Results. Figure 2 shows a typical experiment. The starting resistance of the film was 100 $M\ \Omega\ cm^2$. As indicated in the figure, the addition of enzyme to the aqueous phase does not change the resistance, but as soon as acetylcholine (ACh) is added to the aqueous phase a drastic change can be observed, the resistance dropping by two orders of magnitude. As the substrate is hydrolyzed, the resistance increases slowly again to almost the same magnitude as before. This effect may be obtained over and over again. The limiting factor is the fragility of the membranes.

As a control, the enzyme was inhibited by eserine, so that it was prevented from forming the enzyme-substrate complex. In this case the bilayer becomes insensitive to acetylcholine (see Figure 2).

The experiment was also carried out with enzyme preparations of different specific activities. The extension of the observed effects – namely the number of coulombs passing through the membrane during impedance change – strictly dependent on the specific activity as long as the experimental conditions like protein concentration 1 µg/ml), pH, temperature and the amount of ACh added (5 µg/ml) have been kept constant, thus demonstrating that the change in permeability is closely related to AChase activity.

Another interesting result was the fact that adding the enzyme at one side of the already formed bilayer, then adding the substrate to the other side of the bilayer after 10 min, also produced a drop in resistance. This fact indicates that the enzyme is built into the membrane in such a way that the molecule is accessible from both sides and symmetrically oriented. In other words, these experiments suggest a mosaic structure for the lipid-protein membranes investigated similar to that which GREEN[10] proposes for the living cell.

Discussion. It is becoming more and more evident that the study of the behaviour of membrane-bound enzymes in solution can lead to misinterpretation of their mode of action. These enzymes do not exist in the membrane in a state of autonomy but form an indissociable continuum with the rest of the membrane, and it therefore seemed interesting to study AChase on a definite structure similar to the natural membrane, by incorporating the enzyme into bilayers. Though these bilayers naturally exhibit a much lower order of complexity than natural membranes, it can be assumed that they reflect a general design at least with regard to the basic structural elements of membranes.

The experiments demonstrated that bilayers containing a very small amount of AChase from electrophorus electricus reacted to the addition of ACh by a rapid and transient increase in conductance. In other words, a phenomenon was observed similar to that observable in excitable membranes.

We could further demonstrate the amount of coulombs passing through the membrane during impedance change definitely depends on the enzymatic activity present on the membrane. These findings demonstrate that AChase alone, together with its natural substrate ACh, is sufficient to produce a change in permeability to small ions in artificial membranes.

The assumption of a separate cholinergic receptor molecule independent of the $\alpha_2\ \beta_2$ complex of AChase seems to be unnecessary in these experiments. The data rather support a hypothesis[5] which assumes that α and β subunits of AChase fulfil different functions: the α-unit would represent the catalytic part and the β-unit the receptive part. The whole sequence of events leading to depolarization of the membrane would occur on the same complex, a condition which seems to be important with regard to the extreme rapidity of the processes. The individual steps would be as follows: the β-unit recognizes acetylcholine and interacts with it to form a complex which induces a conformational change that brings the catalytic site of the α-unit into close proximity with acetylcholine bound to the receptive site of the β-unit and is thereby in a position to hydrolyze ACh and reverse the whole effect.

Zusammenfassung. Es konnte gezeigt werden, dass Bilayers, die eine ganz geringe Menge Acetylcholinesterase enthalten, auf die Zugabe von Acetylcholine mit einer Änderung der Permeabilität gegenüber kleinen Ionen reagieren. Dieser Effekt ist reversibel und die Grösse des Effektes hängt bei konstanten Versuchsbedingungen nur mit der spezifischen Aktivität der Enzympräparation zusammen.

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Glycogen Content in Young and Old Rats Liver and Muscles

It was found that in muscles of aged rats the percentage of phosphocreatine to the total creatine is lower than in young animals[1]. Phosphocreatine is used for the rephosphorylation of ADP to ATP in the muscle. It was also found that by an increase of glucose feeding, the phosphocreatine values of old animals muscles return to those of young ones[2]. This may be related to changes in glycogen metabolism, and therefore the glycogen content of muscles and liver in young and old rats was compared.

Materials and methods. Wistar albino rats from the Institute's own colony were used. The preparation of muscles followed the former description[2]. The glycogen content was estimated by the method of VAN DER KLEIJ[3].

