, tissue-culture medium ("TC199," Burroughs Wellcome) without antibiotics, and the patients' own plasma—the latter two in the ratio 4:1. Cultures were all run in triplicate and 0.05 ml phytohemagglutinin P (Difco) was added to the tubes. These tubes and their controls were stopped at 72 hours; 3 μCi of tritiated thymidine was added to the cultures six hours before the addition of ice-cold saline solution to stop the reaction.

The cells were washed three times with ice-cold saline solution (0.9 percent) and tritium-labelled DNA protein was precipitated by the addition of ice-cold 5 percent trichloroacetic acid. The precipitates were dissolved in Nuclear Chicago solubilizer (NCSA) and then prepared for counting by the addition of 10 ml toluene scintillator fluid. Transformation was assessed by examination of cell smears stained with May-Grunwald-Giemsa and also by uptake of tritiated thymidine (liquid scintillation counting). The results are expressed as percent blast cells per surviving cells at 72 hours.

**RESULTS**

Dinitrochlorobenzene (DNCB) sensitization could be induced in 18 of 21 (86 percent) normal subjects, but only in 6 of 21 (29 percent) patients with sarcoidosis reflecting depression of delayed-type hypersensitivity in the majority of the latter (Table 1).

**Lymphocyte transformation** in response to phytohemagglutinin was noted in all but one of the 21 controls. Morphologic examination at 72 hours revealed mean percent blast formation of 84 with standard deviation of eight in all but one of the control subjects but in lymphocytes of only two of the patients with sarcoidosis. The measurement of rate of incorporation of tritiated thymidine in controls revealed high counts ranging from about 500,000 to over three million counts per minute per million lymphocytes. Whereas in only two of 21 patients with sarcoidosis did the count exceed 500,000 counts per minute per million lymphocyte. Poor lymphocyte transformation was therefore an even commoner characteristic than ability to accept DNCB sensitization in this series. The twofold qualities of having responsive lymphocytes and being able to react to DNCB were noted in 86 percent of normal subjects and in only 10 percent of patients with sarcoidosis (Table 2). The absence of both features was noted in seven of ten sarcoidosis patients (Table 2).

**DISCUSSION**

About two-thirds of patients with sarcoidosis have a marked depression of delayed-type hypersensitivity.4 Friou5 (1952) studied the effect of an injection of oidiomycin, trichophytin and mumps antigen in patients with sarcoidosis and in control subjects. He observed that significantly fewer patients reacted to these antigens in sarcoidosis group than in the control groups. Similarly they cannot be sensitized to develop contact sensitivity to chemical agents such as 2:4 dinitrochlorobenzene.6

It is also well-known that the lymphocyte transformation response to phytohemagglutinin (PNA) in patients with active sarcoidosis, is very low.7,8 What we are now recognizing is a close correlation between these two abnormalities, the cutaneous anergy and poor lymphocyte transformation. It seems highly likely that in vivo cutaneous anergy reflects in vitro hyporeactivity of lymphocytes. The immunologically competent lymphocyte is an efficient memory cell for antigens it has already met. When its memory fails it seemingly becomes sluggish and hyporeactive both in vivo and in vitro. How this mechanism is brought about in sarcoidosis remains unknown.

The other characteristic feature of sarcoidosis is the production of noncaseating granulomas in various tissues spontaneously and in skin in response to intradermal injection of extracts of spleen or lymph nodes of other patients with sarcoidosis (Kveim test). This triad of granuloma formation, depression of delayed-type hypersensitivity, and in vitro lymphocyte anergy might be regarded as three hallmarks of sarcoidosis. However, this is not to be, for already there is some preliminary evidence to suggest that the same three hallmarks characterize Crohn's disease9 and primary biliary cirrhosis.10

Table 2—Correlation of in vivo DNCB skin tests in vitro lymphocyte transformation in 21 patients with sarcoidosis and 21 control subjects.

