ileum and the amphibian heart) or recently reported to be cholinergic (the protractor muscle of the sea urchin lantern and the longitudinal muscle of the holothurian).

The longitudinal muscle of *Holothuria grisea,* which is extremely sensitive to acetylcholine (*5*), first responded to 0.5 μg of the pedicellariae extract (in a 15-ml bath). The protractor muscle of the lantern of the sea urchin *Echinometra locunter* is less sensitive to acetylcholine (*3*). It first responded to 0.1 mg of the pedicellariae extract (in a 10-ml bath). The protractor muscle behaves in pharmacological tests like the ileum of the guinea pig (*3*), quickly returning to base line after washing out of the drugs. This permitted us to test the linearity of the curve relating response and dose (expressed on a log scale), using four groups of three doses (0.4, 0.8, and 1.6 mg). The statistical treatment of the data produced a regression line given by the equation:

$$Y = 28.75 + 15.26\ (x - 0.93)$$

The ratio of variances ($F = 601.50$) for the regression line was far above the 99.9-percent level of probability. Deviation from regression, however, was a little above the value for experimental error, although the standard error represented less than 10 percent of the mean for all experiments. This action of pedicellariae homogenates on the protractor muscle is blocked by both atropine and Mytolon, as observed for acetylcholine in this long-fibered smooth muscle (*3*).

The heart of the toad *Bufo ictericus* reacts to 0.1 mg of the pedicellariae homogenate by a slowing down of the rhythm. Treatment with 1 mg induces a diastolic block; this can be prevented by previously atropinizing the heart.

In the case of the guinea pig ileum, we first checked to find whether or not the pedicellariae homogenates had a histaminic action. In a 10-ml bath, 1 μg of Benadryl (which has some atropinic action) totally abolished the response to 0.1 μg of histamine and depressed by about 33 percent the action of 0.8 mg of the pedicellariae homogenate. On washing out of the Benadryl, the recovery of sensibility of the gut to the pedicellariae extract was almost immediate. Treatment with 0.005 μg of Neo-antergan (which has practically no atropinic activity) significantly reduced the response of the ileum to 0.1 μg of histamine but had no effect on the response to 0.8 mg of the pedicellariae extract. Treatment with 0.1 μg of atropine completely abolished the response of the ileum to 0.3 mg of the pedicellariae extract, and a gradual recovery of sensibility was observed on washing out of the drug. Treatment with 0.2 μg of Prostigmin or 0.2 μg of eserine about doubled the response of the gut to 0.2 mg of the pedicellariae homogenate.

To test the linearity of the curve relating the dose and the response of the gut in a log scale, three doses of the pedicellariae extract (0.4, 0.8, and 1.6 mg) were added in four groups. After the slope had been estimated ($b = 27.5$), the regression line was given by the equation

$$Y = 34.0 + 27.5\ (x - 0.8)$$

The ratio of variances ($F = 493.56$) for the regression line was far above the 99.9-percent level of probability. Deviation from regression was below the experimental error, and the standard error of the assay represented less than 10 percent of the mean for all determinations.

The acetylcholine-like material contained in the homogenates is dialyzable and partially inactivated by heating. Complete inactivation was obtained by treatment for 1 to 2 hours with $N/4$ NaOH, followed by neutralization with HCl.

Experiments conducted with the help of D. Valente and G. Maugé showed that the pedicellariae homogenates depress blood pressure in the dog and act on the uterus of the rat.

Thus, the results indicate the presence, in the globiferous pedicellariae of *Lytechinus variegatus,* of a material with many of the characteristics of acetylcholine. On the assumption that it is possibly acetylcholine or a nearly related compound, a four-point assay (*6*) for the ileum of the guinea pig was performed in order to determine the ratio of potency between a standard acetylcholine solution and an unknown pedicellariae homogenate. The doses chosen were within the range of those producing small to submaximal contractions. In the assay, taking doses of the standard (s) and the unknown (u) in the ratio 1:4 (0.1 and 0.4 μg of acetylcholine; 0.1 and 0.4 mg of the pedicellariae extract) and randomizing the doses in five groups of four, we obtained results as follows: $M = U/S = 1.21$; confidence limits, 1.32 and 1.12; real ratio of potency, 1.15.

The parallelism, however, was not strict, perhaps because the homogenate used was crude. But even so the method can afford a reliable indication of the ratio of potency (*7*).

