sors which render it refractory to normal inductive stimuli. On the other hand, the duplicating genome itself may be unable to simultaneously participate in other functions.

The data of these experiments indicate that cells preparing to divide are less able to respond to an hormonal stimulus than are nondividing cells. Therefore, the failure or diminution of enzyme induction by steroids in hepatomas is not a specific property of neoplastic cells but may be common to dividing cells.

Summary. The induction of tryptophan pyrrolase by hydrocortisone has been studied in the regenerating rat liver. An initial depression of response, presumably related to the operative procedure, was observed in both sham operated and partially hepatectomized animals. In partially hepatectomized animals, a second depression was observed during that period in which DNA synthesis has been shown to occur. It has been suggested that alterations in enzyme control mechanisms, such as the failure of tryptophan pyrrolase induction by cortisone in minimum deviation hepatomas, may be a fundamental biochemical alteration in tumors. This study shows that a depressed response to hormonal enzyme induction is found in non-neoplastic hepatic cells preparing to divide.

1. Morris, H. P., J. Nat. Cancer Inst., 1955, Suppl. 15, 1535.

2. Potter, V. R., In Cellular Control Mechanisms in Cancer, P. Emmelot, O. Muhlbock, eds., Elsevier. 1964.

3. Pitot, H. C., Morris, H. P., Cancer Res., 1961, v21, 1009.

4. Knox, W. E., Auerbach, V. H., J. Biol. Chem., 1955, v214, 307.

5. Greengard, O., Smith, M. A., Acs, G., ibid., 1963, v238, 1548.

6. Dyer, H. M., Gullino, P., Morris, H. P., Cancer Res., 1964, v24, 97.

7. Pitot; H. C., Cho, Y. S., Prog. Exp. Tumor Res., 1965, v7, 158.

8. Bucher, N. L. R., Intern. Rev. Cytol., 1963, v15, 245.

9. Goldstein, L., Knox, W. E., Ann. N. Y. Acad. Sci., 1963, v111, 233.

10. Greengard, O., Feigelson, P., ibid., 1963, v111, 227.

11. Becker, F. F., Am. J. Path., 1963, v43, 497.

12. Higgins, G. M., Anderson, R. M., Arch. Path., 1931, v12, 186.

13. Tsukada, K., Lieberman, I., J. Biol. Chem., 1964, v239, 1564.

14. Grisham, J. W., Cancer Res., 1962, v22, 842.

15. Garren, L. D., Howell, R. R., Tomkins, G. M., Crocco, R. M., Proc. Nat. Acad. Sci., 1964, v52, 1121.

16. Salb, J. M., Marcus, P. I., ibid., 1965, v54, 1353.

Received June 29, 1966. P.S.E.B.M., 1966, v123.

In vitro Suppression of the Lymphocyte Response to Tuberculin by Live Measles Virus.* (31465)

ELIZABETH M. SMITHWICK AND SUMNER BERKOVICH†
(Introduced by R. Janet Watson)
Department of Pediatrics, State University of New York, Downstate Medical Center, Brooklyn

It is known that peripheral blood lymphocytes from a tuberculin sensitive individual when exposed to PPD in tissue culture will react by transforming into blastoid cells and by an increased mitotic rate(1). Recent studies have shown that measles-infected tuberculin positive children not only develop a depressed tuberculin skin reaction(2) but also manifest a diminished *in vitro* lymphocyte response to tuberculin(3). Thus, it appeared that in the infected children the measles virus exerted a direct effect on the ability of the peripheral lymphocyte to respond to PPD. To test this hypothesis, both live measles virus, which will multiply in suspensions of human leukocytes(4), and PPD were added to lymphocyte cultures obtained from children

* Supported by Am. Thoracic Soc. and by Health Research Council of City of New York, contract U-1151.

† Career Scientist of Health Research Council of City of New York, contract I-173.

TABLE I. Lymphocyte Response in Six Tuberculous Children.

