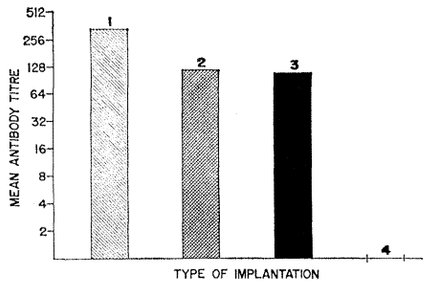

Fig. 1. Mean titers of antibody production in (1) nonbursectomized control chicks; (2) hormonally bursectomized chicks containing bursa; (3) hormonally bursectomized chicks containing bursa-filled diffusion chambers; and (4) hormonally bursectomized chicks containing empty diffusion chambers.

cleansed with alcohol. In each hormonally bursectomized chick either a bursa-filled diffusion chamber or a piece of bursa alone was implanted; for controls, empty diffusion chambers were placed in hormonally bursectomized chicks. A parallel experiment was done in which subcutaneous implants were employed.

On the 9th day after hatching—that is, 1 day after the surgical procedure, all chicks were injected intramuscularly with 1.0 ml of *Salmonella typhimurium* (standardized at 3×10^9 cells per milliliter). Four weeks after the first injection, a second intramuscular injection of 1.0 ml of *S. typhimurium* was given. Two weeks after this second injection blood for bacterial agglutination and serum electrophoretic studies was obtained by cardiac puncture.

Agglutination tests were done by adding 0.25 ml of the standard antigen to 0.25-ml samples of serum in serial dilution from 1 : 2 to 1 : 1025. Tubes were incubated at 45°C for 2 hours and then refrigerated for 24 hours. Agglutination above a titer of 1 : 4 was considered positive—that is, indicating the presence of antibodies to *S. typhimurium*.

A high titer of antibody to *S. typhimurium* (range 1 : 256 to 1 : 512, mean 1 : 330) was obtained from 22 of 30 of the nonbursectomized control chicks (Fig. 1). By contrast, ten chicks hormonally bursectomized and containing empty diffusion chambers were unable to produce demonstrable antibody, this result being in agreement with previous reports for both surgically and hormonally bursectomized chicks which received no further treatment (*1*, *2*). Reconstitution of antibody production (range 1 : 64 to 1 : 256, mean 1 : 105) occurred in 29 of 41 of the hormonally bursectomized chicks bearing bursa-filled diffusion chambers as well as in 15 to 20 of the animals bearing donor bursas not enclosed in diffusion chambers (range 1 : 64 to 1 : 128, mean 1 : 115). No differences were noted in experiments in which the diffusion chambers constructed of 0.45- and 0.1-μ porosity filters were used or in which we used chicks with bursas implanted intraperitoneally or subcutaneously.

Serum electrophoresis added confirmation to our observations in that hormonally bursectomized chicks containing empty diffusion chambers were found to have markedly decreased amounts of γ-globulin, in agreement with reports for bursectomized chicks receiving no further treatment (*9*). In bursectomized chicks containing bursa-filled diffusion chambers and bursas not enclosed in diffusion chambers, γ-globulin concentrations were greater than in the bursectomized chicks, although the concentrations were less than normal.

Our data indicate strongly that in the bursa of Fabricius a noncellular (hormone-like) substance is elaborated which enhances the establishment of immunologic reactivity in bursectomized chicks.

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22 January 1965

Sarcoplasmic Reticulum: Ultrastructure of the Triadic Junction

Abstract. *The appositional region between the intermediate element and adjacent cisternae in the triads of the sarcoplasmic reticulum of striated muscle from humans, copepods, ostracods, and barnacles shows a five-layered construction similar to that of a tight junction. Known functions of tight junctions and cisternal elements suggest that a membrane depolarization, conducted by the intermediate element, is transmitted to the cisternae by way of the triadic junction to cause a release of calcium ions from the cisternae.*

The triad, a constant component of the sarcoplasmic reticulum of vertebrate striated muscle, consists of two cisternae of the sarcoplasmic reticulum flanking a transverse tubule called the intermediate element or T-element. The unit is located either at the level of the Z-band or, as in human muscle, near the edge of the A-band, where it encircles each myofibril. The coapted membranes of the triad, that is, the lateral surfaces of the T-element and the mesial membranes of the cisternae, appear modified by virtue of increased electron opacity in addition to their maintaining a rather uniformly tight spacing. Several authors (*1–3*) have commented recently on peculiarities of this junctional region, pointing out both the existence of electron-opaque material in the space between the T-element and cisterna and the scalloping of the cisternal membrane (*4*); one author has suggested that the differentiated region of the dyad—the morphological equivalent of the triad in some invertebrates—may constitute a tight junction (*5*).

