gave negative results. On the other hand, salmine and dihydrostreptomycin bind zinc ion strongly, the former with an apparent ACTH potency of 275 x on the basis of the calibration of Fig. 2. The binding of zinc ion by salmine is illustrated in Fig. 3. From the m/x intercept of the line, it is estimated that the binding approaches a limit of about 2 zinc ions/arginine residue. Analysis of the polarographic data on the dihydrostreptomycin-zinc complexing reaction by the De-Ford-Hume method (*5*) indicates an equilibrium of the type:

$$(DHS)^{3+} + Zn^{2+} \rightleftarrows (DHS\ Zn)^{5+},$$

with a constant of 43,000 at 25°; this result is, however, subject to a demonstration of reversibility of the reaction.

The only obvious common features of ACTH, salmine, and dihydrostreptomycin are (1) they are all cations under the conditions of study, and (2) they contain guanidine residues, present in the first two as arginine and in the third as streptidine. On the other hand, arginine alone gives no evidence of zinc binding; hence, if guanidine residues are indeed involved, some large common structural feature must be exerting influence. Further speculations do not appear justified at this time.

The binding of zinc by salmine has interesting electrophoretic consequences. The cathodic mobility of salmine (7.5 mg/ml in 0.1 *M* acetate buffer, pH 4.64) was observed to be 29×10^{-5} cm^2 sec^{-1} v^{-1}; addition of zinc at a concentration of 3.3×10^{-3} *M lowered* the mobility to 15×10^{-5} cm^2 sec^{-1} v^{-1}. Since the binding of zinc in these circumstances is favored by low pH, it is necessarily accompanied by an increase in net positive charge of the complexing ion; a *lowering* of cathodic mobility thus suggests a clumping of ions into large aggregates, at least in the case of salmine. The multiplicity of components in the relatively crude ACTH preparations at hand rendered electrophoretic data difficult to interpret, but a general qualitative lowering of cathodic mobilities was likewise observed in the presence of zinc ion.

It is of interest that, while this publication was in process, Holtermann and Heier (*6*) have reported the presence of abnormal amounts of zinc in crude whale corticotropin, and have suggested that the metal may be an inherent and significant constituent. However, in view of the observed binding of zinc ion by substances displaying no adrenocorticotropic activity (salmine and dihydrostreptomycin), there is not necessarily any direct connection between the two properties of ACTH. This gives further force to the warning against attempting any application of a given calibration curve to preparations of history different from those from which the curve was constructed.

Inherent in the application of the Klotz equation to the binding of zinc by ACTH is the assumption that the equilibrium concentration of free zinc ion is being measured; in reality, this may not be entirely the case, since a negative shift of half-wave potential is observed. Thus the wave may be in part due to the reduction of the ACTH-zinc complex; this is of little concern from the practical standpoint, however, provided that measurements are restricted in binding levels of less than 50%.

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"Mixed" Skeletal Muscle Fibers[1]

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In the posterior part of the lingual septum of rabbits, muscle fibers are found which are "mixed" in the sense that they show portions of nonmuscular material. These parts stain pink with hematoxylin-eosin, red with van Gieson's method, and take the aniline blue in Masson's trichrome stain. From these reactions it may be concluded that they are composed of collagen or reticulin, or some closely related substance. Morphologically, they appear finely reticular or, more often, fibrous, not unlike ordinary collagenous fibers. They may occupy only one part of the muscle fiber (Fig. 1) or mingle with the myofibrillae to a lesser or greater extent. This pattern can best be studied in cross sections. In longitudinal sections, the mixed fibers appear to have a core of sarcoplasm surrounded by a coat of collagenlike material of varying thickness. Such pictures are difficult to interpret, however.

When sections of skeletal muscle are stained by the McManus periodic acid–Schiff method (*1*) the sarcoplasm and myofibrillae show only faintly, if at all, but the sarcolemma stands out distinctly, just like basement (limiting) membranes of other structures. In "mixed" fibers of the lingual septum of the rabbit this method brings out the nonmuscular component very clearly, confirming the results obtained with other staining procedures. In cross sections, where the "collagenization" is slight, one can observe that it starts from the sarcolemma (Fig. 2), in that small septa or trabeculae penetrate into the muscle fiber proper. These partitions are either very fine, which may be a first stage, or coarse, until most of the fiber has been "collagenized" (Figs. 3, 4). The diameter of these "mixed" fibers is usually less than that of the ordinary muscle fibers in the tongue.

