This finding prompts the suggestion that ANS binding sites might be related to the active site regions of the enzyme molecule. Yeast GAPD is known to consist of 4 identical subunits[9] and to bind 4 equivalents of NAD^+ per mole of protein[10]. In order to determine whether ANS is capable of binding at the active site of GAPD, the dye was tested as an inhibitor of the enzyme activity. The effect of ANS was studied with varying concentrations of NAD^+. The data in Figure 3 show that ANS is a competitive inhibitor with respect to the coenzyme, suggesting an interaction at a common site. The inhibitor dissociation constant found in these studies ranged from $5 \times 10^{-5} M$ to $6 \times 10^{-5} M$. These results are in good agreement with the fluorescence titration data.

A conclusion may be drawn from these results that ANS bound to GAPD is located at or very near the coenzyme binding site, suggesting the existence of some nonpolar region in this site. However, since ANS is an anion the possible role of ionic interactions between ANS and protein molecule must also be considered. We found, in fact, that inorganic phosphate was rather effective in displacing ANS from its complex with GAPD, presumably due to a competition for a common positively charged group on the protein surface. Such a group must be located in close proximity with the non-polar region in the active site of GAPD which also participates in ANS binding.

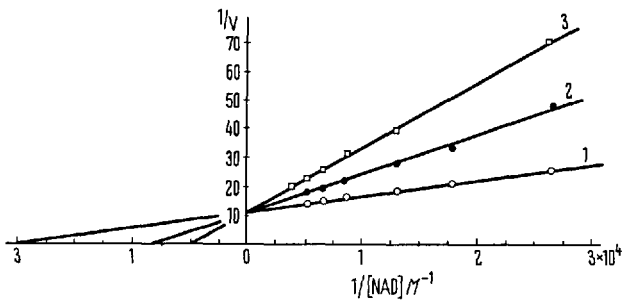

Fig. 3. Inhibition of GAPD activity by ANS with respect to varying NAD concentrations. The reaction mixture (3 ml) contained $0.1 M$ glycine-NaOH buffer pH 8.2, 5 mM EDTA, 5 mM disodium arsenate, $3 \times 10^{-4} M$ glyceraldehyde-3-phosphate, $1.2 \times 10^{-8} M$ GAPD and $0.38 \times 10^{-4} - 2.66 \times 10^{-4} M$ NAD. 1 — no ANS, 2 and 3 — $9.0 \times 10^{-5} M$ and $15.6 \times 10^{-5} M$ ANS respectively, 20 °C. Velocity is expressed in arbitrary units.

Выводы. Связывание 1-анилино-8-нафталин сульфоната (АНС) с дрожжевой глицеральдегид-3-фосфатдегидрогеназой (ГАФД) приводит к возрастанию квантового выхода флуоресценции АНС и сдвигу максимума эмиссии на 30 нм в сторону коротких длин волн. При молярном отношении [АНС]:[ГАФД] < 10:1 на 140000 г белка связывается 3 ± 1 моля АНС с константой диссоциации комплекса $5 \times 10^{-5} M$. АНС угнетает энзиматическую активность конкурентно с НАД, величина Ki при этом соответствует $5 \times 10^{-5} M - 6 \times 10^{-5} M$. Предполагается, что связывание краски происходит в области активного центра ГАФД.

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The ATPases of the Sarcolemma from Skeletal Muscle in Experimental Myotonia

By treating rats with 20,25-diazacholesterol[1,2] or with 2,4-dichlorophenoxyacetate[3] the symptoms of myotonia are induced, i.e. a delayed relaxation of muscle after a contraction and repetitive firings in electromyogram.

It is in general accepted that myotonia is a phenomenon of the muscle membranes. In earlier studies we were able to demonstrate that in rats treated with 20,25-diazacholesterol the calcium pump of the sarcoplasmic reticulum is markedly affected[4]. Rats with induced myotonia also showed a significantly altered fatty acid pattern of the phospholipids and of the cholesterol esters of the sarcoplasmic vesicles[5].

However, these findings cannot explain the repetitive firings in electromyogram, which presumably are due to changes in the external membrane of muscle fibers, the sarcolemma. This membrane is very important for the transmission of electrical activity from the neuromuscular junction over the exterior of the muscle fibers. In the sarcolemma, too, from 20,25-diazacholesterol-treated rats, there are changes in the fatty acid composition of phospholipids and cholesterol ester[5].

The sarcolemma from skeletal muscle of rats contains a Mg^{++} stimulated ATPase and an ATPase stimulated by Na^+ and K^+ in the presence of Mg^{++}[6]. We investigated the activities of these ATPases in the sarcolemma from the skeletal muscles of rats with 20,25-diazacholesterol induced myotonia, and we further determined the inhibition of the ATPases by 2,4-dichlorophenoxyacetate.

Female Wistar rats were given 10 mg 20,25-diazacholesteroldihydrochloride daily for a period of 6 weeks by oesophageal tube. The induced myotonia was demonstrated by electromyogram. The rats – always a myotonic and a normal one at the same time – were decapitated and the sarcolemma was isolated from the muscles of the hind legs according to the method of MCCOLLESTER[7,8] and ROSENTHAL et al.[9] as modified by PETER[6].

