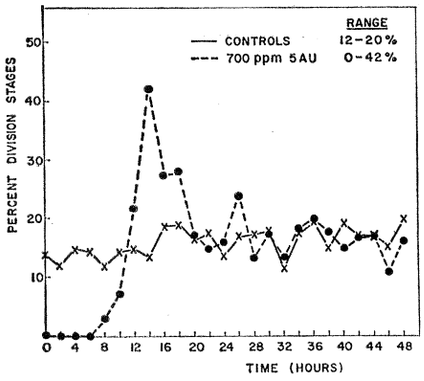


Fig. 1. Frequency of division stages in nuclei synchronized with 5-aminouracil compared with frequency in untreated controls.

1) roots were incubated with 1.5 $\mu$c of tritiated thymidine per milliliter. Secondary root tips were fixed in ethanol–acetic acid (3:1) either immediately after labeling or 14 hours after 5AU treatment, embedded in paraffin, and sectioned at 5 microns. Slides of mid-longitudinal sections were dipped in liquid nuclear track emulsion (NTB), exposed for 12 days, developed, and stained with Delafield's hematoxylin. Autoradiographic grain counts were made over nuclei in interphase and mitotic stages in the meristem.

The results of the synchronization studies with 5AU, graphed in Fig. 1, show the average percentage of nuclei in division stages. Points on the graph represent samples of three to nine root tips collected at 2-hour intervals over a 48-hour period. In the control curve, dividing stages constitute from 12 to 20 percent of the total nuclei counted. In the cells treated with 5AU no divisions were observed for 6 hours after termination of the treatment. The frequency then rose steeply to an average peak of 42 percent at 14 hours after treatment, declined steeply, and reached control levels in 20 to 22 hours. The smaller peak at 26 hours may be an artifact or it may be due to unknown events. Clearly, little synchronization is maintained through a second cycle because 20 to 22 hours after the first peak there is virtually no increase in the percentage of dividing cells above control levels.

Among the six root tips that contributed to the average peak value at 14 hours, the maximum frequency of dividing nuclei was 62.5 percent. This corresponds to the highest percentage obtained in the $H^3$-thymidine experiments (Table 1) and may represent a maximum synchrony achievable in this system without further improvements in technique (*1*).

The data in Table 1 are from two experiments in which root tips were treated with $H^3$-thymidine after removal from 5AU. In experiment A, a treated lot was placed in $H^3$-thymidine for 1 hour immediately after removal from 5AU. In experiment B, one lot of roots (B-1) was labeled from 1.5 to 3 hours after removal from 5AU, and the other lot (B–2) was labeled from 3 to 4.5 hours. Recovery of mitotic activity appeared to be somewhat more rapid in the experiments listed in Table 1 than in the experiments shown in Fig. 1, probably because the added thymidine overcame the effect of 5AU, but in general the two groups of data are similar in showing that a high frequency of division stages is reached 14 hours after removal from 5AU.

More grains were counted over nuclei labeled 3 to 4.5 hours after treatment with 5AU (B-2) than in nuclei labeled 1.5 to 3 hours afterward (B-1). This finding indicates progressive recovery of DNA synthetic rate following release from inhibiting effects of the pyrimidine analog. The result is not unexpected since 5AU interferes with thymine synthesis and folic acid metabolism in *Allium cepa* (*2*). The $H^3$ incorporated into interphase S-stage nuclei, as shown in roots fixed immediately after labeling, was observed later (Table 1, 14-hour fixation) in both dividing and interphase nuclei (*3*).

Table 1. Results from labeling *Vicia faba* root tip cells with tritiated thymidine ($H^3$-thy) after a 24-hour treatment with 5-aminouracil (5AU).

| Time (hr) | | | Fixed immediately after $H^3$-thymidine labeling | | | Fixed 14 hours after 5AU treatment | | |
|---|---|---|---|---|---|---|---|---|
| 5AU to $H^3$-thy | In $H^3$-thy | Stage | Nuclei (%) | Nuclei labeled (%) | Grains per nucleus (No.) | Nuclei (%) | Nuclei labeled (%) | Grains per nucleus (No.) |
| | | | *Experiment A* | | | | | |
| 0 | 1.0 | Interphase | 99.8 | 13.8 | 3.6 | 37.5 | 10.3 | 7.3 |
| | | Division | 0.2 | 0.0 | 0.0 | 62.5 | 28.5 | 5.3 |
| | | | *Experiment B-1* | | | | | |
| 1.5 | 1.5 | Interphase | 96.2 | 36.6 | 5.6 | 48.8 | 36.4 | 7.1 |
| | | Division | 3.8 | 0.0 | 0.0 | 51.2 | 57.6 | 7.0 |
| | | | *Experiment B-2* | | | | | |
| 3.0 | 1.5 | Interphase | 92.5 | 51.0 | 14.2 | 44.1 | 31.8 | 15.9 |
| | | Division | 7.5 | 0.0 | 0.0 | 55.9 | 46.5 | 12.2 |

