beginning is evident at the earlier stage. They occupy positions where, presumably, sand flats had built up. Growth appears to have resulted from the establishment on these flats of islands of intertidal marsh which subsequently become continuous, a process which can be seen to be taking place in the presently existing intertidal islands. The development of these tongues resulted in the separation of the open water into broad sounds which narrowed progressively to define the position of the present major creek systems.

A comparison of Fig. 3, *C–E*, suggests that these sounds narrowed by the spreading of intertidal marsh onto sand flats on which a meandering channel system was already developed. The marsh peat may be expected to have stabilized the meander pattern, which has remained with little alteration, as the peat has built up to the present high marsh level. Confirmation of this supposition is found in relatively young marshes at Provincetown and Wellfleet, Massachusetts, where the creeks at low tide meander between bare sand banks along a "thalweg" formed by relatively straight banks of peat.

At present, high marsh extends to the banks of the present creeks, except in limited local areas. Goldthwait has commented on the stability of the meander patterns of tidal creeks (*11*). A quasi-equilibrium determined by hydraulic forces appears to have been reached between the processes of accretion and erosion. Leopold and Langbein (*12*) and Langbein (*13*) have shown that in a tidal estuary the width, depth, and velocity of flow vary with a power of the mean discharge, Q, such that

Width	$\propto$	Q^b
Mean depth	$\propto$	Q^f
Mean velocity	$\propto$	Q^m

From considerations which include continuity, theoretical relations between velocity, slope, and depth, and the conditions that at equilibrium the total work done in the system be minimal and that energy be dissipated uniformly along the channel, they have deduced theoretical relations between the exponents, b, f, and m, which are shown in Table 1. These values, which define the hydraulic geometry of a tidal stream, agree closely with those obtained by measurements in two tidal estuaries near Alexandria, Virginia. Measurements along Spring Creek, in the Barnstable marsh, yield values which agree equally well with theory.

The reconstructions provide a picture of the ontogeny of the Barnstable marsh which is orderly and plausible. They indicate that the sand spit has grown eastward during a period of about 4000 years. The marsh, which consisted at first of isolated pockets in protected indentations of the upland, became continuous and began to spread into the enclosure from along the upland margin as sediment accumulated in its shallower parts and protection from the sea became more complete. The development of marsh along the margin of the sand spit proceeded more slowly, perhaps because the basin deepened with distance from the upland and more time was required for sedimentation to reduce its depth. The broad sounds between the advancing tongues of marshland became the site of the future creeks, and the meandering channels in the sand which formed their bottom defined the final pattern which these creeks assumed. High marsh has now extended to the margin of these channels and at present the creeks are in quasi-equilibrium with the hydraulic forces which arise from the quantity of water which they must carry in response to the rhythm of the tide.

ALFRED C. REDFIELD
Woods Hole Oceanographic Institution, Woods Hole, Massachusetts

References and Notes

1. N. S. Shaler, *U.S. Geol. Surv. 6th Ann. Rept.*, p. 364 (1886).
2. V. J. Chapman, *Salt Marshes and Salt Deserts of the World* (Interscience, New York, 1960).
3. A. C. Redfield, in *Proceedings of the Salt Marsh Conference 1958* (Marine Institute, University of Georgia, Athens, 1959), pp. 37–39.
4. B. F. Mudge, *Proc. Essex Inst.* **2**, 117 (1858).
5. D. W. Johnson, *The New England-Acadian Shoreline* (Wiley, New York, 1925).
6. J. C. Ayers, *Limnol. Oceanogr.* **4**, 448 (1959).
7. J. B. Woodworth and E. Wigglesworth, *Mem. Mus. Comp. Zool. Harvard* **52**, 1 (1934).
8. A. C. Redfield and M. Rubin, *Proc. Natl. Acad. Sci. U.S.* **48**, 1728 (1962).
9. K. F. Mather, R. P. Goldthwait, L. R. Thiesmeyer, *Bull. Geol. Soc. Amer.* **53**, 1127 (1942).
10. M. Rubin and C. Alexander, *Am. J. Sci. Radiocarbon Suppl.* **2**, 129 (1960).
11. J. W. Goldthwait, *Proc. Geol. Soc. Am.* (for 1936) **73**, 1 (1937).
12. L. B. Leopold and W. B. Langbein, *U.S. Geol. Surv. Profess. Papers* **422-B** (1963).
13. W. B. Langbein, *Bull. Int. Assoc. Sci. Hydrol.* **8**, 84 (1963).
14. This paper was presented at a Conference on Estuaries held at Jekyll Island, Georgia, 31 March–4 April 1964 and will be published as a monograph by the AAAS. The work was supported by the Associates of the Woods Hole Oceanographic Institution and the National Science Foundation (contract GP2042). The assistance of John S. Cobb, E. W. E. Doe, Lincoln Hollister, and Thomas Newbury is gratefully acknowledged.

