lymphocyte transformation. The authors find that the ability of cutaneous nontuberculin reactors to respond to PPD in vitro is suppressed. They cite only Waxman and Lockshin as being unable to detect a depressed response to in vitro PPD in a single patient with miliary tuberculosis as a published work counter to their experience. Unfortunately, they appear to have ignored several other reports in the literature, all of which suggest that tuberculosis or BCG-infected patients who have no intradermal reaction may have lymphocyte transformation studies demonstrating reactivity. Some reports (Matsanotis et al., Rauch, utilized BCG vaccination as a marker for infection; McFarland and Heilman studied skin test negative patients with tuberculosis who had in vitro response to various PPD antigens. Kirby showed in vitro responses to PPD S in patients with less than 10 mm cutaneous reaction. We studied lymphocyte transformation which was positive in seven patients with proved or probable tuberculous disease who failed to react to intradermal PPD. Six of the seven ultimately became reactive to PPD up to three months after transformation was first observed. We noted diminished absolute lymphocyte counts in most of these patients that correlated well with the lymphocyte transformation. It was our conclusion that, contrary to the present authors’ experience, lymphocyte transformation may be useful in the diagnosis of tuberculosis in nonreactive skin test patients. The question arises as to why the difference in our results? We utilized PPD-tuberculin, Tween stabilized and bioequivalent to PPD S without Tween. The antigen supplied to Nash and Douglas from CDC was freeze-dried DT 612, which is not Tween stabilized but is the successor antigen to PPD S. Nash and Douglas used 0.025 micrograms of protein per 0.1 ml, certainly a micromethod, while we used a macromethod (0.1 mg/L) with a heavier concentration of protein. Several of the reports cited in this letter appear to have used larger doses of antigen in their transformation studies.

There is no question that newer methods for in vitro reactivity are available and that lymphocyte transformation may still be tedious. However, I feel that Nash and Douglas’ statement that lymphocyte transformation results correlate well with skin test nonreactivity in tuberculosis patients is misleading and has not been borne out by the literature, our experience or that of other investigators.

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To The Editor:

Dr. Reichman’s letter commenting on our recent publication raises some questions concerning the bioequivalency of the skin test PPD preparation used in the study and an apparent discrepancy between our interpretation and that of other investigators regarding the results of in vitro PPD stimulation of peripheral blood lymphocytes (PBLs).

Dr. Reichman’s remarks on the need to use a standard PPD preparation which is “bioequivalent to PPD-S free of Tween” is certainly justified. The PPD preparation used in our studies (Tubersol, Connaught Laboratories) is indeed bioequivalent to PPD-S in phosphate buffer free of Tween-80 and the product information insert describes, in detail, the standardization tests applied to each lot prior to release.

It was not our intention to suggest that PBLs from patients with negative skin reactivity always demonstrate negative in vitro reactions. There is, however, a strong correlation between in vitro and in vivo reactivity and a highly significant difference in the in vitro responsiveness of PBLs from positive skin test reactors when compared to that observed for PBLs from negative skin test responders.1-4 The papers referred to in Dr. Reichman’s letter4-8 are not unknown to us, but were not included in our discussion because the in vitro tests used (blast cell and mitotic cell counts) are different from the assay technique we used (uptake of tritiated thymidine), and the generally low numbers of individuals tested were considered to be subject to sampling error. It remains clear, however, that these authors also noted a correlation between PPD skin test reactivity and in vitro stimulation with PPD. All the papers referred to did find that a negative or weak skin test response was generally associated with a weak in vitro response. Thus, subjects with negative or questionable skin tests (< 10 mm) demonstrate a suppressed in vitro PPD transformation responsiveness.

One interpretation put forward by the authors referred to by Dr. Reichman was the possibility that any response by PBLs, above background levels, could be a significant observation to be used as an adjunct for diagnosis in the patient demonstrating a negative skin test response. Our experience using thymidine uptake as a measure of activity indicates that although an occasional skin test negative patient reveals strong in vitro activity, the increased cost and time necessary to do the test does not warrant its routine use in the clinical laboratory. It is possible, however, that within individual laboratories, an extensive evaluation using large numbers of negative controls (nontuberculous, nonreactive) could eventually extend the diagnostic usefulness of these in vitro techniques. A number of variables must first be considered such as: the culture system, the number of lymphocytes, the antigen concentration (see below), the source of antigen, etc; many of these would have to be standardized within the individual laboratory setting.