HAROLD H. SMITH
CATHARINE P. FUSSELL
BERTHA H. KUGELMAN
*Department of Biology, Brookhaven National Laboratory, Upton, New York*

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## Reaction of Lymphocytes with Purified Protein Derivative Conjugated with Fluorescein

Abstract. *Buffy coats from the peripheral bloods of persons known to give a positive reaction to purified protein derivative and of those known to be negative were treated with purified protein derivative conjugated with fluorescein isothiocyanate. Lymphocytes from positive reactors took up the labeled antigen in significantly greater numbers than did those from negative reactors. Leukocytes from newborns showed far less affinity for the antigen than leukocytes from adult negative reactors.*

Passive transfer of tuberculin skin hypersensitivity may be accomplished by transfusing leukocytes derived from spleen, peritoneal exudate, peripheral blood, and lymph nodes from tuberculin reactive animals to nonreactive animals (*1*). Transfusion of plasma was found not to be effective in conferring hypersensitivity upon recipients. Moreover, leukocyte extracts, as well as the intact cells, are capable of transferring hypersensitivity (*2*).

Lymphocytes from guinea pigs sensitized to tuberculin take up $I^{131}$-labeled purified protein derivative (PPD) to a somewhat greater extent than do lymphocytes from nonsensitized animals (*3*), but it could not be shown that cells from sensitized animals are concentrated, after passive transfer at the site of PPD injection (*4*). The uptake in vitro of PPD labeled with $I^{125}$ by some of the cells teased from thymus, spleen, and lymph nodes of tuberculin-

sensitive guinea pigs has recently been demonstrated (*5*). Our studies represent an attempt to determine whether lymphocytes from tuberculin-reactive human beings take up PPD antigen conjugated with fluorescein.

Purified protein derivative (*6*) of tuberculin derived from the Corper strain of *Mycobacterium tuberculosis* was conjugated with fluorescein isothiocyanate (*7*) in a ratio of 20 mg PPD to 1 mg of the fluorescein in a total volume of 10 ml carbonate-bicarbonate buffer at *p*H 9.5. The conjugate was dialyzed against phosphate-buffered saline (*p*H 7.4) at 4°C for 14 days. After dialysis the conjugate was transferred to small sterile tubes in 1 ml volumes, and stored at −20°C until used.

Buffy coats were obtained from 16 persons from the hospital patients and staff, demonstrated to be positive on intermediate-strength skin testing, and 13 student technologists demonstrated to be negative by the Tine test. In addition the buffy coats from cord blood (*8*) of eleven newborns were studied.

The buffy coat obtained from 10 ml of venous blood treated with EDTA to prevent coagulation was separated by two centrifugations. Equal volumes of tagged antigen and buffy coat were mixed in a sterile test tube, incubated at 37°C for 30 minutes, washed twice in plasma and resuspended in plasma. Preliminary studies showed that cells washed in saline exhibit more intense autofluorescence than cells washed and suspended in plasma, perhaps because of a quenching effect of substances occurring in plasma. Plasma washing only, therefore, was used. A small drop of each suspension was placed on a slide and covered with a thin cover slip which was then sealed to the slide with Duco cement.

A buffy coat from each subject was examined untreated, except for incubation, as an autofluorescence control. Blocking preparations were made by incubating buffy coats with unconjugated PPD, washing and reincubating with the fluorescein-conjugated PPD. Specificity preparations consisting of buffy coats from PPD reactors previously shown to concentrate the conjugate were treated with fluorescein-conjugated bovine albumin.

By means of a microscope, equipped with visible wave length and ultraviolet light sources (OSram HBO 200), with a darkfield condenser the preparation was scanned for mature lymphocytes under oil immersion at 1000-fold magnification. Each lymphocyte found under visible light was examined under ultraviolet illumination and the presence or absence of apple-green fluorescence in the cytoplasm noted. The percentage of such "positive" cells was recorded. Some of these cells were not intensely bright, while others showed intense apple-green fluorescence.