E. G. MENDES
L. ABBUD
S. UMIJI
Department of General and Animal Physiology and *Institute of Marine Biology, University of São Paulo, São Paulo, Brazil*

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Erythrocyte Acid Phosphomonoesterase and Glucose-6-Phosphate Dehydrogenase Deficiency in Caucasians

Abstract. *Caucasian patients with erythrocyte glucose-6-phosphate dehydrogenase deficiency also have a deficiency in erythrocyte acid phosphomonoesterase. This acid phosphomonoesterase deficiency is not present in Negroes with the glucose-6-phosphate dehydrogenase deficiency.*

Deficiency of glucose-6-phosphate dehydrogenase (G-6-PD) in erythrocytes is now a widely recognized hereditary defect which occurs in both Negroes and Caucasians. Accumulating clinical evidence suggests that there may be biochemical differences between enzyme-deficient patients in these two racial groups. For example, there is no report of the occurrence of congenital nonspherocytic anemia in Negroes with G-6-PD deficiency although there are several references to this condition in enzyme-deficient Caucasians (*1*). Also, the ingestion of fava beans by enzyme-deficient Negroes has not been found to result in acute intravascular hemolysis as it does in Caucasians with a G-6-PD deficiency (*2*).

In an attempt to demonstrate enzy-

Table 1. Erythrocyte acid phosphomonoesterase (AP), glucose-6-phosphate dehydrogenase (G-6-PD), and reduced glutathione (GSH) in Caucasians and Negroes with deficiency of G-6-PD. One unit of activity is the amount of enzyme that will liberate 1 mg phenol per 100 ml red cells from 0.046*M* solution of disodium phenylphosphate in ½ hour at a *p*H 6.0 at 37°C under conditions of this experiment.

AP units/100 ml packed erythrocytes	G-6-PD units/100 ml packed erythrocytes	GSH mg/100 ml whole blood
Normal (Negroes and Caucasians)		
336.0 to 519.0	180.0 to 300.0	60
	Negroes	
447.0	13.5	35.4
408.0	0.0	41.0
384.0	30.3	
372.0	18.7	36.0
360.0	62.1	
	Caucasians	
210.0	0.0	
242.0	38.4	
282.0	0.0	68.2
294.0	0.0	47.2
318.0	0.0	45.0

matic differences between these two groups, several enzymes including erythrocyte acid phosphomonoesterase were measured in Caucasians and Negroes deficient in G-6-PD, as well as in patients with a variety of other hematologic disorders.

A deficiency of erythrocyte acid phosphomonoesterase accompanies deficiency of G-6-PD in Caucasians but not in Negroes. This appears to be a consistent biochemical difference between the two groups. This is the first demonstration of an erythrocyte acid phosphomonoesterase deficiency. King, Wood, and Delory (*3*) and Valentine *et al.* (*4*) found no such deficiency states in the wide variety of hematologic disorders they studied.

Erythrocyte acid phosphomonoesterase was measured by a modification of the method of King *et al.* (*3*), whereby 0.046*M* disodium phenylphosphate (1 g disodiumphenyl phosphate per 100 ml of water) solution was used instead of the 0.01*M* solution of the original method; the cells were washed three times in cold isotonic saline before assay, and they were incubated with the substrate at a *p*H of 6.0, rather than at the original *p*H of 4.9 because this was found to be closest to the physiological range at which maximum activity could still be demonstrated.

The glucose-6-phosphate dehydrogenase was assayed by a modification (*5*) of the method of Glaser and Brown (*6*). The reduced glutathione content of erythrocytes was measured by the method of Beutler (*7*). Glutathione-stability tests were performed with either the addition of acetylphenylhydrazine or of vitamin K.

The mean value of erythrocyte acid phosphomonoesterase for the normal controls whose ages were 3 months to 82 years was 417.0 ± 45.1 units (range 336.0 to 519.0 units). Within this normal group there were 48 Caucasians and 21 Negroes. The mean values of these two normal population groups were essentially the same; the mean value for the Caucasian group was 417.0 ± 45.6 units (range 342.0 to 519.0 units) and for the Negroes, 417.6 ± 43.8 units (range 336.0 to 498.0 units). There was no difference between the sexes and the values for erythrocyte acid phosphomonoesterase did not increase or decrease with age.

Five Negro males with G-6-PD deficiency showed values for erythrocyte acid phosphomonoesterase within the normal range. The value of the mean for these five patients was 394.2 units (range 360.0 to 447.0 units).