To test the influence of work, the animals were transferred into a narrow container with water at 25 °C[1]. After exhausting swimming, they were immediately killed and the glycogen content of liver and white and red muscles was estimated. The significance of the differences of results between young and old animals, and red and white muscles was checked by Student *t*-test.

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Results. Results obtained from resting animals are summarized in Table I for young and in Table II for old animals. Males and females, and also white and red muscles, and livers are differentiated.

Table I. Glycogen content in muscle and liver in young rats at rest

Age (Month)	Sex	Body weight (g)	Glycogen content in g/100 g wet tissue weight		
			White muscle	Red muscle	Liver
5	♀	250	0.28	0.13	4.76
5	♀	200	0.21	0.13	5.44
5	♀	260	0.28	0.20	6.54
5	♀	295	0.21	0.16	3.40
5	♀	420	0.32	0.15	4.96
6	♀	200	0.29	0.24	7.01
6	♀	380	0.30	0.07	5.62
Mean $p<0.001$			0.27±0.05	0.17±0.03	4.53±1.46

Table II. Glycogen content in muscle and liver in old rats at rest

Age (Month)	Sex	Body weight (g)	Glycogen content in g/100 g wet tissue weight		
			White muscle	Red muscle	Liver
20	♀	470	0.11	0.08	1.96
20	♀	390	0.19	0.23	1.53
20	♀	300	0.06	0.20	2.50
23	♀	260	0.10	0.10	1.43
23	♀	485	0.06	0.09	1.49
23	♀	560	0.17	0.05	1.32
23	♀	760	0.12	0.09	0.54
23	♀	400	0.09	0.04	2.02
24	♀	380	0.16	0.05	1.94
24	♀	230	0.03	0.01	0.13
25	♀	350	0.18	0.21	2.23
26	♀	455	0.14	0.05	1.29
27	♀	250	0.09	0.10	2.47
29	♀	255	0.05	0.02	0.18
30	♀	200	0.12	0.04	1.54
Mean $p<0.05$			0.11±0.05	0.09±0.07	1.51±0.75

Table III. Changes in glycogen content in white muscle after exhausting work

Age (Month)	Sex	Body weight (g)	Glycogen content in g/100 wet weight
5	♀	260	0.15
5	♀	270	0.12
5	♀	250	0.16
5	♀	260	0.12
			Mean 0.14
26	♀	320	0.06
26	♀	325	0.05
26	♀	260	0.08

Table I shows that in young and upgrown animals white muscles contain significantly ($p < 0.001$) more glycogen than red muscles. This confirms the results of GUTMAN[4] and LÉONARD[5]. However, in our experiments this difference is not significant in the animals older than 20 months. Table I shows further that in muscle, as well as in liver, the glycogen content in old animals is smaller. The differences are significant ($p < 0.05$).

Since one possible objection may be that the absorption from the intestine for glucose may decrease in old rats, we repeated the experiments of KLIMAS[6] and found, in accordance with him, no difference between glucose absorption in 5 young (5 months) and 3 old (22–26 months) animals. Thus the difference of glycogen content between young and old animals cannot be caused by a diminished absorption of glucose.

After exhausting work (Table III) the glycogen content in white muscle decreases both in young and old animals, as a comparison of Tables I, II and III shows.

Discussion. The decrease of liver glycogen may be caused by a decreased fixation of glycogen in this organ. LOHMANN and BOCK[7] observed a smaller increase of blood sugar in old age in the test for glucagon and supposed that it is explained by a decreased capacity of liver for fixation of glycogen.

According to NIKITIN et al.[8] the quantity of glycogen in muscles decreases in old age, while BERTOLINI[9] and HORVATH[10] found no differences in different ages. It may be supposed that the lower content of glycogen in the muscle fibers is caused by a diminished entrance of glycose through the cell's membrane, for which insulin is necessary. SHOCK and ANDRES[11] concluded that in old men a decreased tolerance for glucose is the result of a decreased sensibility of the beta cells of the pancreas, and BULLOCK et al.[12] reached the conclusion that in aged rats an atrophy of the pancreas is generally seen. It will need further experiments to explain the decrease of glycogen in liver and muscles in old age.

Zusammenfassung. Der Glykogen-Gehalt von jungen und alten, weissen und roten, ruhenden und arbeitenden Muskeln sowie in der Leber von Ratten wurde bestimmt. Dieser vermindert sich im Alter sehr stark und sinkt weiter bei Arbeit der Muskeln.

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