Cell donor	Total reactive cells, % PPD	Medium and PPD	Measles and PPD	ECHO 9 and PPD
Measles-immune*				
J.M., 12 yr	18.9	11.5	7.0	14.1§
Ja.M., 9 mo‖	14.9	8.9	2.3‡	—
C.B., 2 yr	8.7	4.6	1.7	7.6
Measles-susceptible†				
U.C., 6 mo	12.0	9.0	2.4‡	—
A.R., 2 yr	10.1	4.2	3.5‡	5.4§
R.B., 8 mo	5.3	9.6	2.6	11.3
Mean	11.7	8.0	3.3	9.6

* With measles hemagglutination inhibiting (HI) antibodies.
† Without HI antibodies at lowest dilution tested (1:2).
‡ Ten- to 100-fold increase in measles virus titer after 5 days in culture.
§ Ten- to 1000-fold decrease in ECHO 9 titer after 5 days in culture.
‖ Simultaneous cultures were also done using PHA in the test system with the following results: 1) PHA alone: 68.6%; 2) Medium and PHA: 67.2%; 3) Measles and PHA: 69.2%.

with tuberculosis. A marked diminution was demonstrated in the reactivity of these cells to PPD.

Materials and methods. Peripheral leukocytes from 6 tuberculous children were cultured according to the method described by Bach and Hirschhorn(5). Each culture tube contained 2.5×10^6 cells (approximately 80% lymphocytes) in 3.5 ml of Eagle Minimum Essential Medium (MEM) supplemented with fetal calf serum, l-glutamine and antibiotics. For each child, a series of cultures was set up containing the following: (1) no additive: to serve as control for spontaneous transformation; (2) tuberculin-PPD (Merck Sharp & Dohme), 2.5 μg (125 TU): to determine degree of cellular reactivity; (3) measles virus, Edmonston strain (Enders), prepared in human embryonic renal cell cultures, 0.1 ml containing 10^{-1} $TCID_{50}$: to detect any transformation due to measles virus; (4) maintenance medium (MEM with 2% fetal calf serum) from uninfected human embryonic renal cell cultures, 0.1 ml: to determine whether response was related to the diluent rather than to the virus; (5) measles virus and PPD: to demonstrate a stimulating or depressing effect of the live virus on the response to PPD; and (6) renal cell maintenance medium and PPD: to detect an enhancing or inhibiting action of the medium alone on the PPD response. As a further control, tubes containing prototype ECHO 9 virus (10^{-6} $TCID_{50}$/0.1 ml) alone and with PPD were included in several of the experiments; this virus will also propagate in human leukocyte cultures(6). In addition, the effect of phytohemagglutin (PHA Difco) and PHA plus measles virus was tested in one set of cultures.

Culture tubes, except those containing PHA, were incubated at 37°C for 5 days; PHA cultures were incubated for 3 days. Aliquots were then removed from several virus-inoculated cultures for viral assay. Vinblastine sulfate (Velban, Eli Lilly & Co.) was now added to the cultures, and in 2 to 4 hours the cells were fixed, stained with 1% aceto-orcein, and examined under oil immersion using a light microscope. At least 1000 mononuclear leukocytes were examined by 2 experienced observers and the percentage of transformed and mitotic cells was determined. Final results are expressed as per cent total reactive cells (%TRC). This value is obtained by subtracting the percentage of reactive cells in the appropriate control culture from the corresponding percentage in the test preparation.

Results. The outcome of lymphocyte studies done on 6 PPD positive children with active tuberculosis is summarized in Table I. The mean lymphocyte response to PPD was 11.7% as compared to a mean value of —1.9% in 20 PPD negative children previously tested(3). When either renal cell maintenance medium or ECHO 9 virus was added to the PPD cultures, the mean per cent of total reactive cells decreased; yet the differ-

ence between each of these means and the PPD mean was not significant. However, in the presence of measles virus, the mean lymphocyte response to PPD was markedly reduced, 11.7% to 3.3%. The difference between these two means is significant at the 0.01 level. The inhibiting effect of the measles virus was similar in all the cultures even though 3 of the children studied were immune to measles. In one child tested in this series, the percentage of cells stimulated by PHA was 68.6% which is in our usual range (55-86%). Thus, the action of PHA on the lymphocytes was not influenced by the presence of either renal cell maintenance medium (67.2%) or measles virus (69.2%).