5 November 1964

Acid Phosphatase-Rich Granules in Human Lymphocytes Induced by Phytohemagglutinin

Abstract. *Human lymphocytes, cultured in the presence of phytohemagglutinin, undergo morphologic transformation and subsequent mitosis. Before mitosis (48 to 72 hours), a sharp increase in acid phosphatase activity occurs in cells stimulated with phytohemagglutinin. Histochemical examination of these cells demonstrates that innumerable granules containing acid phosphatase develop in the cytoplasm before mitosis. It is possible that enzymes present in granules which stain for acid phosphatase activity (lysosome-like) may play a role in phytohemagglutinin-stimulated cell division.*

Human lymphocytes, obtained from either peripheral blood or thoracic duct lymph, can be cultured in vitro. When phytohemagglutinin (PHA), an extract from the kidney bean *Phaseolus vulgaris*, is added to the cultures, such cells clump and promptly begin the synthesis of RNA (*1*) and protein, a variable proportion of which is gamma globulin (*2*). After approximately 36 hours in culture, these lymphocytes begin to synthesize DNA (*1*); after 48 to 72 hours in culture they undergo mitosis (*3*). However, before cell division, 80 to 95 percent of the cells have already enlarged (*2*), becoming basophilic and pyroninophilic (*4*). Since a perinuclear clear zone (Golgi apparatus?) and acidophilic granules were observed in over 50 percent of these cells by means of conventional staining procedures and phase contrast microscopy, it appeared possible that new lysosome-like structures might have been formed. Novikoff (*5*) had previously suggested that lysosomes, organelles containing acid hydrolases such as acid phosphatase, may take their origin from the small vesicular bodies of the Golgi apparatus.

Cultures of lymphocytes, some of which were stimulated with PHA, were grown as previously described (*6*), but the lymphocytes were harvested before the addition of a mitotic arresting agent and were resuspended in calf serum, smeared on glass slides, and dried by air. They were stained for acid phosphatase by a modification of the Gomori method (*7*), incubation times of 4 to 8 hours being used. Over 90 percent of the cells from stimulated cultures (including all the cells within aggregates) showed abundant cytoplasmic granules containing acid phosphatase (Fig. 1*A*).

These granules could be demonstrated as early as 12 hours after the addition of PHA to the culture. They were occasionally concentrated in the perinuclear area. We have observed similar granules containing acid phosphatase in lymphocytes obtained from tuberculin-sensitive individuals and cultured in the presence of tuberculin, but only in the smaller percentage of cells (10 to 20 percent) transformed in the presence of the challenging antigen (*8*). In contrast, non-stimulated cultures showed no such granules (Fig. 1*B*) except for an occasional cell with two to three granules containing acid phosphatase.

Cells and their nutrient media were assayed to determine whether a net increase in the activity of acid phosphatase or of another lysosomal enzyme, β-glucuronidase, had been provoked. Lymphocytes from the thoracic duct and peripheral blood were washed and cultured as described previously both with and without PHA. The cultured cells were centrifuged, washed in 0.25*M* sucrose, and resuspended in 0.34*M* sucrose to a final cell concentration of 2 million cells per milliliter. They were subsequently frozen and thawed six times in a mixture of dry ice and acetone. The activities of acid phosphatase (*9*), β-glucuronidase (*10*), and the protein content (*11*) were determined; incubation with appropriate substrates was for 3 to 7 hours. The enzyme activity of the medium in which the cells had been cultured was also determined.

