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Some Histochemical Observations on Human Dystrophic Muscle

A number of histochemical techniques have been applied to biopsy specimens of human muscle taken from 32 patients suffering from muscular dystrophies and a variety of other muscular diseases. Control muscle was obtained partly by biopsy, at post mortem, and within 1 hour of death from two young, healthy men.

The following histochemical tests were employed: succinic dehydrogenase, cytochrome oxidase, cholinesterase, 5-nucleotidase, acid and alkaline phosphatase, Periodic acid-Schiff reaction, fat, disulfide and sulfhydryl groups, and amino groups. Figure 1 shows some of the results.

Succinic dehydrogenase activity varied considerably between different control muscles and between individual fibers in any one muscle. It varied independently of the cytochrome oxidase reaction. In pathological muscle there was a decrease in activity of succinic dehydrogenase, suggesting a lowered aerobic metabolism. Where substantial replacement of muscle by connective tissue occurred, there was, as might be expected, a considerable reduction of the over-all reaction for this enzyme.

Gomori's (*1*) modification of Koelle and Friedenwald's (*2*) method showed, both in control and in pathological muscle, positive classical end-plates, "terminaisons en plaque" (*3*), and the elongated scattered type of end-plate described by Coërs as "terminaisons en grappe." We were also able to confirm the findings by Couteaux in 1953 and Gerebtzoff in 1956 (quoted by Gerebtzoff, *4*) that there were cholinesterase-positive reticulated or parallel-guttered structures sitting like caps over the ends of the muscle fibers—that is, at the musculotendinous junctions. Another cholinesterase-positive structure consisted of a series of parallel gutters situated in various positions along the length of the muscle fibers. We call these "cake-frill" endings. Their appearance suggests that they, like the endings of the musculotendinous junctions, are stretch receptors. Muscle spindles gave a positive reaction, mainly in the end-plates situated at the poles of the spindles, but occasionally also in some of the spirally arranged sensory nerve fibers.

There was very little evidence of decrease of cholinesterase activity in dystrophic muscle, but where there was considerable replacement of muscle by connective tissue, as for example in facioscapulohumeral or familial dystrophy, isolated and apparently intact end-plates attached to remnants of muscle fibers were seen which gave a normal cholinesterase reaction. Atrophying muscle fibers showed a strong positive reaction in the muscle substance itself.

We could obtain little evidence from our work that muscular dystrophy and related diseases were associated with either physical or enzymatic breakdown of neuromuscular transmission.

Nerves and some blood vessels are the only positive elements in normal human muscle. In dystrophies characterized by atrophy of muscle fibers and their replacement by connective tissue, cells, fibers, and capillaries of the latter gave a strong 5-nucleotidase reaction and appeared to be actively invading and destroying the substance of the muscle fibers. Atrophying fibers also gave a strong reaction for the enzyme.

Since 5-nucleotidase dephosphorylates adenosine monophosphate (which is a starting point for the resynthesis of adenosine triphosphate), this result may be of some significance. Inhibitors of 5-nucleotidase may be of use in the treatment of these conditions, and so might also the anti-snake-venom serums prepared from venoms containing 5-nucleotidase.

Acid phosphatase in normal muscle is present in peripheral nerves, Golgi material and nuclei of both muscle fibers and connective tissue cells, and in adipose tissue. The small amount of acid phos-