Discussion. Although many viral agents may affect tuberculin skin sensitivity, only measles does so with great regularity. Studies recently completed(3) indicate that, in addition to changes in skin reactivity, the *in vitro* lymphocyte response to PPD is also depressed during measles. Both factors return to pre-infection levels within 1 to 3 weeks. To date, the mechanism responsible for these changes during measles has not been delineated.

Delayed sensitivity of the tuberculin type is probably mediated by the peripheral lymphocyte; this sensitivity can be transferred from the reactive to the non-reactive individual by intact human peripheral white blood cells or by dialysates from such cells(7). Since the measles virus has been recovered from leukocyte fractions obtained from measles patients during the first 2 days of the exanthem (8), it is possible that the changes in delayed sensitivity during measles are due to a direct effect of this virus on the lymphocytes. Results from the present study support this hypothesis. The titer of measles virus, a highly labile agent, recovered after 5 days from aliquots of lymphocytes tested was 10 to 100 times higher than measured in the original inoculum. It is obvious that the measles virus had multiplied in the cultures. The number of PPD stimulated cells was significantly reduced only in the suspensions inoculated with measles virus. By contrast, lymphocytes in cultures inoculated with ECHO 9 virus responded normally to PPD; however, this agent probably had failed to multiply during the observation period. Of further interest is the finding that measles virus *in vitro* did not interfere with the stimulating action of PHA; nor have we found a suppression of the lymphocyte response to PHA in measles-induced tuberculin negative children.

Very few data are available concerning the effect of the measles virus on lymphocyte function; yet, it would be of great importance to know precisely how this virus alters the lymphocyte response to PPD. It has been reported(9) that the measles virus induces chromosomal abnormalities in human leukocytes *in vivo* and *in vitro*. Thus, it is probable that this RNA virus interferes with cell processes vital for the expression of delayed hypersensitivity. Persistence of the lymphocyte response to PHA when the reactivity to PPD has been suppressed suggests that these two substances stimulate different mechanisms in the cells. However, additional data are needed to confirm this postulate.

Summary. The addition of live measles virus to PPD sensitive lymphocytes has been shown to reduce significantly the mean response of these cells to PPD. The stimulatory effect of PHA in a similar system was not influenced by the measles virus.

We are indebted to Mrs. J. D. Quick for technical assistance and to Dr. S. Fikrig for helpful criticism.

1. Pearmain, G., Lycette, R. R., Fitzgerald, P. H., Lancet, 1963, v1, 637.

2. Starr, S., Berkovich, S., New Eng. J. Med., 1964, v270, 386.

3. Smithwick, E. M., Abstract, Soc. for Ped. Res., Atlantic City, N. J., 1966, p152.

4. Berg, R. B., Rosenthal, M. S., Proc. Soc. Exp. Biol. and Med., 1961, v106, 581.

5. Bach, F., Hirschhorn, K., Science, 1964, v143, 813.

6. Berg, R. B., Proc. Soc. Exp. Biol. and Med., 1961, v108, 742.

7. Lawrence, H. S., Al-Askari, D. J., Franklin, E. C., Zweiman, B., Trans. Assn. Am. Phys., 1963, v76, 84.

8. Gresser, I., Chany, C., Proc. Soc. Exp. Biol. and Med., 1963, v113, 695.

9. Nichols, W. W., Am. J. Human Genet., 1966, v18, 81.

Received July 1, 1966. P.S.E.B.M., 1966, v123.