The results of representative experiments are listed in Table 1. Expressed as the activity per initial cell inoculum, acid phosphatase in PHA-stimulated cultures increased by 120 percent to 615 percent over that in control cultures. Enzyme activity was expressed as a function of initial cell count since clumping of cells through the action of PHA introduces inaccuracies in cell counts after culture. In experiments where cell counts on PHA-stimulated cultures were done, there was a similar increase in acid phosphatase activity per cell; the acid phosphatase activity per initial cell inoculum of the culture medium from PHA-stimulated cultures also increased (Table 1). Because of the "background activity" introduced by the varying numbers of granulocytes which contaminate cultures from peripheral blood, there was considerable variation among the control values.

The increase in the enzyme activity expressed as a function of protein concentration, or "relative specific activity" (ratio of activity per hour per amount of protein in stimulated cultures to activity per hour per amount of protein in control cultures), was less than that expressed as the percentage increase per cell, the increase ranging from 128 to 405 percent. This may be due to the known increase in cellular protein and the small increase in cell number caused by divisions before the cultures are harvested.

The discrepancy between the large amount of acid phosphatase activity shown histochemically, and the relatively smaller increases in activity of this enzyme demonstrated chemically, may reflect the high "background" activity in control cultures; such activity may be due to the presence of varying numbers of degenerating polymorphonuclear leucocytes. Control cultures in the biochemical experiments were replicate cultures of cells grown without PHA; it is therefore not possible to make categorical statements about net synthesis in the system described. However, since both the control cultures and the cultures treated with PHA were obtained from the same sample of blood or lymph, it would appear that total enzyme activity had increased in the PHA-treated cells.

Table 1. Enzyme activities in lymphocytes and their nutrient media with the addition of phytohemagglutinin (PHA) and without such addition (controls), and the percentage increase in activity (Increase %) of PHA-stimulated cells over cells not stimulated with PHA.

Duration of culture (hr)		Cells: Activity per protein unit*	Cells: Activity per cell†	Medium: Activity per cell
	Acid phosphatase			
36	Control	5.51	1.87	3.90
	PHA	8.33	2.24	7.00
	Increase (%)	151	120	180
72	Control	5.13	1.54	3.80
	PHA	6.54	2.32	6.75
	Increase (%)	128	146	178
72	Control	2.40	0.48	
	PHA	9.80	2.99	
	Increase (%)	405	615	
72‡	Control	4.90	0.56	1.19
	PHA	8.00	1.56	5.55
	Increase (%)	174	275	466
	β-Glucuronidase			
36	Control	8.87	3.76	0.72
	PHA	12.2	3.28	0.78
	Increase (%)	138	87	105
72	Control	10.2	3.18	0.58
	PHA	8.58	3.05	1.12
	Increase (%)	84	96	193
72	Control	10.3	2.05	
	PHA	8.40	2.56	
	Increase (%)	82	125	
72‡	Control	2.78	0.397	0.475
	PHA	3.49	0.795	0.560
	Increase (%)	126	200	118

* Micrograms of phenolphthalein released per hour per million cells in the inoculum. † Micrograms of phenolphthalein released per hour per microgram of protein × 10^{-3}. ‡ Thoracic duct lymphocytes.

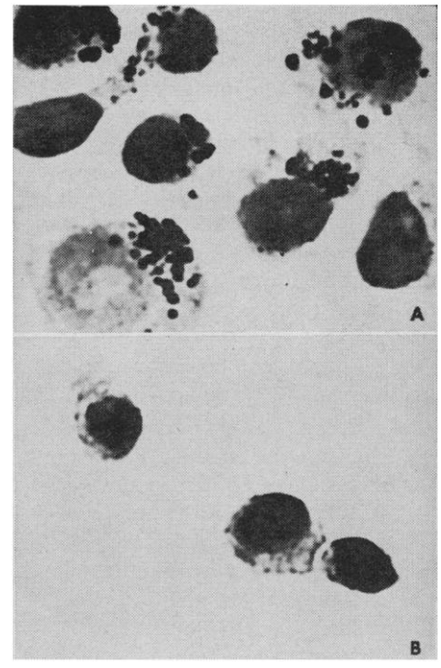

Fig. 1. Lymphocytes cultured for 72 hours and stained for acid phosphatase (phase contrast, oil objective × 3600). (*A*) Stimulated with phytohemagglutinin. (*B*) Not stimulated.