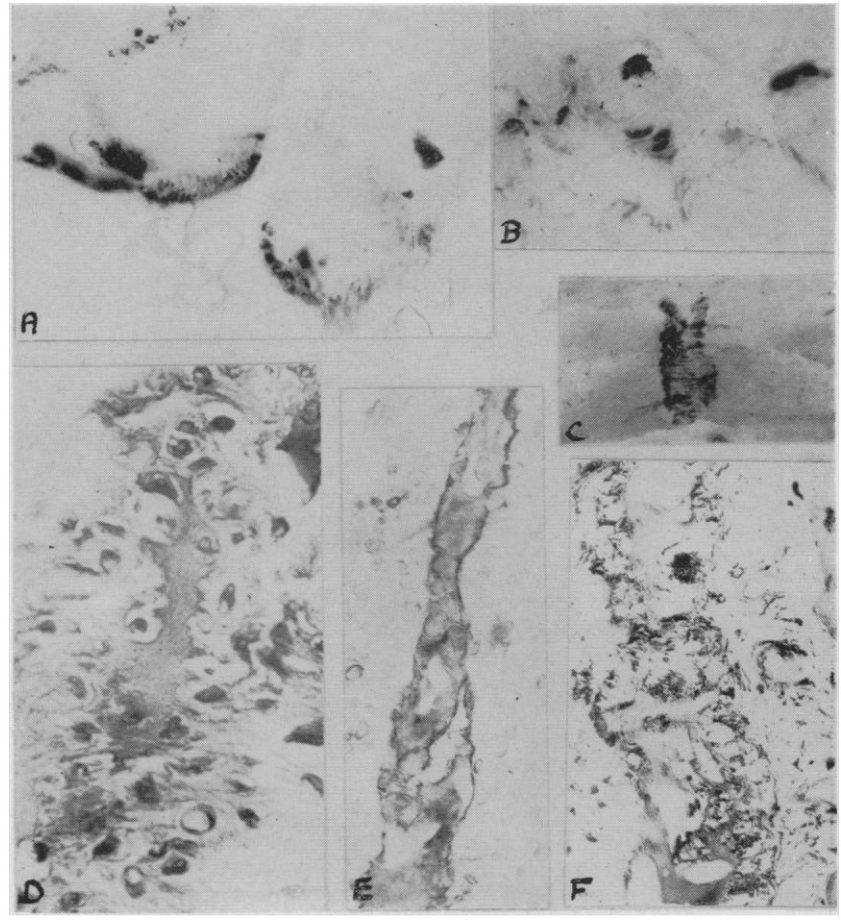

Fig. 1. *A*, Acetyl cholinesterase in structures situated at musculotendinous junctions; *B*, acetyl cholinesterase in numerous small end-plates from a case of dystrophia myotonica; *C*, acetyl cholinesterase in two "cake-frill" type structures in adjacent muscle fibers; *D*, 5-nucleotidase in an atrophied degenerating fiber and in the cells involved in its destruction, from a case of doubtful diagnosis (polymyositis or pseudohypertrophic muscular dystrophy); *E*, alkaline phosphatase in a degenerating muscle fiber and in fine connective tissue fibers wound around it, from a case of familial dystrophy; *F*, acid phosphatase present in high concentration in a muscle fiber undergoing necrosis and in the cells involved in this necrosis (the muscle was taken from a case of familial dystrophy).

phatase in the substance of the muscle fibers is increased in atrophied fibers. Where muscle fibers are being replaced by connective tissue, the invading cells show a high level of acid phosphatase activity.

Alkaline phosphatase in normal human muscle is limited to the capillaries and the intima of larger vessels. In dystrophies, the enzyme occasionally appears in the atrophying muscle fibers and in the very fine connective tissue fibers found surrounding them.

The increase of phosphatase activity (including 5-nucleotidase) in the connective tissue of muscles in some types of dystrophy suggests that phosphatases may play an important part in the destruction of muscle fibers associated with these diseases. Even if they are not directly concerned with the breakdown of muscle substance, they may cause functional failure through excessive breakdown of high-energy phosphate compounds. A similar process may be involved in aging (*5*).

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6 May 1957

Resistance to Desiccation in Intertidal Barnacles

The resistance to desiccation on exposure to air is recognized to be an important factor in the zonation of intertidal animals, and it is achieved in a variety of ways by different species. In spite of the work of Monterosso (*1*) on *Chthamalus stellatus* var. *depressus*, it is frequently stated that barnacles that live relatively high up on the shore achieve their resistance to desiccation by enclosing a small quantity of water in the mantle cavity as the tide recedes. This is not so.