In the present study of human rectus abdominis muscle, further details of the junctional region were revealed by means of double-staining with potassium permanganate and lead citrate (*6*). The apposed membranes of the T-element and the cisterna have an average thickness of 45 to 60 Å and are separated by a space of about 110 Å. This gap, in turn, is bisected by a third layer, averaging 40 Å in thickness (Fig. 1). In a tangential section of the triad, this intermediate membrane follows the gap for as long a distance as the triad stays in the plane of section; in transverse section the intermediate membrane occupies the full width of the gap between the flattened, apposed

sides of the T-element and cisterna. Depending on the variable diameter of the T-element and the degree of its flattening, the width of the intermediate membrane may measure between 200 and 600 Å. With ordinary staining techniques the intermediate line is invisible. I have observed an intermediate line in the dyads or triads of *Cypridopsis vidua* (Crustacea, Ostracoda), *Macrocyclops albidus* (Crustacea, Copepoda), and *Balanus cariosus* (Crustacea, Cirripedia) after application of the same double-staining technique. In *M. albidus* and *C. vidua* the differentiated junctions occur only near the edges of the A-band, regions in which the apposed membranes show increased electron opacity with ordinary stains (*5, 7*), but not in the remainder of the sarcomere. As this specialization exists in widely divergent species, it may be a constant feature of the dyad or triad. Its possible functional significance merits some discussion.

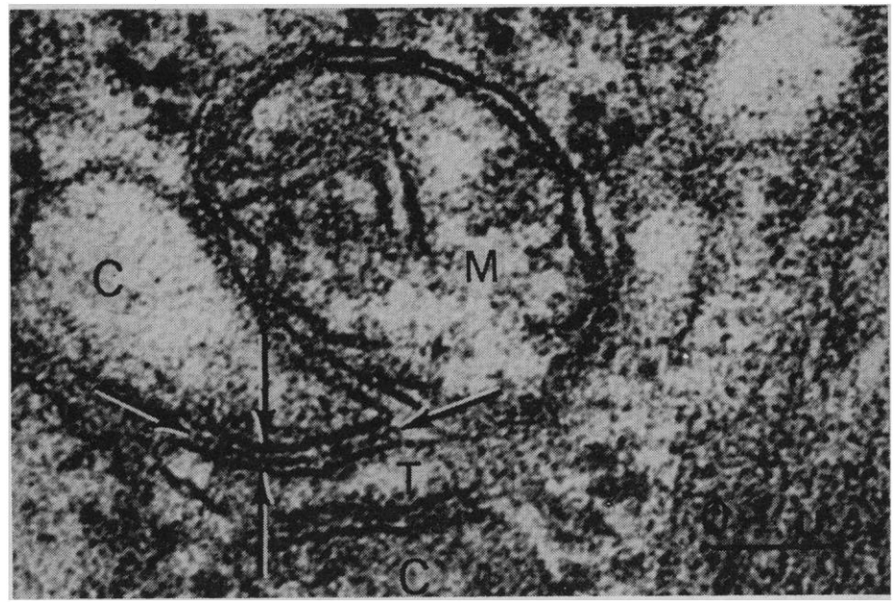

Fig. 1. Human striated muscle (rectus abdominis), fixed in OsO_4 buffered with *s*-collidine, embedded in Araldite, sectioned at about 450 Å, and stained with potassium permanganate and lead citrate. A triad composed of the intermediate element (*T*) and two cisternae (*C*) is shown. The apposed membranes are indicated by vertical arrows and the intermediate membrane by slanted arrows. Mitochondrion (*M*) (× 180,000).

