8,20 (1 H, d, $J \sim 1$ Hz) H der Formylgruppe; 8,09 (1 H, s) H im Oxazolring; 7,42 (1 H, d, $J = 8$ Hz) und 7,08 (1 H, d, $J = 8$ Hz) aromat. Protonen.

Mit Brenztraubensäure-methylester[12] reagierte IX zum 6gliedrigen Oxazin-analogen III; (±)-2-Methyl-3-oxo-1-aza-4-oxaöstra-1,5(10),6,8-tetraen-17β-ol, $C_{17}H_{19}NO_3$, besitzt den Smp. 156–157°. NMR ($CDCl_3$): 7,09 (2 H, s) aromat. H; 3,92 (1 H, m) H an 17α; 2,52 (3 H, s) CH_3-Gruppe im Oxazinring; 0,62 (3 H, s) CH_3-Gruppe an C-18.

Anellierungsreaktionen mit dem 8-Aminophenol XI führten zu Anthrasteroidderivaten. So resultierten zum Beispiel aus XI und *Ortho*ameisensäure-triäthylester das Benzoxazol XIIa und das entsprechende 17β-Formiat XIIb. (±)-2,3,3a,4,5,10b-Hexahydro-3β-hydroxy-3a-methyl-trans-1H-indeno-[4,5-f]benzoxazol (XIIa). $C_{15}H_{17}NO_2$, schmilzt bei 115–117°. NMR ($CDCl_3$): 8,04 (1 H, s) H im Oxazolring; 7,47 (1 H, s) und 7,38 (1 H, s) aromat. Protonen; 3,98 (1 H, m) H an 3α; 0,62 (3 H, s) CH_3-Gruppe. Der entsprechende Formylester XIIb zeigte Smp. 158–165°. NMR ($CDCl_3$): 8,16 (1 H, s) H der Formylgruppe; 8,03 (1 H, s) H im Oxazolring; 7,46 (1 H, s) und 7,38 (1 H, s) aromat. Protonen.

Biologische Wirkung. Die neuen Equileninanaloga I–III sowie einige ihrer Derivate zeigten in der pharmakologischen Untersuchung keine oder nur unbedeutende östrogene Aktivität[13].

Summary. The synthesis of heterocyclic equilenin-analogues is described. The new compounds are devoid of any significant estrogenic activity.

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Distribution of Proteins in the Extracellular Space of Muscles and the Possible Role of Mucopolysaccharides

It has been difficult to estimate the volume of the extracellular space of muscle because the uptake of various solutes which are generally assumed not to penetrate into the fibers varies widely. It is desirable, therefore, to study the problem by a more direct approach. Information on this question is important also to solve an apparent paradox. It is generally accepted that the ground substance between tissue cells contains mucopolysaccharides. As shown by OGSTEN and PHELPS[1] these substances exclude sterically large molecules such as proteins and inulin in concentrations as low as 0.2%, but it is also known that proteins, even molecules as large as those of ferritin, readily diffuse into the extracellular space. To study these problems muscles were equilibrated with concentrated solutions of proteins and their distribution was determined with the electronmicroscope.

Most of the work was done with the stomach of the frog, but the same results were obtained with the sartorius and ventricle of the frog and with the taenia coli of the guinea-pig. The muscles were immersed in Ringer solution containing about 20% hemoglobin (Hb), myoglobin (Mb) or ferritin for 45–120 min. In solutions of Hb and Mb normal spontaneous contractions of stomach muscle continued for more than 2 h, demonstrating that muscles were not injured. Ferritin solutions were prepared as described by HUXLEY[2]. The muscles were fixed in a stretched condition in glutaraldehyde and osmium tetroxide and embedded in Dow epoxy. Thin sections were stained in uranylacetate and lead citrate and examined with a Hitachi electronmicroscope.

That proteins penetrate into muscles is shown by the fact that after immersion in solutions of Hb or Mb most of the extracellular space appeared uniformly darker than the fibers in stained sections. However, in all types of muscles studied long filaments of uniform diameter remained unstained (Figures 1, 2 and 3), probably because they contain mucopolysaccharides and, therefore, exclude proteins. They will be called mucofilaments. They differ from other known structures of the extracellular space, specifically from collagen fibrils, by their appearance, size and distribution as shown by the following description.

(1) Collagen fibrils take up stain and have sharp outlines, while mucofilaments remain unstained and appear fuzzy. (2) The diameter of the filaments is about 500 Å if shadowed with Hb (single measurements varying between 450 and 600 Å) and 300 Å (with a range of 250 and 350 Å) when Mb is used. The difference between Hb and Mb evidently is due to partial penetration of Mb into the filaments. Collagen fibers of the frog stomach, on the other hand, generally have a diameter of about 220 Å, never more than 300 Å, confirming a previous report[3]. (3) In frog stomach muscle collagen fibrils are mainly present near the surface and near blood vessels, while mucofilaments are found throughout the extracellular space, except in regions less than 1000 Å wide. Due to their staining, collagen fibrils generally cannot be seen in Hb and Mb treated muscles, but in underdeveloped micrographs both structures can sometimes be seen side by side (Figure 2).