Although morphologically they are very similar, littoral and sublittoral barnacles behave very differently when they are removed from water. In general, the intertidal species such as *Chthamalus stellatus, C. fragilis, C. dalli, Balanus balanoides* and *B. glandula* show what may be termed a "controlled" behavior pattern that leads to adjustment to the new environment. On the other hand, sublittoral species such as *Balanus crenatus* or *B. balanus* struggle in an erratic fashion and soon become desiccated.

The most detailed observations have been made on the intertidal *B. balanoides*. When these are first removed from water, the cirri, in a collapsed state, are partially extruded several times, from the mantle cavity with the expulsion of water. Such movements are repeated at intervals during the first few minutes of exposure to air. Very soon this activity decreases. The valves, still far from being fully retracted, then come together in such a way that the underlying, uncalcified folds of the operculum form a small, diamond-shaped, micropylarlike opening. There is, therefore, direct access for air to the mantle cavity.

Subsequent macroscopic movements of the valves may be accompanied by further expulsion of small droplets of water; such droplets are often found on the valves of shore animals during intertidal periods. In between such macroscopic movements, and subsequent to them, the micropylar orifice is open and, from time to time, appears to be subject to regular pulsation—some 40 pulsations per minute. After several hours the valves may completely close from time to time; this is accompanied by reformation of the micropylar opening, whose size decreases with time. Over a period of 24 hours the valves are not fully retracted within the shell; complete retraction takes place only when the valves are touched (this is doubtless a defense mechanism against predators, which usually attack the animal at the opercular valves).

Although extremes of desiccation attributable to high temperature or long exposure to air may result in complete closure, the evidence indicates that, during an intertidal period of several hours, the barnacles are utilizing atmospheric oxygen in their respiratory activity. That this is the case is also supported by the fact that we have found that, after the barnacles have been exposed to air for several hours, there is no significant accumulation of lactic acid and no accumulated oxygen debt such as might be expected if anaerobic processes were involved.

When animals, after exposure to air for some time, are reimmersed, the operculum is quickly opened (in *B. balanoides*, this occurs within from 10 to 15 seconds), and bubbles of gas escape from the mantle cavity. This precedes extrusion of the cirri, amongst which gas bubbles are often entangled, and resumption of normal cirral activity (*2*).

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Adsorption-Hemagglutination Test for Influenza Virus in Monkey Kidney Tissue Culture

In the course of investigations of influenza viruses in monkey kidney tissue culture, it was observed that addition of erythrocytes directly to tubes with viral cytopathic effect resulted in adsorption of erythrocytes onto the monocellular epithelial sheet. Further studies indicated that this is a specific phenomenon dependent on the hemagglutinating property of the virus. Besides contributing to clearer understanding of the mechanism of hemagglutination, the reaction may be of practical importance in diagnostic work. The purpose of this preliminary report is to describe our technique because of its possible value to rapid diagnosis of suspected cases of influenza. This is pertinent to studies of the influenza epidemic which has already involved major portions of the Far East.

Monkey kidney cells in monolayer sheets were prepared by a modified Youngner technique (*1*), grown in a medium recommended by Melnick (*2*), and maintained in a nutrient mixture (*3*) of 75 per cent Earle's balanced salt solution, 24 percent bovine serum ultrafiltrate, and 1 percent heated horse serum with appropriate antibiotics. Calf serum was not used in the maintenance medium because of its possible anti-viral effect. Most of the experiments employed an influenza virus strain recently isolated in this laboratory directly in tissue culture; this strain was provisionally designated A/Md/1542/57 and was found to be closely related to A/Md/1/55.

Our current technique is as follows. Supernatant fluids are removed from infected and control monkey kidney tubes. Two-tenths of a milliliter of 0.4-percent suspension of washed, citrated guinea pig erythrocytes are added to each tube (results with influenza A were not as consistent using chicken erythrocytes). The tubes are placed in horizontal position for about 1 minute, and the initial reading is made by microscopic examination under low power for clumping of erythrocytes in the fluid and visible adsorption-hemagglutination. With experience, this examination is often sufficient to determine whether the test is positive or negative. Because the settling of erythrocytes onto the monolayer sheet during standing, especially if it exceeds a period of a few minutes, may resemble a