

Short Communications and Preliminary Notes

ELECTROPHORETIC STUDY OF THE "EASILY SOLUBLE" PROTEINS OF NORMAL AND ATROPHIED RABBITS' MUSCLES AND OF NORMAL HUMAN MUSCLES

by

A. M. F. H. HAAN

*Laboratory of Physiological Chemistry, The University,
Utrecht (Netherlands)*

The composition of the mixture of the "easily soluble" proteins of various rabbits' muscles and of human skeletal muscles—*viz.* the proteins extracted by KCl-phosphate of ionic strength 0.13 and pH 7.15—was determined by electrophoresis as described by BOSCH¹.

The experiments on rabbits were carried out with the vastus lateralis and the gastrocnemius. The differences between two normal contra-lateral muscles were always scarcely perceptible, even when they were removed with a week interval. Fifteen rabbits were subjected to different treatments causing muscular atrophy. As a control the normal contra-lateral muscle was always used.

All atrophical muscles investigated showed the same electrophoretic pattern, independent of the cause of atrophy, but differing very much from the normal pattern. In accordance with CREPAX² we found that in atrophy, caused by dissection of the motoric nerve, the percentage of component II (called myoalbumin by the DUBUISSON school) had increased, while the percentages of components VII, VIII and IX had decreased (percentages of the total surface of the diagram). It further appeared that also the percentage of the level part of the diagram between components II and V had distinctly increased. Similar results were obtained after vitamin E deficient feeding, starvation and immobilisation.

Independent of the cause of the atrophy an atrophied muscle which had lost about half its original weight contained per g about twice the original amount of protein II and of the proteins indicated by the level part of the diagram, while

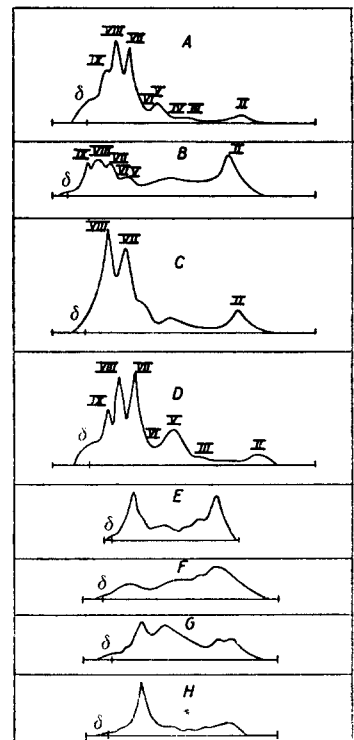


Fig. 1. Electrophoretic patterns of extracts of different muscles. Buffer, used for extraction, dialysis and electrophoresis: 0.05 M KCl + 0.023 M Na_2HPO_4 + 0.01 M KH_2PO_4 ($\mu = 0.13$, pH = 7.15). Ascending boundaries.

- A. normal rabbit's skeletal muscle
- B. atrophied rabbit's skeletal muscle
- C. normal human skeletal muscle
- D. rabbit's skeletal muscle in rigor
- E. rabbit's heart muscle
- F. rabbit's diaphragm
- G. rabbit's stomach muscle
- H. rabbit's uterus

the total amount of easily soluble proteins per g had decreased to some extent. Thus it appeared that protein II and the components of the level part of the diagram had only increased in relative proportion to the other components, and that their absolute amounts in the total muscle had remained constant.

Rigor mortis also caused a distinct change in the diagram as compared to the fresh contralateral muscle. The percentage of component V had increased 2 to 3 fold, while the percentages of components VIII and IX had decreased. The protein composition of muscles in rigor mortis thus differs essentially from that of normal as well as from that of atrophic muscles.

As an introduction to the study of patients suffering from muscle diseases, twelve normal human muscles were investigated. The electrophoretic diagrams closely resembled the diagrams obtained with rabbit's skeletal muscles. The latter diagrams, however, differed very much from the diagrams obtained from rabbit's heart, uterus, diaphragm and stomach, while the diagrams of these latter muscles were also mutually different.

This work forms part of the investigations on the chemistry of muscle diseases by H. G. K. WESTENBRINK and collaborators. It has been communicated in the Meeting of the Netherlands Society for Biochemistry on December 1st, 1951. Full details will be published. The support of the Netherlands Organisation for Pure Scientific Research (Z.W.O.) is gratefully acknowledged.

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² P. CREPAX, *Experientia*, 5 (1949) 167.

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QUATERNARY AMMONIUM SALTS AS INHIBITORS OF ACETYLCHOLINE ESTERASE

II. pH DEPENDENCE OF THE INHIBITORY EFFECTS OF QUATERNARY AMMONIUM SALTS, AND THE DISSOCIATION CONSTANT OF THE ANIONIC SITE

by

FELIX BERGMANN AND ARELA SHIMONI

*Department of Pharmacology, The Hebrew University - Hadassah Medical School,
Jerusalem (Israel)*

Quaternary ammonium salts, in which one part or the other of the ester grouping $R-O-C-R'$



is missing, were compared as inhibitors for ACh esterase. Whereas the inhibitory effect decreases in the series prostigmine > acetophenone trimethylammonium iodide > choline chloride > tetraethylammonium bromide, all members of the series show a very similar relative decrease of activity with decreasing pH. Near pH 5 the activity of all quaternary ammonium salts approaches zero. On the other hand, over the whole range of pH 7-10.5 their inhibitory efficiency remains practically constant. The progressive inactivation with decreasing pH can not be ascribed to the inactivation of the nucleophilic group G_1 in the esteratic part of the active surface¹, as claimed by WILSON², since *e.g.* the tetraethylammonium ion has no "specific" chemical affinity to such a group (its alkyl chains are attached to the active surface by unspecific VAN DER WAALS forces). The dependence of inhibitory effects on pH changes must be due to the combination of the anionic site with the hydrogen ion, which inactivates both G_1 in the esteratic site and the negative charge of the anionic site. The dissociation constant $K_{EH_2^+}$, determined previously as characteristic for G_1 , thus appears now to be incorrect and requires redetermination.