Since the replacement of the sarcoplasm starts apparently from the sarcolemma, it may be worth while to consider what sarcolemma really is. According to some authorities, it is composed of collagenous or

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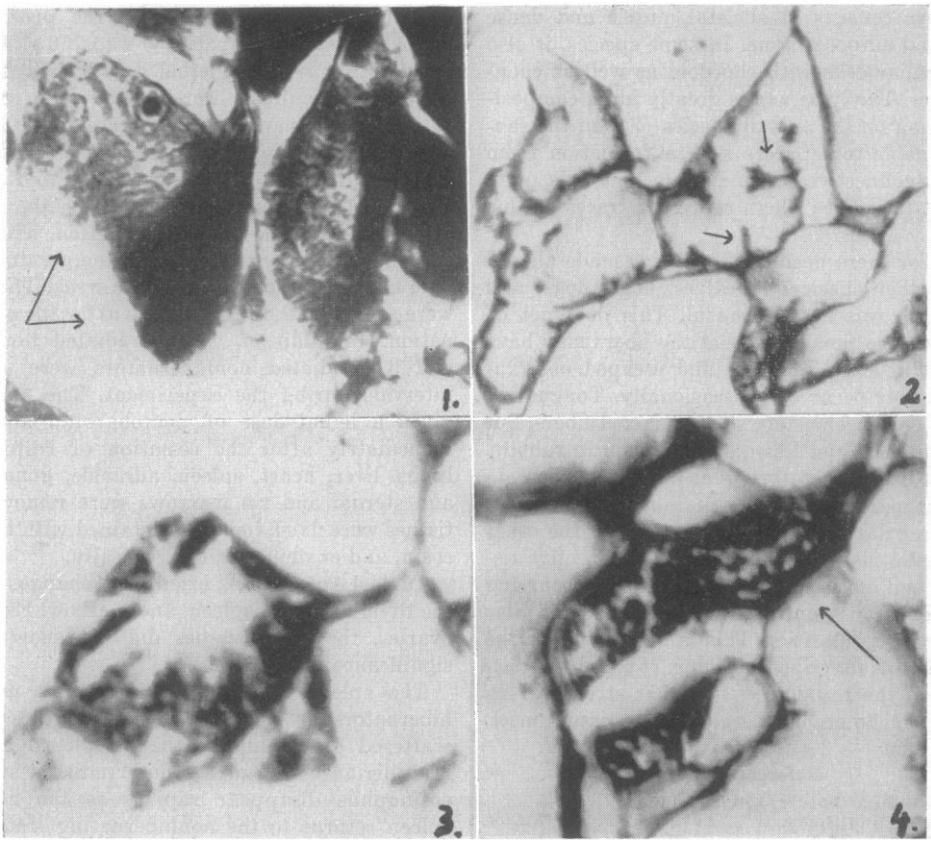

FIG. 1. Masson's trichrome stain. Arrows indicate a muscle fiber, the dark part of which is "collagenized" (blue). × 1275.
FIGS. 2, 3, and 4. McManus' periodic acid–Schiff stain. In Fig. 2 the arrows indicate invasion of a muscle fiber by collagenous partitions. In Fig. 3 the invasion is more marked, and in Fig. 4 (arrow) the muscle fiber is strongly collagenized. × 1500.

reticular fibers embedded in an amorphous matrix. For other workers, it is composed of two layers, an outer reticular and an inner plasmatic; for still others, of three layers, an outer amorphous, a middle of collagenous fibers, and an innermost granular layer (Draper and Hodge [2]). Pease and Baker (3) believe that sarcolemma is nothing but an amorphous cement layer, and Conte and Rieser (4) conclude that it is probably a lipoprotein and not collagen.

In the case of the "mixed" fibers, it should also be determined whether these may not represent only the musculotendinous junction. The morphology of this area has aroused great interest. There are two main views, one that the myofibrillae extend into the connective tissue of the tendon, the other that no such continuity exists, but that the tendon inserts on the sarcolemma or on the endomysium. According to several workers, notably Goss (5), projections of the reticular sarcolemmal net frequently invaginate the end of the muscle fiber proper in the form of slender spikes, spiral threads, or narrow septa. It would not, therefore, be unreasonable to assume that my observations of mixed fibers simply refer to musculotendinous junctions. Although at the present time such a possibility cannot be ruled out entirely, the following observations do not support it: (*a*) Often the sarcoplasmatic (interfibrillar) substance is almost completely replaced by material which gives staining reactions of collagen, but embedded in it are typical myofibrillae and muscle nuclei, the latter surrounded by some myogenous endoplasm. In many other fibers the collagenlike material occupies an entire sector of the fiber without mingling with, or projecting between, the myofibrillae. (*b*) Similar structures are not found elsewhere in the muscles of the tongue. (*c*) From longitudinal sections it appears that the collagenlike material is not restricted to those areas which could reasonably be regarded as muscular attachment but occurs throughout the whole or most of the fiber. Furthermore, (*d*) similar collagenization has been observed in degeneration atrophy of skeletal muscle of the limbs (6), where it constitutes a regressive phenomenon; and (*e*) the mixed fibers are frequently found far from any likely attachment, lying in adipose tissue, in which the septum is very rich.