If the filaments consist mainly of mucopolysaccharides it must be assumed that these substances are arranged in a highly oriented form. That they are normally in this

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[2] H. E. HUXLEY, Nature *202*, 1067 (1964).
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state in the ground substance of tissues, not in an amorphous form, has been previously suggested by K. MEYER[4] because of the regular spacing of their repeating units.

In Hb treated muscles a layer about 150 Å thick directly above the plasma membrane also appears light (Figure 3), probably because it also contains mucopolysaccharides and, therefore, excludes proteins. This layer is smooth and uniform and appears as an integral part of the cell wall, but it is absent in the pinocytotic vesicles which are completely filled with the protein.

Ferritin was found to penetrate into the extracellular space of the sartorius, confirming HUXLEY[2], also into stomach muscle and taenia coli. The molecules are not distributed at random in the extracellular space, but are excluded from some regions, probably corresponding to the mucofilaments.

It has been previously suggested that the mucopoly saccharides might determine the distribution of some solutes in the extracellular space. Therefore the cross-sectional area of mucofilaments of Hb treated muscles the volume of the filaments was determined in suitable sections. It was tentatively estimated to be about 26% of the extracellular space, or 6% of muscle volume. If the filaments excluded not only protein but also inulin completely while smaller molecules, such as sugars, could penetrate, some of the difficulties in estimating the volume of the extracellular space would find a simple explanation. However, the fact that the filaments appear thinner when shadowed with Mb than with Hb makes it unlikely that they provide a complete explanation of the differences in the distribution of various solutes in muscle[5–8].

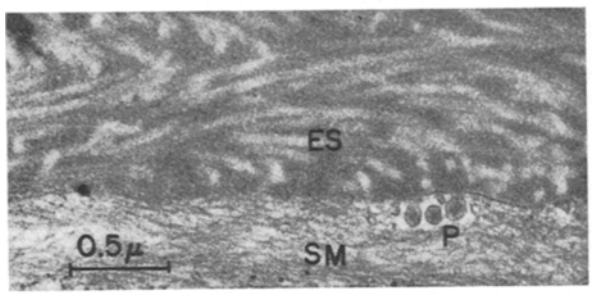

Fig. 1. Stomach muscle of frog immersed in Hb Ringer solution for 45 min and sectioned longitudinally shows many mucofilaments in extracellular space (ES). P, pinocytotic vesicles; SM, smooth muscle fiber. × 44,000.

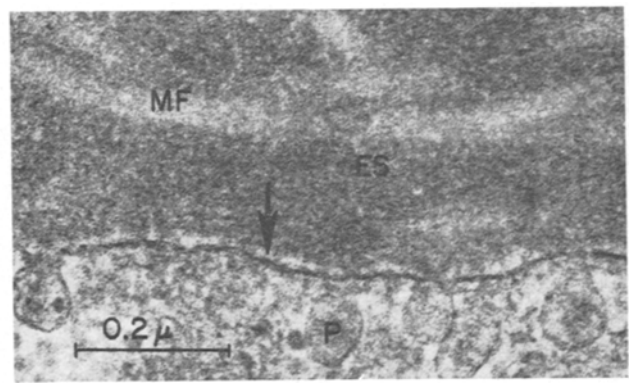

Fig. 3. A portion of a longitudinal section of frog stomach muscle immersed in Hb Ringer solution for 45 min. ES, extracellular space with mucofilaments (MF); P, pinocytotic vesicles. Arrow points to plasma membrane with coat. × 126,000.

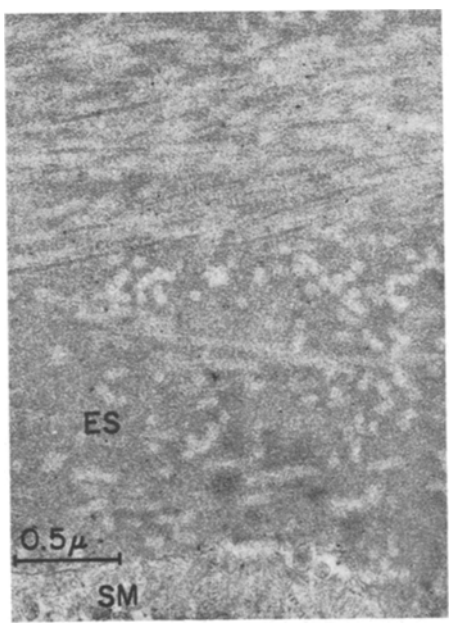

Fig. 2. Stomach muscle of frog immersed in Hb Ringer solution for 30 min. Crossections near surface. Lower part of extracellular space shows collagen fibers (dark) and mucofilaments, upper part mainly the latter. SM, smooth muscle fiber. × 55,000.

Zusammenfassung. Muskeln wurden in Ringerlösung mit 20% Hämoglobin oder Myoglobin equilibriert. In gefärbten Schnitten erscheint der extrazelluläre Raum im Elektronenmikroskop dunkel, aber lange Filamente, Mukofilamente genannt, bleiben ungefärbt. Diese haben einen Durchmesser von 500 oder 300 Å, je nachdem ob das erste oder zweite der Proteine verwendet wurde. Diese Struktur unterscheidet sich deutlich von Kollagenfibrillen durch ihre diffuse Oberfläche, ihre Dicke, ihre Färbung und andere Eigenschaften und besteht wahrscheinlich aus Mukopolysacchariden.

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