β-Glucuronidase activity showed a variable response, but, in those cultures with a maximum rise in acid phosphatase activity, a rise in β-glucuronidase activity was also seen.

These observations are compatible with several other studies reported previously. Robbins and Gonatas (*12*) have observed, during mitosis of HeLa cells in culture, an accumulation of osmiophilic, acid phosphatase–staining organelles which are most prominent during metaphase, but are readily distinguishable in prophase. The appearance of these bodies in their published photomicrographs closely resembles that of lysosome-like structures in other cells. In phase-contrast and electron microscopic studies of PHA-stimulated lymphocytes, Tanaka *et al.* (*8*) found cytoplasmic granules in the form of multivesicular bodies and small lipid vacuoles appearing in the transformed cells preceding mitosis. Although these were not identified by histochemical techniques, a study of the published plates (*8*) strongly suggests that the

single membrane-bounded osmiophilic vacuoles and granules are identical with those we have observed. Many of the new organelles described in these early studies were in close proximity to the Golgi apparatus; since acid phosphatase staining in some of our cultured cells was also localized in the perinuclear area, it is possible that these newly formed, lysosome-like structures arise from the Golgi apparatus, as suggested by Novikoff (*5*).

Cellular enlargement and mitotic activity can also be induced in 5 to 40 percent of human lymphocytes by culturing them in the presence of specific antigens to which the donor of the cells has been sensitized (*2*); such cells contain granules and vacuoles (*13*) resembling those described by Tanaka. From these studies and from our observations of tuberculin-stimulated cultures it would appear, therefore, that both nonspecific (PHA) and specific (antigen) stimulants induce the formation of lysosome-like structures before mitosis.

If these granules containing acid phosphatase resemble the lysosomes of other tissues, it may be that such organelles participate in the remodeling processes immediately preceding cell division.

ROCHELLE HIRSCHHORN
J. MARTIN KAPLAN
ARTHUR F. GOLDBERG
KURT HIRSCHHORN
GERALD WEISSMANN

Department of Medicine, New York University School of Medicine, New York, New York

References and Notes

1. M. A. Bender and D. M. Prescott, *Blood* **20**, 103 (1962).
2. K. Hirschhorn, F. Bach, R. L. Kolodny, I. L. Firschein, N. Hashem, *Science* **142**, 1185 (1963).
3. P. C. Nowell, *Cancer Res.* **20**, 462 (1960).
4. W. H. Marshall and K. B. Roberts, *Quart. J. Exptl. Physiol.* **48**, 146 (1963).
5. A. A. Novikoff, in *Lysosomes*, A. V. S. de Rueck and M. P. Cameron, Eds. (Little, Brown, Boston, 1963), p. 36.
6. F. Bach and K. Hirschhorn, *Science* **143**, 813 (1964).
7. A. F. Goldberg, *Blood* **24**, 305 (1964).
8. Y. Tanaka, L. Epstein, G. Brecher, F. Stohlman, Jr., *ibid.* **22**, 614 (1963).
9. C. Huggins and P. Talalay, *J. Biol. Chem.* **159**, 399 (1945).
10. P. Talalay, W. H. Fishman, C. Huggins, *ibid.* **166**, 757 (1946).
11. O. H. Lowry, N. J. Rosebrough, A. L. Farr, R. J. Randall, *ibid.* **193**, 265 (1951).
12. E. Robbins, and N. K. Gonatas, *J. Cell Biol.* **21**, 429 (1964).
13. K. Hirschhorn, unpublished observations.
14. Supported by grants from USPHS (HD-00542, AM-08363, CA-07178), New York City Health Research Council (U-1030), Association for the Aid of Crippled Children, and the Life Insurance Medical Research Fund.

