by Appella and Markert (*6*). The final concentration was 0.1*M* tris, 0.036*M* L(+) lactic acid sodium, or L(−) malic acid, NAD (0.3 mg/ml), nitro-blue tetrazolium (0.8 mg/ml), and phenazine metholsulfate (0.14 mg/ml), all adjusted to *p*H 8.3. In some experiments the reaction mixture described by Allen (*7*) was used with identical results.

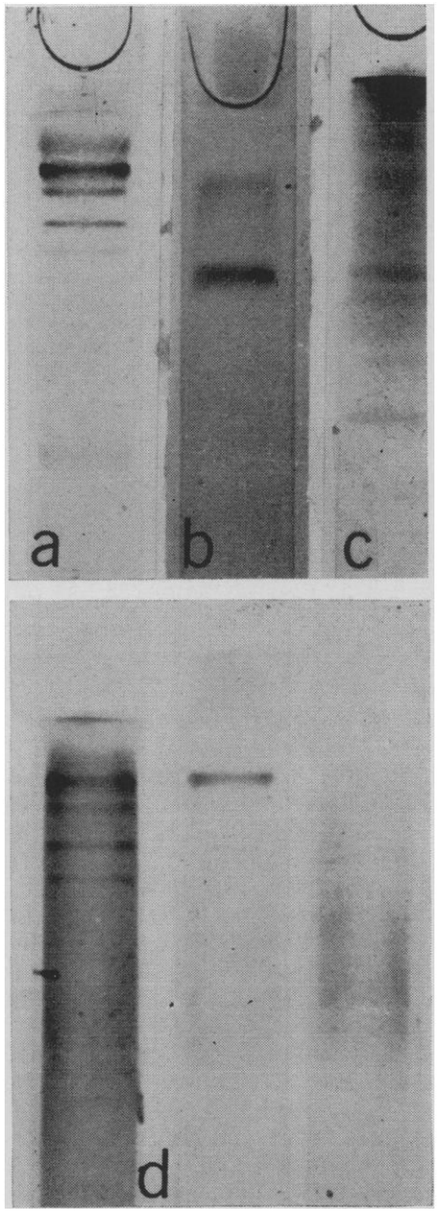

Fig. 1. Electrophoresis patterns of LDH and MDH, and protein distribution. The anode was at the bottom of the gel. (*a*) LDH pattern. (*b*) MDH pattern. (*c*) Gel stained for protein. (*d*) LDH zymogram with NAD (left), the 3-acetyl pyridine analog of NAD (center), and the pyridine-3-aldehyde analog of NAD (right) in the incubation mixture. These gels were simultaneously subjected to electrophoresis and incubated in the appropriate reaction mixture for the same length of time.

Sites of dehydrogenase activity on the gel were localized by the precipitation of the reduced tetrazolium salt which was purple in color.

Five electrophoretically distinct lactic dehydrogenases (Fig. 1*a*) and two malic dehydrogenases (Fig. 1*b*) were demonstrable in solutions obtained from the sonification of these cells. Duplicate gels stained for protein showed 16 well-defined bands separated by this procedure (Fig. 1*c*). If, for convenience, we number the sites of dehydrogenase activity from I to V in order of migration toward the anode, it appears that LDH_{IV} and MDH_{I} have the greatest activity if judged by size of band and intensity of stain: sites V, III, II, and I follow in order of decreasing activity.

These data were compared with the results of Blanco and Zinkham (*8*) on pre- and post-puberal testis. Specimens of human testis from autopsy, supplied to me by Zinkham, were assayed here by disc electrophoresis. In my system LDH_{IV} corresponds to the "band X" which they report is unique to spermatozoa. In addition, I have determined that isozymes 1, 2, and 3 from human erythrocytes and testis are identical electrophoretically to the isozymes in sperm, designated LDH_{I}, LDH_{II}, and LDH_{III}; LHD_{V} would be the same as isozyme 4 in testes, and therefore presumably in other human tissues.

When the 3-acetylpyridine or pyridine-3-aldehyde analogs of NAD were used in the incubation mixture only a single LDH band was observed on the gels; this band coincided with LDH_{IV}. These data, in Fig. 1*d*, show that there is greatest activity with NAD; the 3-acetyl analog of pyridine is capable of serving as hydrogen acceptor, and there is no reaction with the 3-aldehyde analog. This result would further support the suggestion that "band X" and LDH_{IV} are comparable, since this isozyme has a strikingly high ratio of the 3-acetyl pyridine analog of NAD to NAD (*8*). Spectrophotometric assay of the activity of LDH in the solutions from disrupted cells revealed in two experiments that the reaction, pyruvate to lactate, oxidizes $NADH_2$ at the rate of 1.22 and 1.09 μM/min per milligram of protein. These values obviously reflect the composite activity of the five molecular species of LDH.