A final comment concerning the antigen concentration used in our in vitro studies. The assay system requires a final PPD concentration of 2.0 μg/ml in a total of 2 ml of culture media containing 2.5 × 106 cells. This concentration represents two times the concentration necessary to obtain a maximal re-
sponse by PBLs in 90 percent of a group of positive skin test reactors free of active disease. Increasing the concentration increases the risks of in vitro stimulation in the same way that increasing the skin test dose increases the number of positive reactors, i.e., the number of false positives also increase. Although the antigen we used for the in vitro studies differed from the skin test antigen, all tests were run simultaneously with positive and negative controls. The correlation between results obtained by in vitro reactivity and in vitro reactivity remained true throughout the course of the study.

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Tricuspid Valve Fluttering

To the Editor:

In "Tricuspid Valve Fluttering: Echocardiographic Features of Ventricular Septal Defect" (Chest 1980:77; 517-520) Alam et al reported four patients with ventricular septal defect with and without associated cardiac abnormalities who showed systolic fluttering of the tricuspid valve on echocardiogram and concluded that "the presence of fuzzy fluttering systolic echoes of the tricuspid valve is suggestive of a membranous ventricular septal defect. . . ."

We have recently made a similar observation. An eight-month-old girl with ventricular septal defect was referred to the echocardiographic laboratory. The diagnosis was based on clinical grounds and cardiac catheterization was not indicated at this stage. There was no evidence of tricuspid incompetence or ruptured sinus of Valsalva aneurysm. The echocardiogram showed hyperdynamic left ventricle and systolic fluttering of the tricuspid valve (Fig 1).

Reviewing the literature we found it difficult to agree with statements by Alam et al. Systolic fluttering of the tricuspid valve was first described by Nanda et al in 1974 in two patients with congenital left ventricular-right atrial communication through a defect involving the tricuspid valve. A year later they published a paper in which they stated that "this tricuspid valve fluttering may be caused by the passage of the left ventricular jet of blood into the right atrium through a deficiency in the tricuspid valve, the margins of which are deformed by fibrosis and fused to some extent to the membranous septal defect." They also studied 14 cases of isolated ventricular septal defect, as well as other abnormalities and 12 normal children and failed to demonstrate evidence of systolic fluttering of the tricuspid valve. Ventricular septal defects with communication between the left ventricle and the right atrium are rare but should be carefully searched for, particularly from the standpoint of surgery since some of the defects may be repaired through the right atrium without performing ventriculotomy. It is difficult to understand why Alam et al did not discuss this important entity and neglected the initial observations by Nanda et al and one wonders whether they missed this clinically difficult diagnosis.

Secondly, these authors stated in their introduction, as well as in discussion, that similar systolic fluttering and anterior motion of the tricuspid valve were reported in patients with ventricular septal aneurysms associated with ventricular septal defects, disputed this report and proposed "a different mechanism for this finding." However, they appear to have missed the point of the important paper by Snider and colleagues who did not discuss anything about systolic fluttering of the tricuspid valve. They discussed "coarse fluttering echoes with abrupt systolic anterior motion anterior to the tricuspid valve echo. . . . most easily separated from the tricuspid valve in the region of the right ventricular outflow tract" (see their Fig 3 which shows coarse systolic fluttering of the echoes anterior to the tricuspid valve but no fluttering of the tricuspid valve). Systolic fluttering of the tricuspid valve might indeed be seen in some patients with ventricular septal aneurysm associated with ventricular septal defects; or fluttering echoes from the ventricular septal aneurysm might, at times, superimpose on the tricuspid valve echo, depending on site and size of the aneurysm and associated tricuspid valve anomaly. Confirmation must await further extensive