Action of Cardiac Glycosides on the Polymerization of Actin

ACCORDING to Szent-Györgyi[1], muscular contraction involves the following reaction: at certain salt concentrations, actomyosin, formed from myosin and polymerized actin, loses water and contracts by the action of adenosinetriphosphate. Actin may be present in two forms: in a globular and a fibrous form. In the presence of salts, globular actin is transformed into the fibrous form, that is, polymerization takes place. Only at this stage is actin able to form with myosin the highly viscous actomyosin. Straub *et al.*[2] showed that adrenaline increases, chinine and veratrine diminish, the speed of polymerization.

We thought that heart glycosides, which have an elective action on heart muscle, might influence the polymerization of actin prepared from cardiac muscle, although they have no effect, or only at very high doses, on actin obtained from skeletal muscle.

Actin was prepared from heart muscle of the dog by a slight modification of the method of Straub *et al.*[2]. We have observed that the protein fractions can be extracted faster from the muscles of animals as they go down in phylogenetical order[4]. Further, protein fractions can be extracted faster from animal muscles as they become less and less differentiated. As a result, we decreased the time and temperature of extraction and also that of the volume of extraction fluids. The actin solution obtained from the acetone dry powder has a dry material content of 3–4 mgm. Viscosities were measured in Ostwald viscosimeters.

Our investigations show that the polymerization of actin prepared from heart muscle is accelerated even by 0·2–0·3 γ/ml. of crystalline strophantine-*G* and by the same amount of digitoxin in a salt concentration of 0·001 *M* magnesium chloride and 0·1 *M* potassium chloride, when the solution of actin has a dry content of 2–3 mgm./ml. Maximal effect is obtained by 2–4 γ/ml. strophantine-*G* and digitoxin. On the other hand, even 100 γ/ml. strophantine had no effect on the polymerization of actin prepared from skeletal muscle.

These observations support Straub's view that the polymerization of actin is an integral part in the mechanism of muscular contraction. Further, these findings suggest that in the mechanism of action of cardiac glycosides on heart muscle, their effect on the polymerization of actin may play a part.

I. HORVÁTH
C. KIRÁLY
J. SZERB

Department of Pharmacology,
University of Budapest.
May 21.

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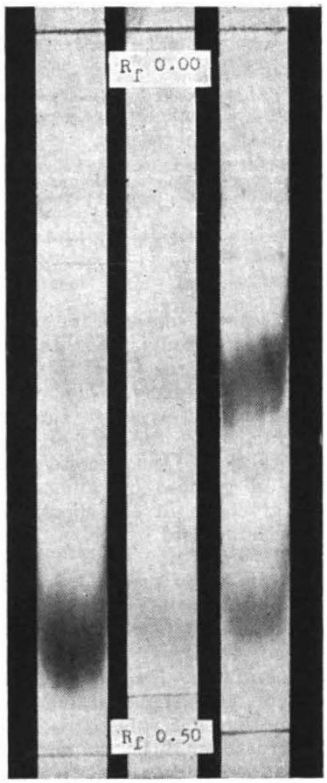
Keto-Acid Content of Human Blood and Urine

THE common procedure used to detect the keto-acid content in a biological material is usually confined to the isolation of the dinitrophenylhydrazone derivatives from a deproteinized sample, followed by the photometric evaluation of the red colour which they develop with addition of alkali. The results are expressed as milligrams of pyruvic acid, which is believed to be the main biological constituent of this class of compounds. Actually, the method is regarded as a method for pyruvic acid determination[1,2]. This method is, of course, not specific for pyruvic acid, since other keto-acids may be present in the sample giving the same reaction as pyruvic acid. It is not accurate, either, for the keto-acids as a class of compounds, since different phenylhydrazones with alkali show different colour intensities for each one of them[3]. Furthermore, some carbonyl compounds are partially extracted, so increasing the inaccuracy. In order to improve the specificity towards pyruvic acid, the procedure has been modified[4–6]; but it is not yet satisfactory.



Centre: Filter-paper chromatography of keto-acids in human blood. Left: The same with pyruvic acid added. Right: The same with α-ketoglutaric acid added

Recently, we reported on the successful separation of some dinitrophenylhydrazone of keto-acids of biological interest by making use of filter paper chromatography[7,8]. This fact enabled us to analyse the mixture of keto-acids, extracted by the usual method, and to carry out their quantitative determination. The purpose of the present communication is to describe the procedure adopted, and report the first results obtained by its application to normal blood and urine.

A volume of 10 ml. of blood, collected according to Long[9], is deproteinized by 30 ml. 5 per cent metaphosphoric acid. To a 20-ml. aliquot of the filtrate is added 2 ml. of 0·2 per cent 2,4-dinitrophenylhydrazine in 2 *N* hydrochloric acid, keeping the mixture for 20 min. at 38°. The hydrazones are extracted as usual[2] four times with 4 ml. ethyl acetate, centrifuging