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Table I. Activities (μmoles P_i × mg protein^{-1} × min^{-1}) of the ATPases of the sarcolemma from normal and 20,25-diazacholesterol treated rats

	n	$(Na^+ + K^+)$ ATPase + (Mg^{++}) ATPase	(Mg^{++}) ATPase
Control rats	14	0.241 (0.206–0.260)	0.164 (0.135–0.200)
Myotonic rats	14	0.158 (0.152–0.165)	0.123 (0.088–0.146)

Conditions of assay: 3 m*M* [*Tris*-ATP], 20 m*M* [TES] pH 7.4, 1 m*M* [$MgCl_2$], 65 m*M* [choline chloride] and 0.1 mg sarcolemmal protein/ml in a total volume of 2 ml. T = 37 °C. For the determination of the $(Na^+ + K^+)$ ATPase + the (Mg^{++}) ATPase 60 m*M* [NaCl] and 5 m*M* [KCl] were added to the solution instead of the choline chloride[6]. After 5 and 10 min, aliquots were pipetted into an equal volume of 10% trichloroacetic acid and the P_i was determined according to FISKE and SUBBAROW[10]. n = number of experiments; the extreme values are shown in parentheses.

Table II. Inhibition of the ATPases of the sarcolemma from normal and 20,25-diazacholesterol treated rats by 2.5 m*M* 2,4-dichlorophenoxyacetate

	n	$(Na^+ + K^+)$ ATPase + (Mg^{++}) ATPase	(Mg^{++}) ATPase
Control rats	10	54% (34–79%)	64% (49–73%)
Myotonic rats	10	61% (44–73%)	58% (36–73%)

n = number of experiments; the extreme values are shown within parentheses.

Table I gives the results of ATPase determinations for control and myotonic rats. The values for the control rats are in the same region as the data given by PETER[6]. For myotonic animals, however, a decrease is found in the activities of both the (Mg^{++}) ATPase and the $(Na^+ + K^+)$ + (Mg^{++}) ATPase. The activity of the former is 75% that of the control, while the activity of the $(Na^+ + K^+)$ + (Mg^{++}) ATPase is only 66% that of the control. From these results it can be concluded that the $(Na^+ + K^+)$ ATPase in myotonic rats is more inhibited than the (Mg^{++}) ATPase. In further experiments we measured the inhibition of the sarcolemmal ATPases by 2,5 m*M* 2,4-dichlorophenoxyacetate. As can be seen from Table II, this concentration, which is able to induce myotonia in rats, causes a 60% inhibition of the ATPases from the sarcolemma of normal and of myotonic rats.

This results give further evidence that the induction of myotonia by 20,25-diazacholesterol and by 2,4-dichlorophenoxyacetate is connected with alterations in the membrane system of muscle fibers. It remains to be proved whether there are comparable changes in human hereditary myotonia.

Zusammenfassung. Die Aktivität der (Mg^{++})- und der $(Na^+ + K^+)$-stimulierten ATP-ase des Sarkolemm von Ratten mit Myotonie durch 20.25-Diazacholesterin ist gegenüber den Kontrollratten eindeutig erniedrigt. 2,5 m*M* 2,4-Dichlorphenoxyacetat im Bestimmungsansatz hemmt beide ATP-asen um ungefähr 60%.

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A Structure Resembling Proteinpolysaccharide Complexes in Preparations of Mitochondrial DNA from Mouse Liver

The presence of polysaccharides as a common contaminant in phenol-extracted DNA has been reported by various authors[1–3]. This also applies to mitochondrial DNA (M-DNA), which after phenol-extraction and centrifugation in CsCl gradients has been found to contain a contaminant of high UV-absorbancy[4–6]. On the other hand, it is also known that phenol-extracted polysaccarides are always contaminated by DNA[7]. After isopycnic centrifugation in CsCl density gradients, this material accumulates together with M-DNA at a buoyant density of 1.68–1.71 g/cm^3. However, separation of polysaccharides from M-DNA has been achieved either by the addition of ethidium bromide to CsCl density gradients[8], or by purification of M-DNA through MAK-columns (MAK = methylated albumin on Kieselguhr)[9].

During our work on the electronmicroscopical characterization of DNA extracted from mouse liver mitochondria, we found at a frequency of about 0.5% 'lampbrush-like' structures showing a characteristic configuration. They consist of a central filament, 50 Å thick, and unbranched side chains, 80–110 Å thick, which are fully extended and insert at the central filament at inervals of 200–300 Å. The side chains which possess a terminal thickening of 170–210 Å have a rather constant length 1574 ± 135 Å (S.D.). On the other hand, the central filaments vary in length (Figure 1), but there is a close correlation between the length of the central filament and the number of attached side chains (Figure 2). Since the method of spreading leads to a two-dimensional configuration of the molecules, their normal configuration is unknown. Likewise, it is impossible to deduce from the electron micrographs whether or not the filamentous struc-

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