In contrast, the erythrocytes from all five Caucasian males with G-6-PD deficiency showed markedly decreased activity of acid phosphomonoesterase. The values for this group ranged from 210 to 318 units with a mean of 265.2 units. All values were below two standard deviations from the mean of the normal group and in no instance was there an overlap in values between those of the deficient Caucasians and those of the normal group.

There was no such deficiency in patients with any of the other hematologic disorders studied. These included congenital and acquired aplastic anemias, congenital hypoplastic anemia, hereditary spherocytosis, iron-deficiency anemia, folic and iron-deficiency anemia, sickle-cell anemia, and thalassemia major and minor.

The acid phosphomonoesterase appeared to be intracellular in origin. Stroma, prepared by the method of Tishkoff (*8*), neither showed acid phosphomonoesterase activity nor did it appear necessary as an activator for the intracellular enzyme. No difference could be demonstrated in the acid phosphomonoesterase activity of a group of predominantly young erythrocytes from that of a group of older cells.

Sterile erythrocytes incubated for 24 hours at 37°C in the absence of glucose showed a steady fall in enzyme activity. The acid phosphomonoesterase fell from 432.0 units at the start to 120 units at 24 hours. Only a slight fall from 432 units to 414 units occurred in identical preparations of erythrocytes incubated in the presence of glucose (200 mg per 100 ml). Acetylphenylhydrazine also caused a decrease in the activity of the enzyme when it was incubated with washed erythrocytes for a period of 2 hours in the absence of glucose. The addition of glucose prevented this fall.

Simultaneous determinations of erythrocyte acid phosphomonoesterase and reduced glutathione were made at intervals during incubation of normal red cells and varying amounts of vitamin K (0.02 to 0.15 mg/ml). It was not until the reduced glutathione had dropped to approximately 5 mg per 100 ml that the activity of the acid phosphomonoesterase declined. The suggestion that this enzyme required small amounts of reduced glutathione as a cofactor also was supported by demonstrating that the addition of reduced glutathione to the hemolyzate could partially restore the activity of the acid phosphomonoesterase after sterile incubation for 12 hours.

Within the groups deficient in G-6-PD there was no correlation between the values for G-6-PD and the value of the acid phosphomonoesterase (Table 1). Although in the Caucasian group the activity of G-6-PD was lower or even absent there was one Negro patient (the second case in Table 1) who showed a normal erythrocyte acid phosphomonoesterase but no measurable activity of G-6-PD. There also appeared to be no correlation within the deficient groups between the value of the erythrocyte acid phosphomonoesterase and the initial value of reduced glutathione.

This significant difference in the activity of erythrocyte acid phosphomonoesterase between the patients of the two racial groups supports the belief that this G-6-PD deficiency is not identical in the two races.

Angeletti and Gayle (*9*) have recently isolated three distinct acid phosphomonoesterases from normal erythrocytes by column chromatography. Whether our patients with deficiency of acid phosphomonoesterase show an absence of one or more of these fractions or merely a quantitative deficiency of all three fractions is under investigation.

FRANK A. OSKI
NASROLLAH T. SHAHIDI
LOUIS K. DIAMOND
Children's Hospital Medical Center, Harvard Medical School, Boston, Massachusetts

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Cell Guidance by Alterations in Monomolecular Films

Abstract. *Monolayers of stearic and behenic acids were transferred to quartz slides by the Blodgett technique. Troughs of different depths were cut into these multilayers (substrata), and additional monolayers were superimposed. When cultured and fresh embryonic cells were grown on these substrata, the cells were entrapped within troughs whose depths were as small as 60 angstroms. The results demonstrate cellular responses to molecular changes in contact surfaces.*

The classical experiments of Harrison (*1*) were among the earliest demonstrations of cell guidance. He showed that cells in tissue culture would follow the threads of a spider web. Weiss (*2*) found that spindle cells in a blood plasma clot would orient in the direction of the lines of tension of the coagulum. He suggested that the guiding cue consisted of submicroscopic oriented aggregations of fibrin molecules. Since that time Weiss and coworkers (see *3*) have carried out a systematic study of the effects of physical configuration of the contact substratum on the morphology and behavior of cells. He has grouped these phenomena under the conceptual heading "contact guidance."