Table 1. Percentage uptake of PPD, conjugated with fluorescein isothiocyanate, by lymphocytes from PPD positive and PPD negative reactors. In each case 200 lymphocytes were counted.

| Positive skin reactors | | Negative skin reactors | | Cord blood | |
|---|---|---|---|---|---|
| Subject | Positive*† (%) | Subject | Positive *‡ (%) | Subject | Positive* (%) |
| FJ | 0.0 | WI | 0.4 | 1 | 0 |
| OS | 4.0 | ST | 0.4 | 2 | 0 |
| HO | 8.8 | MS | 0.0 | 3 | 0 |
| AG | 4.4 | DD | 2.0 | 4 | 0 |
| JA | 6.0 | ES | 0.4 | 5 | 0 |
| JS | 6.5 | JS | 0.0 | 6 | 0 |
| WJ | 5.5 | EA | 0.5 | 7 | 0 |
| AS | 4.0 | CH | 0.5 | 8 | 0 |
| BM | 3.6 | JK | 0.5 | 9 | 0 |
| LH | 4.5 | SH | 0.0 | 10 | 0.5 |
| VA | 4.5 | SV | 2.5 | 11 | 0.5 |
| JC | 2.5 | MK | 0.0 | | |
| OA | 2.0 | CL | 1.3 | | |
| BL | 1.5 | | | | |
| LW | 4.0 | | | | |
| OC | 4.0 | | | | |

*$P = .001$. †Mean $4.11 \pm 2.08$. ‡Mean $0.65 \pm 0.79$.

Autofluorescence was commonly found in buffy coats whether or not treated with fluorescein-conjugated antigen. The granules of polymorphonuclear neutrophils are the brightest objects in an unstained preparation. Autofluorescence is manifested as pale, soft, ice-blue luminescence which contrasts sharply with the hard yellowish-green color, characteristic of fluorescein.

Table 1 shows the results of lymphocyte counts of buffy coats from 16 individuals having positive tuberculin skin reactions, 13 subjects having negative skin reactions, and from cord bloods of 11 newborn infants. The PPD positive reactors showed an average of 4.11 percent positive lymphocytes, while the negative reactors showed only 0.65 percent, nearly a seven-fold difference between the means. The values for the two adult groups differ from each other, $p \leq 0.001$. Two positive lymphocytes occurred in 2200 lymphocytes counted from cord blood.

In the specificity studies, with conjugated bovine albumin, ten subjects were used. Fifteen hundred lymphocytes were counted and three (0.2 percent) were recorded as positive.

Conjugation of antigens with fluorescein has not been extensively used. In discussing the possibility of locating antibodies by such a method, Coons *et al.* (*9*) have suggested that the chemical diversity of antigens is limiting and the larger number of immunologically reactive groups in an antigen compared to those in an antibody molecule would probably make the detection of antibody less sensitive than the detection of antigen.

The relatively low luminescence in the "positive" cells encountered in this study is in keeping with the above prediction. However, that lymphocytes containing the conjugated antigen are easily identified and easily distinguished from lymphocytes showing only pale-blue autofluorescence seems worth emphasizing. We have no explanation to offer for the failure of the cells of patient F. J. (active tuberculosis) to take up the antigen. Repeated examinations of his buffy coat failed to show positive cells. In this connection we note that Pearinan, Lycette, and Fitzgerald (*10*), studying the mitogenic activity of PPD—an adaptation of the phytohemagglutinin transformation (*11*)—on leukocyte cultures from Mantoux-positive patients, observed absence of this activity on the cells from two patients with active tuberculosis. The individuals D. D. and S. V., listed as negative reactors, showed 2.0 and 2.5 percent positive cells. They were skin tested only with the Tine test and were not available for second-strength testing. It is possible they would have been second-strength positive. The statistical difference in the frequency of such cells derived from PPD reactors from their frequency in PPD nonreactors seems to lend credence to the notion that these cells may be the ones participating in the skin reaction.

The relatively low proportion of "positive" cells to the total number of circulating lymphocytes is consistent with the clonal selection theory enunciated by Burnet (*12*). That these cells become concentrated in the cellular exudate from the skin reaction is suggested by the finding ( preliminary unpublished observations) that the inflammatory lesion produced in tuberculin-positive rabbits by injecting the conjugated PPD contained a high proportion of fluorescent leukocytes.

Polymorphonuclear neutrophils took up the antigen nonspecifically in most cases. However, there was obvious variation in the numbers of neutrophils engulfing the antigen and in the brightness of polymorphonuclear cells under

ultraviolet light. No counts were made, but we have the impression that neutrophils from positive skin reactors had a greater affinity for the antigen than the neutrophils from negative reactors (*5*).

Neither neutrophils nor lymphocytes from cord blood showed affinity for the antigen. This suggests the possibility that the polymorphonuclear neutrophil, as well as the lymphocyte, may in some way take part in the response to tuberculin skin testing.