12 November 1964

Foveal Receptors of the Monkey Retina: Fine Structure

Abstract. *The outer segments of the foveal cones of the rhesus monkey are about 40 microns long and 0.9 microns wide. They consist of stacks of membrane-limited, transverse discs about 140 Å thick, surrounded by a plasma membrane. The inner segments are about 30 microns long and 2.5 to 3 microns wide at the base, and they taper gradually to a tip diameter of about 1.5 microns. They contain many long mitochondria which are oriented lengthwise and are concentrated in the distal portion of the segment. The terminal pedicles show many synaptic contacts, probably as many as 36 per pedicle.*

The central portion of the primate fovea contains receptor cells of only one type, classified physiologically as cones (*1*). The gross structure of the foveal cone-cells presents something of an enigma, however, since they appear more like rods than cones when observed with the light microscope (*1*, p. 249). They have thin, cylindrical outer segments which show no taper and are the longest outer limbs in the retina, extending some 40 to 60 μ in length (*1*, p. 448). The inner segments of the foveal cones are likewise thin and elongated and also resemble rod inner segments. No detailed studies of the fine structure of the foveal receptors have been reported as yet, although there have been several studies of the fine structure of rods and extrafoveal cones in primates (*2*). This report describes the foveal receptors in the rhesus monkey.

Eyes were obtained from small rhesus monkeys (*Macaca mulata*) anesthetized with nembutal. The cornea and lens were cut away, along with the more peripheral retina, and the back of the eye was immersed in 2-percent osmium tetroxide buffered to *p*H 7.8 with veronal acetate and containing 1 percent calcium chloride and sucrose at 45 mg/ml. The eyes were fixed for 1 hour, dehydrated in graded acetone-water mixtures, and embedded in Araldite in a flat aluminum pan. After hardening, the pan was cut away and the clear plastic disc containing the tissue was examined under the dissecting microscope to locate the fovea.

The fovea was not easily distinguished in the densely-staining tissue, but the optic disc, retinal blood-vessels, and nerve fibers on the surface of the retina were easily seen and provided marks to locate the fovea. When the approximate foveal position was found, thick sections were cut with a razor blade until the fovea was found. Then thin sections were cut on a Porter-Blum microtome, stained with lead citrate, and examined in an RCA EMU-3F electron microscope.

A portion of a typical outer segment of a central foveal cone is shown in Fig. 1. The diameter of the foveal cone is approximately 0.9 μ, and no tapering of the outer segment structure is evident throughout its length. As is the case with rods and cones from all vertebrates (*3*, *4*), the internal structure of the outer segments of the foveal cone consists of a stack of flattened, membrane-limited discs piled one atop the other. Each disc is approximately 140 Å thick, the bounding membranes are about 50 Å wide, and the intra-disc space is about 40 Å. The inter-disc space

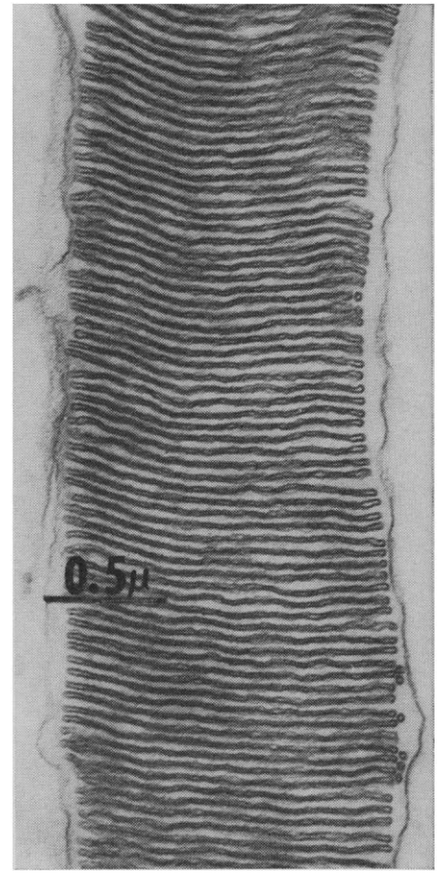

Fig. 1. Portion of the outer segment of a cone from the central fovea of a rhesus monkey. The outer segment consists of a pile of membrane-limited discs piled one atop the other. (× 52,000)