The research data and analysis presented below resulted from experiments designed to elucidate this phenomenon, especially the role of molecular or submicroscopic mechanisms. The experiments were suggested by my earlier work (*4*) regarding the interactions between tissue culture cells and multimolecular layers of fatty acids, wherein it was found that the rates of cellular attachment and spreading are functions of the number of monolayers underlying the cells. In these earlier experiments, monolayers of barium stearate–stearic acid were transferred by the Blodgett technique (*5*) to quartz slides from a Langmuir trough such that nonpolar methyl groups faced the cell surface. Increments in thickness of these substrata could be made as small as 50 Å. In general, the time necessary for attachment and spreading of cells was increasingly lengthened as the number of subjacent monomolecular layers was increased. Similar results have since been obtained for behenic acid–barium behenate multilayers.

From these results it was predicted that if a random population of cells were grown on substrata of varying thickness a statistically significant sample should migrate to and be entrapped in the nether regions. To test this prediction a predetermined number of monolayers both of stearic and behenic acid was transferred to quartz base slides. Troughs 10 to 100 μ wide were cut into the layers and additional monolayers were superimposed. The resulting substrata had troughs 25 to 1000 Å deep, whose floors were 25 to several thousand angstroms distant from the quartz slide. Single cell suspensions were randomly dispersed on these hydrophobic surfaces. The cells then attached, spread and migrated in a random manner on these surfaces. Figure 1 illustrates the entrapment of these cells in a groove in the form of a cross, roughly 180 Å deep, in a multimolecular film of behenic acid. Cells have been entrapped in similar depressions only 60 Å deep.

This marked sensitivity of cells to alterations in substrata led to some further studies. Dissociated cells were grown for 10 hours in standard culture medium on grooved substrata of barium stearate–stearic acid. The distances to the top and bottom of the troughs or grooves were varied to determine the physical conditions under which alignment similar to that shown in Fig. 1 could be observed. Figure 2 summarizes the results for three cell types, a tissue culture strain of human conjunctiva cells, fresh embryonic chick heart (10 to 11 days) cells, and fresh embryonic chick lung (10 to 11 days) cells. The distance to the top of the trough is plotted as the ordinate, and the distance to the bottom as the abscissa.

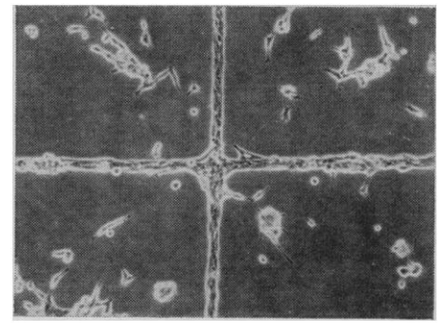

Fig. 1. Alignment of tissue culture cells within a trough cut in multilayers of behenic acid.

When the distance to the top and bottom of the trough satisfied conditions indicated by coordinates to the left of the straight-line graph, cell alignment was observed; otherwise, no entrapment or alignment occurred. As shown in Fig. 2, the depth of the trough must be increased as the height of its floor is increased. In addition, the results are suggestive of differential sensitivities of cells of different origins.

Under conditions favorable for the differential response of cells, the trough serves as a trap. Cells adhere and spread more rapidly within the trough and accommodate to its dimensions. In narrow troughs, spreading is polarized and individual cells demarcate the region by lengthwise alignment and

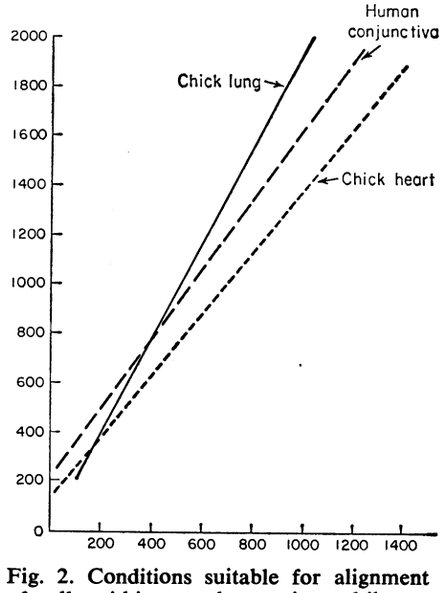

Fig. 2. Conditions suitable for alignment of cells within troughs cut in multilayers of stearic acid. *Abscissa*: Distance in angstroms from surface of slide to bottom of trough. *Ordinate*: Distance in angstroms from surface of slide to top of trough.