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LYMPHOCYTE TRANSFORMATION IN SARCOIDOSIS

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Reprint requests: Dr. Sharma, Department of Medicine, USC School of Medicine, 2025 Zonal Avenue, Los Angeles 90033

Cardiorespiratory Hazards of an Age-Old Euphoriant

From religious symbolism, empirical medicinal application to sumptuous Lucullan debauchery, alcoholic beverages have gained popular part in the design for living of mankind. Epithets bestowed upon various alcoholic drinks are of interest. Osler called whiskey the milk of old age, Pasteur acclaimed wine as the most wholesome of all beverages. On the other hand, Winck of Duquesne University asserted in 1967 that all of those ethyl alcohol tops the list in seriousness of its consequences. Possibly, the latter incrimination was based on rather shocking findings: over 150,000 annual deaths throughout the world attributable to alcoholic cirrhosis of the liver; over 25,000 annual deaths in the United States, which result from drunk-driving. According to Terhune (New York State J Med 64:2041, 1964) 10 percent of the United States population are alcohol dependent and 5 percent are alcoholics. Alcoholic myocardiopathy was reported by Bollinger almost one hundred years ago (Deutsch med Woehenschr 10:180, 1884). During the past decade convincing experimental studies revealed the direct effect of alcohol on the myocardium: impaired cardiac contractility, decline in ventricular output, rise in left ventricular and diastolic pressure. Several reports on histologic and electron microscopic findings are of interest. Some of the muscle fibers show degenerative changes while adjacent ones are hypertrophied. There is deposition of lipid material in the muscle fibers; also, there are minute foci of myocardial necrosis and scarring. Edema of arterioles is followed by destruction of the vessel walls. Mitochondrial and microsomal oxidative enzymes are decreased or lost. With electron microscope one can visualize mitochondrial swelling, distorsion of cristae, swelling of sarcoplasmic reticum, myofibrillar fragmentation, increased glycogen and fat deposits (Hibbs et al, Am Heart J 69:766, 1967; Alexander, C S, Am J Med 41:229, 1966). In their classic review of the subject, Burch and De Pasquale (Am J Cardiol 23:723, 1969) emphasize that alcoholic heart disease is due to the direct effect of alcohol on the myocardium and that it may occur in well-nourished persons of all socio-economic groups. They discuss the essential features of the early and late phases of alcoholic cardiomyopathy. In the early stage there are tachycardia, atrial fibrillation, premature atrial and ventricular fibrillation, bundle branch block. The early stage is reversible by bed rest for at least 90 days and by complete abstinence from alcohol. In the late stages, the patient is likely to complain of vague chest pain, palpitation, unexplained weakness, cough, fatigue and dyspnea, orthopnea and nocturnal dyspnea. There are diffuse cardiomegaly, proteidostial gallop sound, distended neck veins, frequently blowing apical systolic murmur, and possibly pulsus paradoxus. X-ray shows a globular enlarged heart, engorged pulmonary veins, perhaps small pleural effusion. In the electrocardiogram one notes sinus tachycardia, atrial or ventricular extrasystole, and atrial fibrillation or flutter in 15-20 percent, together with findings characteristic of underlying pathologic changes. Alcoholic intoxication depresses the respiratory center, inhibits the normal protective mechanism of the respiratory tract, decreases immunity and interferes with phagocytic activity. Consequently, there is a relatively high incidence of pneumonia, lung abscess and aspiration of foreign bodies. Occurrence of allergic bronchial asthma may be attributable to alcohol as an allergen, to alcohol as a solvent which may release and render absorbable otherwise inert allergens, to some of the non-alcoholic ingredients of alcoholic drinks. As a corollary, perhaps it may be permissible to point out in less well known sequel of alcoholic cirrhosis of the liver, namely sexual insufficiency in the male. It is presumably due to overproduction of circulating estrogen (Lederer, J, 1968).

Andrew L. Banyai, MD.