Still another factor seems worthy of consideration. In the tongue of many animal species there occurs a core of tissue, separated from the septum proper and called "lyssa," because early anatomists erroneously believed it to have some connection with hydrophobia.

This structure consists of skeletal muscle and dense connective and adipose tissue. In some species, it also contains peculiar cells with chordoid as well as chondroid features. The lyssa varies greatly in its composition, depending on the animal species (*7*). In the rabbit, I was unable to separate such a formation from the ordinary connective tissue septum, but it is quite possible that the mixed fibers represent traces of this structure.

A search for these peculiar fibers was made also in tongues of other species. In calves, hogs, dogs, and rats the search was not successful. This does not of course exclude the possibility that the fibers may have been present in small numbers and escaped observation, or that they occur only occasionally. Tongues of mice and of golden hamsters showed mixed fibers, but in smaller numbers and less distinctly than in rabbits.

It appears likely, therefore, that the mixed fibers do not represent merely a musculotendinous junction, but rather an irregularity in morphogenesis. On the other hand, it may be that the projections of connective tissue into the end of the muscle fiber proper, occurring at musculotendinous junctions elsewhere, should also be considered irregularities. This is supported by the morphogenesis as described by Long (*8*) and is in accordance with the recognized fact that structural irregularities tend to occur where different tissues meet.

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Histological Changes in the Tissues of the Hibernating Marmot Following Whole Body Irradiation

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The delay in the lethal processes following x-irradiation of the hibernating marmot has been described in another paper from this laboratory (*1*). Cytological studies of the blood of irradiated hibernating marmots failed to reveal any significant changes until after the termination of hibernation (*2*). The present study was undertaken to investigate the histological changes in the tissues of the irradiated hibernating marmot.

The induction and maintenance of hibernation, as well as the method of irradiation, have already been described. The only change in the procedure is an increase in the dose from 650 r to 750 r. This exceeds a previously reported lethal dose by about 100 r (*1*).

Seventeen animals were placed in a cold room at 4.5° C. Eight marmots were irradiated after they had been in hibernation for about 1 week, and the others were left unmolested. Two irradiated and 2 nonirradiated marmots were sacrificed after the fourth and after the eighth week of hibernation. The remaining animals were returned to room temperature to terminate hibernation after the eighth week. These marmots were sacrificed 2, 5, and 7 days after the end of hibernation. In addition, 4 nonirradiated nonhibernators and 3 irradiated nonhibernators were sacrificed at intervals during the experiment. The marmots were given a lethal dose of pentobarbital and autopsied immediately after the cessation of respiration. The lungs, liver, heart, spleen, adrenals, gonads, thymus, and sternal and rib marrows were removed, and the tissues were fixed, imbedded, stained with hematoxylin-eosin, and examined microscopically.

Table 1 summarizes briefly the changes observed in the thymus, testes, spleen, and marrow. Except for the ovaries, the other tissues did not show changes of significance.

The spleens of the irradiated and nonirradiated hibernators are similar except for the presence of scattered eosinophils in the peripheral areas of the Malphigian bodies of the nonirradiated spleen. These eosinophils disappear rapidly as the nonirradiated spleen returns to the nonhibernating status after the end of hibernation.

The bone marrows of the irradiated and nonirradiated hibernators are similar except for the presence of a high percentage of eosinophils in the nonirradiated marrow. This eosinophilia disappears as the marrow returns to the nonhibernating condition.

The thymus glands of the irradiated and nonirradiated hibernator are similar during hibernation. The nonirradiated thymus resumes the nonhibernating condition rapidly after the end of hibernation, whereas the irradiated thymus remains unchanged.

The testes of the nonirradiated hibernator are similar to those of the nonhibernator, whereas those of the irradiated hibernator show loss of spermatogonia and spermatocytes. The ovarian changes are omitted from the table because of an insufficient number of specimens. In general, the changes parallel those of the testes, with loss of the ovogonia and ovocytes.

The retardation in the development of radiation damage in the hibernating marmot resembles that observed in poikilothermic animals at low temperatures, but it is not comparable. Benedict and Lee (*3*) found that a rectal temperature of less than 3° C is fatal to the marmot, but that poikilotherms frequently survive near zero temperatures. They also determined that the heat production of the hibernating marmot is more than twice that of a large poikilotherm at the same temperature.

The seasonal variation in the marmot testes has