Since the triadic junction appears to constitute the first instance of an intracellular junction between membranes, comparisons must be made with intercellular junctional complexes. The dyad or triad is an area of firm mechanical cohesion as observed in sectioned muscle (*5*) and in the isolated sarcotubular fraction (*8*). If a purely mechanical function of the junction is assumed, the intermediate line could be equated to the medium stratum (*9*) of the desmosome, or macula adhaerens (*10*). However, the intermembranous gap of the desmosome measures usually more than twice the width observed in the triadic junction. Also, other modifications associated with the desmosome are absent.

The triadic junction may be interpreted, on the other hand, as a tight junction, or zonula occludens (*10*). Most of the dimensions of the triadic junction are only slightly larger than those quoted (*10*) for epithelial tight junctions. Separation of the intermediate line into the two lamellae of the adjacent contributing unit membranes has not been observed clearly. The demonstration of such fusion is made difficult partly by the tenuous nature of the cisternal membrane and partly by the curvature of all involved surfaces in all planes. Mechanical cohesion of the coapted tubules might be served by a tight junction even more effectively than by a desmosome (*11*).

The possible existence of a tight junction also has some physiological implications in relation to the impulse-contraction coupling mechanism. The T-element is continuous with the sarcolemma in muscle of a number of arthropods (*1, 5, 7, 12*), in fish muscle (*2*), in mammalian heart muscle (*13*), and, as shown by an indirect approach, in frog muscle (*3*). Such membrane continuity most probably exists in striated muscle of higher vertebrates. The T-element also has been implicated in the inward conduction of excitation in both arthropod and vertebrate muscle (*14*). This tubule was considered originally to be the site of storage and release of Ca^{++}, the physiological activator of muscular contraction. However, recent histochemical studies indicate that the cisternal elements concentrate Ca^{++} (*15*). This finding is supported by the fact that Ca^{++} transport is dependent on adenosine triphosphate (*16*), by the histochemical demonstration of adenosine triphosphatase activity in the cisternae but not in the T-element (*17*), and by quantitative considerations (*3, 18*) which suggest that a considerably larger volume of vesicles for storing calcium ions is required than is provided by the T-elements alone. It was suggested previously that electrical activity is transmitted in some way from the T-element to the cisternae (*3, 5*); that such transmission might be achieved by a tight junction is indicated by parallel physiological and morphological studies on several species (*19*). Hence, if it is assumed that the triadic junction constitutes a zonula occludens, membrane depolarization could progress from the sarcolemma along the T-element into the cisternal elements with virtually no delay. Calcium release from the intracellular sarcoplasmic reticulum would, in this manner, remain coupled directly to the electrical surface activity of the muscle cell.

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16 December 1964

Synthesis of Block Oligonucleotides

Abstract. *The concentration of sodium chloride strongly influences primed polymerization of nucleoside diphosphates by polynucleotide phosphorylase. High concentrations of NaCl allow the addition of only a few nucleotide residues onto the 3′ end of the primer. When pure oligonucleotides are used as primers and when the product is fractionated, pure block oligonucleotides can be obtained.*

Highly purified preparations of polynucleotide phosphorylase from *Micrococcus lysodeikticus* show a nearly absolute requirement for oligonucleotide primer in the polymerization of nucleoside diphosphates (*1*). Singer, *et al.* (*2*) have shown that primers serve as chain initiators in the following manner:

$$\text{NDP} + \text{NDP} \longrightarrow \text{little or no reaction}$$
$$(\text{pN})_n + \text{NDP} \rightleftharpoons (\text{pN})_{n+1} + \text{P}_i$$

where NDP is nucleoside diphosphate, $(\text{pN})_n$ is polynucleotide, and P_i is inorganic phosphate. At saturating primer concentrations virtually every polynucleotide chain that is formed contains a primer residue at the 5′ end (*3*). Thus, in principle, this reaction should provide a simple method for the synthesis of short two-component block copolynucleotides. However, there are two major obstacles to the general application of this method which have until now restricted its practical use to the synthesis of very long homopolynucleotide chains containing two or three heterologous bases at the 5′ end of the chain (*4*).

