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Electrophoretic and Immunological Studies of Squid Axoplasm Proteins

Abstract. *By disc electrophoresis of the axoplasm of* Dosidicus gigas, *14 protein bands have been resolved. Antibodies to the intra-axonal proteins and to squid blood proteins were produced in rabbits. By Ouchterlony's technique, six antigenic components can be demonstrated in axoplasm; the combined use of disc electrophoresis and immune diffusion in agar resolves seven antigenic components in axoplasm; none of these components is detectable in squid blood.*

The giant axons of the squid permit the isolation of macroscopic quantities of cytoplasm of peripheral nerve. Studies on certain proteins in the axoplasm from *Loligo pealii* obtained at Woods Hole, Massachusetts (*1*), and from *Dosidicus gigas* caught near Valparaiso, Chile (*2*), were reported previously from this laboratory. *Dosidicus* offers the technical advantage of being larger; its average giant axon is 1.2 mm wide and 20 cm long; moreover, the axon can be freed from the sheath of connective tissue and thus obtained within only a thin layer of Schwann cells (*3*). By extrusion of the axoplasm from such a clean preparation it is possible to isolate intracellular material which we believe is practically free from nonneuronal contaminants. In addition to characterizing the structure and function of these intracellular proteins, studies were made to determine the number of protein species present in the axoplasm and to determine which of these proteins are specific to the neurons.

Davison and Taylor (*2*), by moving-boundary electrophoresis, showed that the axoplasm of *D. gigas* contains at least two different components: the major and fastest migrating fraction at neutral *p*H probably includes the proteins which make up the neurofilaments; a minor and slower-moving boundary appeared inhomogeneous. However, they were not able to define further the number of protein species present. We have made subsequent studies of the intra-axonal proteins from *D. gigas* by means of a variety of electrophoretic techniques, using paper, cellulose acetate, glass paper, agar gels, and polyacrylamide gels as support media. Electrophoresis on the latter medium has resolved at least 14 components.

In the experiment reported here, electrophoresis on polyacrylamide gels was performed as described by Ornstein and Davies (*4*). A 7.5-percent acrylamide gel at *p*H 9.5 was used as an anticonvection and sieving medium. The only departure from the original method described (*4*) is that the sample was held down by a piece of dialysis membrane (cellophane) placed between the spacer gel and the cathode buffer reservoir. This modification was introduced to avoid further dilution of the protein solutions with the acrylamide solution and also to cope with difficulties in polymerizing the acrylamide solution when it was mixed with the sample. Figure 1 shows a stained gel after electrophoresis of *Dosidicus* axoplasm.

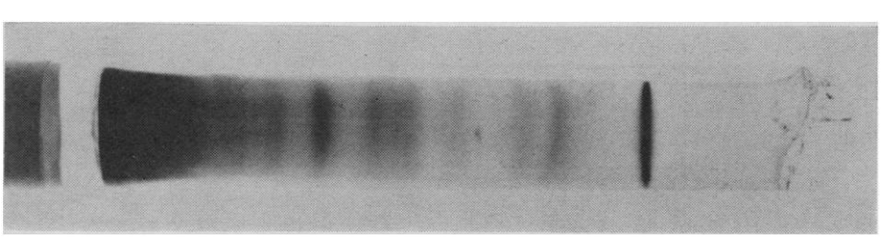

Fig. 1. Disc electrophoresis of squid axoplasm proteins. Cathode at the left, migration toward the right. Stained with amido black.

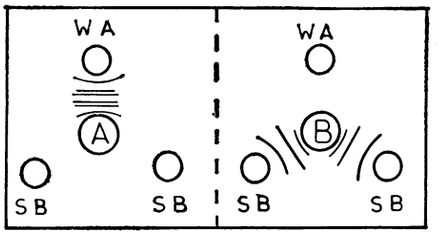

Fig. 2. Two-dimensional immune diffusion. Outer wells: *WA*, whole axoplasm; *SB*, squid blood. Center wells: *A*, axoplasm antiserum; *B*, squid blood antiserum.

To study the specificity of the intra-axonal proteins and to distinguish any which are common to axoplasm and to *Dosidicus* blood, some rabbits were immunized with proteins from whole axoplasm and other rabbits with squid blood. The lack of cross-reactivity between the antigenic proteins in axoplasm and those in squid blood, as studied by Ouchterlony's method, is illustrated

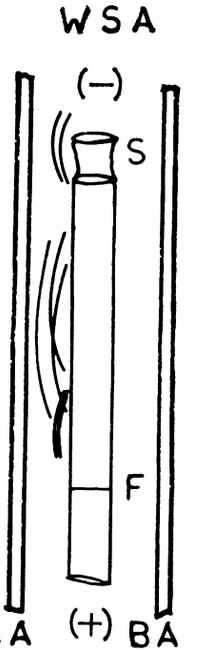

Fig. 3. Combined use of disc electrophoresis and immune diffusion in agar gel. *WSA*, Whole squid axoplasm; *S*, spacer gel; *F*, migration front; *AA*, axoplasm antiserum trough; *BA*, squid blood antiserum trough.

in Fig. 2. Six precipitin lines appear between the wells containing whole axoplasm (*WA*) and axoplasm antiserum (*A*); no lines appear at the wells containing squid blood (*SB*). Conversely, no precipitin lines appear between whole axoplasm (*WA*) and squid blood antiserum (*B*), but three lines appear with squid blood and its own antiserum.