Several studies suggest that multiple forms of enzymes occur within a single cell (*9*). Such work, however, has dealt with cells capable of division, or in different stages of division, or of different ages. The present report demonstrates conclusively that a single cell type possesses its own complement of "isozymes." A recent report describes five lactic dehydrogenases and two malic dehydrogenases in a number of human fetal tissues (*10*).

On the basis of these studies it is not possible to establish the role of molecular heterogeneity of enzymes within the metabolic framework of the cell. It seems probable that LDH_{IV} (or "band X") represents the most important isozyme for lactate metabolism and even glycolysis in the human spermatozoa.

ERWIN GOLDBERG
Department of Zoology,
North Dakota State University, Fargo

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19 December 1962

Ripple Marks Show That Countercurrent Exists in Florida Straits

Abstract. *About 60 percent of the area shown in photographs taken at the axis of Florida Straits exhibits well-defined current ripple marks. These ripples indicate a flow of water of at least 0.2 to 0.6 knots from the north. This current is in the opposite direction from the surface currents of 2 to 4 or more knots.*

The floor of Florida Straits is a smoothly graded valley that has its head near the northern end of the straits (*1*). The valley extends south and then west and empties at grade onto the floor of the Gulf of Mexico. In the area north

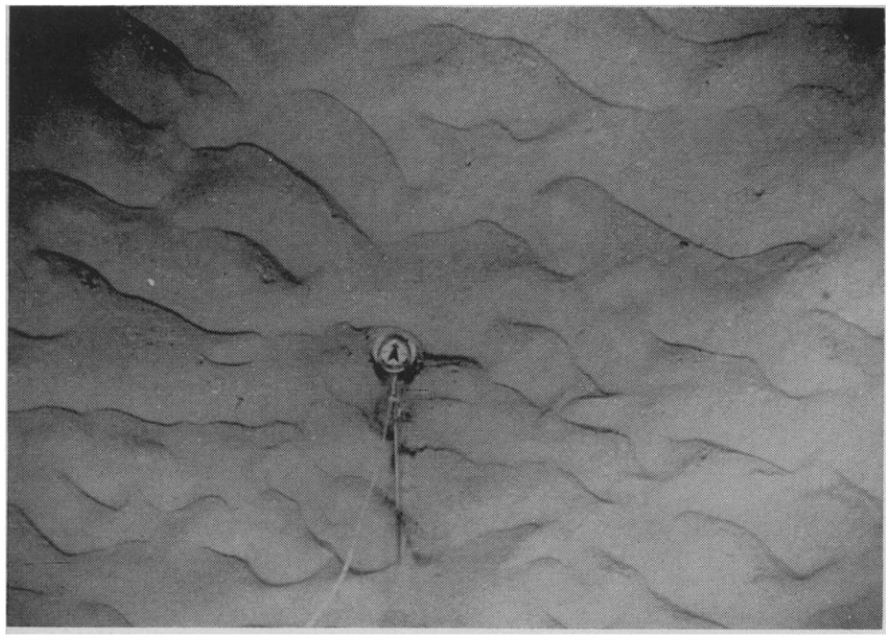

Fig. 1. Ripple marks at 24°55′N, 79°35′W, 24 September 1962. Time: 19:06:33; depth: 462 fathoms. North at top. Compass 13.75 inches long.

of Cay Sal Bank it includes a 60-mile-long plain, presumably of ponded sediment, at 462 fathoms. The photographs of the sea floor discussed here were taken on the plain near its northern end. Asymmetrical ripple marks found in these photographs indicate a bottom current flowing from the north (Figs. 1 and 2).

Exposures were obtained over a period of 2 hours at heights varying from about 2 to about 20 feet above the bottom. During the period the camera was operating the ship drifted at approximately 1.65 knots. Exposures were made at 12-second intervals (corresponding to about 33 feet).

Table 1 shows the relative numbers of exposures exhibiting ripple marks. If it is assumed that ripples appear in one-half the area of the photograpns showing both rippled and unrippled sea floor, about 60 percent of the photographed area exhibits ripple marks and 40 percent does not. In all of the photographs showing ripples, the crests trend east and west and in every case where the camera height is small and asymmetry is detectable, ripples indicate a current flow from the north.