T. A. WITTEN
W. L. WANG
M. KILLIAN
*Veterans Administration Hospital, Denver 20, Colorado*

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## Bilateral Differences in the Human Occipital Electroencephalogram with Unilateral Photic Driving

Abstract. *Differential activity was induced in the electroencephalograms of the occipital lobes by limiting intermittent photic stimulation to the right or left halves of the two retinas. The results indicate that the driving of one hemisphere also affects the opposite hemisphere and that the amount and pattern of the effect is determined by the hemisphere being directly stimulated.*

Lindsley (*1*) was the first to suggest an independence of the alpha activity of the two hemispheres. Regions of synchronous, in phase, activity in the resting state were interpreted as reflecting a focus of alpha activity. Lindsley found eight such foci, two in each occipital area, one in each parietal area, and one in each temporal area of the two hemispheres. Because he found differences in the frequencies of the foci, the independent variation of their patterns and magnitudes, and blocking in one region and not in others, Lindsley concluded that the origins of the alpha waves were independent in these bilaterally homologous areas of the brain.

Sperry (*2*) and Myers (*3*) have been more directly concerned with independent functioning of the two hemispheres. Myers found that animals with optic chiasms sectioned in the midline performed discriminations learned with one eye equally well with input to the other eye. In other words, with a lateralized input, the information or engram is presumably laid down in both hemispheres of the animal if the corpus callosum is intact. Thus, the higher functions such as learning and memory do not occur in one hemisphere independently of the other.

The independent action of Lindsley was based upon observations of the human electroencephalogram (EEG) in the passive normal resting state. The dependent action of the two hemispheres found by Myers and Sperry was based on operated animals actively engaged in learning and transfer. Although the two studies are not directly comparable, they do suggest an interesting set of questions. Can differential activity be stimulus-induced in the two hemispheres of the intact human by driving the electrical activity of one hemisphere with intermittent light while the other hemisphere remains unstimulated? If this differential electrical activity is achieved, do both hemispheres show stimulus following or does only the hemisphere stimulated? If, as inferred from the work of Lindsley and Myers and Sperry, active hemispheres act dependently upon each other, while passive resting hemispheres are independent of each other, what would happen to the independence of the resting hemisphere if the other were made active through the use of photic stimulation?

In an investigation of these questions, subjects were exposed to a stroboscopic light (*4*), flashing at a rate of 8 flashes per second through the translucent portion of a pair of goggles, specially designed to achieve differential stimulation. The goggles were made out of halves of table-tennis balls, cut to fit snuggly over each eye socket. They were nearly equidistant from the pupil at all points. Slightly more than half of each eyepiece was painted inside and out with a flat black paint. Thus, either the temporal or the nasal hemiretinas could be shielded from light exposure, depending upon where the painted side was placed. A small fixation hole was drilled 4 mm inside the opaque portion and a "grain-of-wheat" light bulb was cemented over the hole so that the subject would have a constant fixation point. The outside of the bulb was painted black to prevent a field lighting of the translucent portion. With the subject gazing at the fixation point, intermittent photic stimulation through the translucent portion of the goggles fell onto the retina in a wide arc from the peripheral limit of the retina to a point about 19° short of the fovea. Thus, with the translucent portion of the goggles toward the subject's right, the stroboscope persumably stimulates the left hemiretinas and is "reported" on the left occipital lobe.

The EEG's of four subjects were recorded (*4*) from two bipolar scalp parieto-occipital pairs of leads. Eye movements were recorded on a third channel in order to monitor fixation. The signals from the three channels were simultaneously recorded on magnetic tape (*4*), from which representative 10-second epochs were selected on the basis of good fixation. The analysis of the taped EEG epochs was by means of a type of baseline crossover system sometimes called the zero-crossing technique. Thus, EEG potentials were analyzed in terms of their wavelength, wave by wave, by means of an electronic tachometer circuit arranged to trigger as the wave crossed the zero voltage point in the positive direction. The height of a zero-crossing readout was directly proportional to the wavelength and therefore inversely proportional to the frequency. A count was subsequently made of the number of waves having a wavelength in a class interval between 7.5 and 8.5 cy/sec, since an 8-flashes-per-second stimulus was the driving frequency.

Three conditions of stimulation were investigated. The unstimulated condition, (I), was a control condition in which the subject was resting with his eyes open, wearing unpainted goggles, and in the presence of a steady, full-field light source. Since the frequencies of the EEG in the two hemispheres may differ within a given subject in the resting state, the difference in the amount of 8-cy/sec waves may then be used as a correction factor for the subsequent driving conditions at that frequency. In