When polyacrylamide disc electrophoresis is combined with immune diffusion on agar, a form of high-resolution immune electrophoresis is achieved, which takes advantage of the good electrophoretic separation obtained in polyacrylamide and the rapid diffusion occurring in agar. After the electrophoretic run the unfixed and unstained acrylamide gel is placed in a bed previously formed on an agar gel. Troughs for the antiserum are then cut in the agar, parallel to the acrylamide gel.

Figure 3 shows the result after separating electrophoretically on acrylamide the proteins of whole squid axoplasm (*WSA*) and allowing axoplasm antiserum (*AA*) and squid blood antiserum (*BA*) to diffuse from the troughs. By using this technique, seven antigenic proteins have been resolved thus far, none of which is present in a detectable amount in squid blood. No attempts to obtain quantitative immunological results have yet been made. The fractionation and identification of some of the axoplasm proteins will be reported later.

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Homeostasis of the Nonfat Components of Migrating Birds

Abstract. *Obesity in migratory birds appears to differ from obesity in man in that gains and losses in body weight do not involve changes in the tissue structure of the body; fat is added to and used from preexisting tissue spaces without appreciable change in the water content or the nonfat dry weight of the body as a whole. Evidence is presented which supports the hypothesis that the nonfat body is essentially homeostatic during migration despite very large scale changes in total body weight.*

Many species of small land birds, even though relatively slow and weak fliers, migrate between eastern North America and Central or South America by crossing the long stretches of open water of the Gulf of Mexico or the Caribbean. Just prior to long flights, individuals of these species build up huge temporary stores of body fat equal to two or three times the weight of the total nonfat dry weight of the body (*1*). These stores apparently are used as the sole energy source for nonstop flights which may last for many hours and cover distances of 1000 kilometers or more (*2*). Our studies of specimens accidentally killed by tall television towers during nocturnal migration revealed that migratory fat deposition is quite different from obesity in man, laboratory rats, or other nonmigratory species. In man, for example, gains and losses of body weight in obesity usually include gains and losses of water and nonfat tissue components as well as lipids. Thus, a gain or loss of a gram of body weight has been variously estimated to include a gain or loss of 5.8 to 7.8 kcal, depending on the nitrogen balance and the amount of water present (*3*). Pitts (*4*) summarizes a recent study on guinea pigs by stating that "changes in fatness are followed by changes in the same direction of fat-free adipose tissue and adipose-free body." In contrast, gains and losses of weight in migrating birds do not include addition or subtraction of water or nonfat tissue components, but merely gains and losses of dry lipids which have an energy value of at least 9 kcal/g. In other words, components of the nonfat body remain essentially homeostatic despite very large and rapid changes in total body weight. The migratory bird is thus analogous to the airplane in that "high octane" fuel (that is, fat) is added to and used from preexisting "tanks" (tissue spaces) without appreciable change in the tissue structure of the body as a whole. The purpose of this report is to outline briefly the five different lines of evidence we have to support this hypothesis.

Frozen specimens of birds killed by television broadcasting towers during nocturnal migration provided the principal material for our studies. Total lipid, water, and nonfat dry weights were determined by vacuum-drying chopped-up whole specimens which were then extracted in alcohol-ether. For studies on the adipose tissue itself, samples were removed from frozen specimens and were either prepared for histological sectioning, or were extracted with chloroform after the tissue had been freeze-dried and homogenized.

At the tissue level of organization, we would expect the percentage of water and nonfat residue in adipose tissue to decrease as the bird became fat—if water, blood vessels, connective tissue, and other nonfat components are not added while the fat is being deposited prior to migration. Table 1 shows that this is indeed the case. Adipose tissue of birds at the peak of lipid deposition just prior to long flights con-

Table 1. The percentage of water, nonfat residue, and total nonfat material in samples of adipose tissue dissected from red-eyed vireos (*Vireo olivaceus*) killed by a television tower during nocturnal migration. The nine birds are arranged in a series from lean to very fat condition. Percentages are means of samples from several body locations totaling an estimated 10 to 20 percent of "depot" fat tissue in each individual. Data from Walker (*8*).

Water (%)	Nonfat dry residue (%)	Total nonfat (%)
*Lean spring (April) migrant (fat index = 0.2)**		
40.2	24.0	64.2
Moderately fat early fall (Aug.–Sept.) migrants (fat index 0.5–1.5)		
23.7	15.2	39.9
18.7	10.0	28.7
12.4	5.5	17.9
7.5	4.1	11.6
*Very fat late fall (Oct.) migrants (fat index > 2.0)**		
6.5	3.1	9.6
6.0	3.5	9.5
6.0	2.3	8.3
4.9	2.2	7.1

* Fat index = grams of fat per gram of non-fat dry weight.