In several photographs, the rippled areas end abruptly. The boundaries of rippled areas nearly always trend at right angles to the ripple crests. In a few cases it is clear that unrippled areas are in strips elongated parallel to the current direction. It is not possible to determine whether the rippled areas are at a different level from unrippled areas. Dark objects, presumably debris of plants such as *Thalassia,* are generally much more abundant in unrippled areas. The ripples have crests varying in length from 10 or more to only 1 or 2 wavelengths. Careful measurements by photogrammetric techniques were made to estimate the dimensions of the ripple marks. The long, crested ripple marks (not illustrated) have wavelengths averaging 6.2 inches with a standard deviation of 0.5 inch in 13 measurements. The short crested ripples have wavelengths averaging 5.6 inches with a standard deviation of 0.7 inch in 14 measurements. The shorter and more variable wavelengths of the short crested ripples suggest that these ripples are in an active state of flux. The height or amplitude of the ripple marks is very uncertain. We have estimated height between 0.5 and 2.0 inches and most probably about 1.0 inch. The ripples appear roughly trochoidal in form, and a tendency for oversteepening of the upper lee side of the crests is frequently observed.

Efforts to estimate the velocity of the current causing these ripple marks depend largely on the nature of the sediment. Repeated attempts were made to obtain core samples before and after the camera was lowered. These attempts were largely unsuccessful, recovery being limited to a few grains of foraminiferal sand washed into the corer during recovery. From this and from an examination of all the photographs it is most likely that the sediment is a noncohesive, biogenous, calcareous muddy sand. Small round white objects seen resting on the sediment surface are thought to be pteropods. Data on minimum velocity for the initiation of ripple mark formation, summarized by Inman, suggest that a velocity of 0.4 to 1.0 ft/sec (0.2 to 0.6 knots) is required (*2*).

While there is no doubt that a water current along the sea floor has produced the current marks, we have no direct

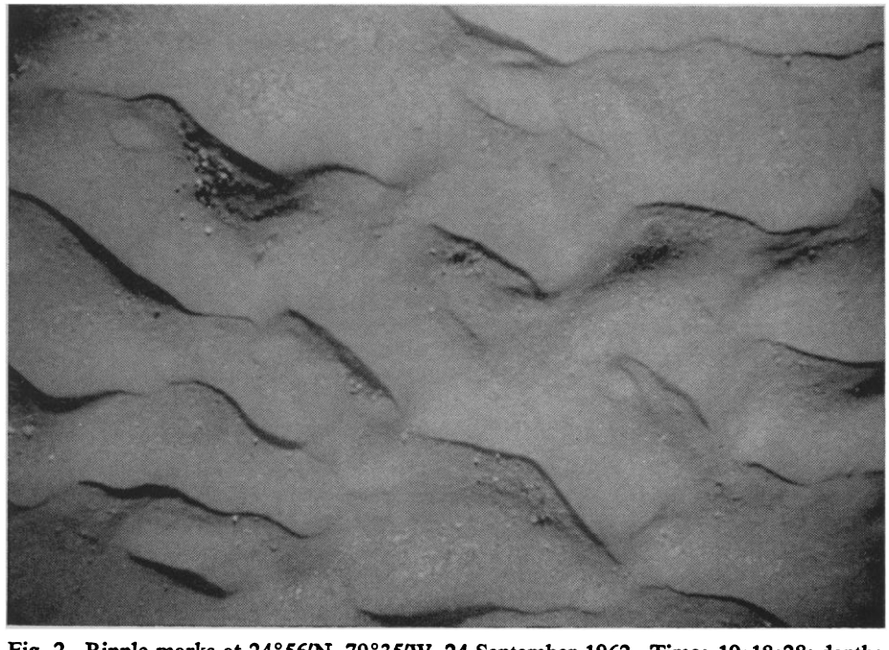

Fig. 2. Ripple marks at 24°56′N, 79°35′W, 24 September 1962. Time: 19:18:28; depth: 462 fathoms. North at top.

Table 1. Relative numbers of exposures exhibiting ripple marks.

Condition	No. of photos	Total No. of photos (%)
All rippled	138	47.5
Rippled and nonrippled	71	24.5
All nonrippled	81	28
Total	290	100

information about the nature of the current. One possibility is that we sampled one, southbound, phase of an oscillating tidal current; however, the well-developed, very long crested ripples suggest a more uniform flow regime (*3*).