The first obstacle is that the reaction is readily reversible. Thus the partial phosphorolysis of any of the primer oligonucleotides present in the reaction mixture must be prevented. The second difficulty arises from the fact that under normal conditions chain initiation is much slower than chain growth (*5*). As a result, the short, primed oligonucleotide intermediates which we seek are present only in very small amounts throughout the course of the polymerization (*3*, *6*). Both these problems can be eliminated simply by choosing the proper set of reaction conditions.

Polynucleotide phosphorylase from *M. lysodeikticus* was purified 90-fold (*1*). The fractions used in our experiments were virtually free of nuclease and contained negligible phosphatase activity. A nearly absolute requirement for primer was observed under normal reaction conditions, polymerization rates being increased 15 to 20 times by the addition of saturating amounts of primer (*7*, *8*). We have found that the scanty endogenous enzymatic activity which remains after extensive purification (*1*) is completely inhibited by high concentrations of NaCl (above 0.4*M*), a condition which prevails throughout all the polymerization reactions described below. This inhibition is observed not only at *p*H 8.1 but also at *p*H 9.3, and it is found even with relatively crude enzyme preparations which, in the absence of salt, are not stimulated by primer at the higher *p*H.

Oligocytidylic acid $[\text{C}(\text{pC})_5]$ was labeled with C^{14} and used for priming the polymerization of uridine diphosphate (UDP) at two different NaCl concentrations. The course of polymerization was followed by removing samples from the reaction mixture at intervals and spotting them on chromatography paper. The chromatograms were then developed in a solvent composed of 95 percent ethanol and 1*M* ammonium acetate (60 : 40 by volume). In this solvent unincorporated primer migrated away from the origin, while chains longer than eight residues were immobile (*9*). After the chromatograms were dried, origin spots were eluted, and the amount of polymer was determined spectrophotometrically; the amount of incorporated primer was determined by measurements of radioactivity. The ratio of these two parameters, (nucleotide residues) / (primer residues), gave the number-average degree of polymerization of the product.

The progress of the polymerization can now be followed in three ways (Fig. 1). The higher NaCl concentration (i) depresses the rate of UDP polymerization, (ii) leaves unaffected the complete utilization of primer, and (iii) yields a product of quite short chain length. Thus NaCl terminated chain growth at a very early stage so that primed oligonucleotides accumulated. This NaCl effect is not simply a general noncompetitive inhibition of the enzymatic active site; the rate of primer incorporation is, if anything, slightly increased, whereas the rate of chain growth is drastically reduced. Moreover, when the reaction is completed, only 5 percent of the nucleoside diphosphate originally present has reacted to

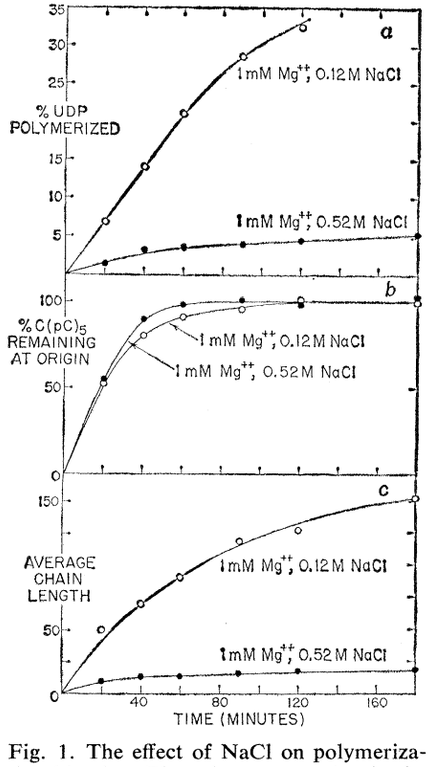

Fig. 1. The effect of NaCl on polymerization kinetics. The salt concentration in the reaction mixture was varied by the addition of NaCl. Incorporation of radioactive primer was determined by adding a portion of the eluates from the origin spots of the chromatogram to 20 ml of scintillator gel (Bray's solution (*12*), made 6 percent in Cab-O-Sil) and counting in a liquid-scintillation counter. The average chain length is calculated as the mole ratio of polymer residues to incorporated primer residues.