ROBERT J. HURLEY
L. KENNETH FINK
Institute of Marine Science,
University of Miami,
Miami 49, Florida

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23 November 1962

Cerebral White Matter: Selective Spread of Pneumococcal Polysaccharides

Abstract. *Pneumococcal polysaccharides were implanted in rat brain and their distribution was studied by immunofluorescence. The polysaccharides spread selectively in white matter, and frequently extended from anterior to posterior poles. The results suggest that selective localization of experimental and natural leukoencephalopathies may be related to an innate property of white matter that permits or facilitates spread of noxious agents.*

Demyelinating diseases and cerebral edema are characterized by selective localization of lesions in white matter. Recently, three experimental lesions (leukoencephalopathies) localized in the white matter of rat brain have been described: edema that follows implants of certain inorganic or organic chemicals; edema and leukocytic infiltration that follows implants of purified protein derivative of tubercle bacilli, or bacterial endotoxin; vacuolation, with little edema or leukocytic reaction, which results from implants of capsular polysaccharides from pneumococcus or cryptococcus (*1*). The common pattern of distribution of these histologically different lesions suggests that a mechanism is present in white matter which permits or facilitates the spread of exogenous substances. Support for this hypothesis is presented in this report.

Pneumococcal polysaccharides of types 2 or 3, mixed with an equal weight of graphite, were implanted as solid pellets in the anterior end of the right cerebral white matter (callosal radiation) of the rat brain (*1*). At the appropriate time the rats were exsanguinated and their brains were removed and fixed in Rossman's fluid (*2*) or acetate-buffered 10 percent Formalin. Horizontal slices were embedded in paraffin. Sections were deparaffinized with xylene, and passed through 100, 95 and 70 percent ethanol into saline. Preparations of frozen sections mounted on slides from an alcohol bath, gave results comparable to those obtained with paraffin-embedded tissue. Slides were stained for 30 minutes at room temperature with antipneumococcal serum conjugated with fluorescein isothiocyanate (Sylvana), washed in saline for 10 minutes, and mounted in buffered glycerol (*2*). Observations were made with ultraviolet light and dark-field illumination on Leitz and on Reichert fluorescence microscopes.

Brilliant green fluorescence was observed in the sections from the brains implanted with type 2 polysaccharide when they were stained with conjugated type 2 antiserum (Fig. 1) but not with conjugated type 3 antiserum. Sections of brains implanted with type 3 polysaccharide showed fluorescence after staining with type 3 conjugated antiserum but not with type 2 conjugated antiserum. This type specificity of fluorescence was further substantiated. If the antibodies from the type 2 conjugated antiserum were removed by specific precipitation with type 2 polysaccharide, the residual antiserum no longer caused the staining of sections of brains implanted with the same or a different preparation of type 2 polysaccharide. If type 3 polysaccharide was added to type 2 conjugated antiserum there was no precipitation and no alteration of staining. Similarly, fluorescent staining of sections of brains implanted with type 3 polysaccharide by type 3 conjugated antiserum was prevented if the antiserum was specifically precipitated with type 3 polysaccharide but was unaffected by addition of the type 2 polysaccharide. Blocking the reaction sites with type specific but unconjugated antiserum for 30 minutes at room temperature before the conjugated antiserum was applied give slight but definite reduction of fluorescence. Four consecutive pretreatments resulted in much greater reduction of fluorescence. Neither one nor four pretreatments with unconjugated antiserum of a noncorresponding type reduced the subsequent fluorescent staining with conjugated antiserum of type corresponding to the implant. Only mild autofluorescence was observed in sections of brains with implants of unrelated materials such as silver nitrate and purified protein derivative.

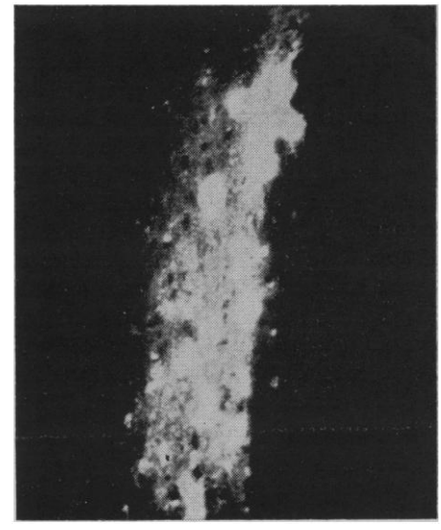

Fig. 1. Horizontal section of posterior portion of rat brain stained with fluorescein-labeled type-specific antipneumococcal serum. There is bright fluorescence in the white matter (callosal radiation), but not in the cerebral cortex (left) or corpus striatum (right), indicating pneumococcal polysaccharides spread selectively in white matter (× 125).

Fluorescence was distributed selectively in the white matter, and corresponded to the distribution of the vacuolar lesions (*1*). Fluorescence in white matter adjacent to the implant was detected 6 hours after implantation; at 12 hours fluorescence extended along the callosal radiation, and at 24 to 48 hours the entire callosal radiation was fluorescent (Fig. 1). The fluorescence reaction was reduced 1 week after implantation; preparations of brain made 2 and 3 weeks after im-