and col.[1] showed the competitive nature of this type of inhibition using segments of sheep carotid artery. Similar results have been obtained on guinea-pig ileum[2]. The experiment of Figure 2 has been made using ergotamine as inhibitory substance. It represents the inhibitory effect of ergotamine on contractions with serotonin, while contractions with histamine and thrombocytolysine are present.

From all these experiments it can be deduced that thrombocytolysine, a substance liberated from the platelets by the anti-platelet serum is not identical with 5-hydroxytryptamine.

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Instituto Oswaldo Cruz, Rio de Janeiro, January 4, 1954.

Zusammenfassung

In einer früheren Arbeit wurde das Thrombozytolysin beschrieben, eine Substanz, die aus Pferde-Thrombozyten nach Einwirkung des homologen Thrombozytenantikörpers freigesetzt wird. Die in dieser Arbeit berichteten experimentellen Resultate berechtigen zur Schlussfolgerung, dass das Thrombozytolysin mit Serotonin (5-hydroxytryptamin) nicht identisch ist.

[1] E. SHAW and D. W. WOOLLEY, J. Biol. Chem. *203*, 979 (1953).
[2] H. MOUSSATCHÉ, unpublished results.

Non-Specificity of the Effect of Cardiac Glycosides on the Polymerization of Actin

A few years ago HORVÁTH, KIRÁLY, and SZERB[1] reported that the polymerization of actin from heart muscle is promoted by cardiac glycosides. This finding was confirmed in this laboratory by SNELLMAN and GELOTTE[2]. In view of the selectivity of action of the cardiac glycosides on the heart the further observation of HORVÁTH *et al.*[1] that these drugs had no effect on actin from skeletal muscle seemed of particular interest. The existence of differences in the reaction of preparations of cardiac and of skeletal muscle actin to steroid substances was again brought to the fore in recent experiments with cortisone[3].

The action of the cardiac glycosides *in vivo*, besides being largely confined to the heart, is specific in a second respect in that it is strictly dependent upon certain distinctive structural features of the active molecules. Slight structural modifications may suffice to bring about complete or partial inactivation. As has been emphasized before[4], it seems reasonable to expect the correlation between chemical structure and cardiac activity to hold for any effect of these drugs *in vitro* having a bearing on their action on the living heart muscle fiber. The experiments reported below indicate that the enhancing effect on the polymerization of actin does not fall into this category.

G-actin was obtained from calf heart muscle by the method of FEUER, MOLNÁR, PETTKÓ, and STRAUB[5]. For polymerization 0·25 volumes of a salt solution were added to the aqueous extract of the acetone-dried heart powder containing the protein. The composition of the salt solution was as follows: 0·5 M KCl, 0·01 M $MgCl_2$, 0·1 M potassium phosphate of pH 7·0. Polymerization was measured viscosimetrically. The following steroid glycosides, which had previously been tested by us in the heart-lung preparation of the dog, were used: The cardio-active emicymarin and its cardio-inactive isomer alloemicymarin, which differs from the active compound in the spatial configuration of the substituents at carbon 17; and the cardio-active scillaren A and its cardio-inactive derivative hexahydroscillaren A. The latter possesses a saturated lactone ring and has no nuclear double bond. The glycosides were added to the actin solutions immediatly before addition of the salt as 0·2% solutions in methanol. In the amounts added the alcohol itself had no significant effect on the actin in the presence of salt.

The results of an experiment in which the final concentration of glycoside was 20 μg per ml are presented in the Table. It is seen that the cardio-inactive compounds increased the viscosity of the salt-treated acetone powder extract to the same extent as did the cardio-active compounds. None of the compounds had a significant effect at concentrations below 5 μg per ml.

Extracts of acetone heart powder usually contain variable amounts of tropomyosin. This protein is polymerized in water and depolymerizes on addition of salt, with a resulting decrease in the viscosity of its solutions[1]. The glycosides had no appreciable influence on the depolymerization of tropomyosin in the concentrations used in this study. The high viscosities of the glycoside-containing solutions in the experiment shown in the Table can, therefore, be attributed to increased polymerization of actin.

Table

Effect of Cardio-Active and Cardio-Inactive Steroidal Glycosides on the Polymerization of Actin from Heart Muscle.

Final concentration of protein 3·0 mg/ml. Final concentration of glycosides 20 μg/ml. Viscosity measured at 22°C, 30 min after addition of salt.

Addition to acetone powder extract	η spec.
0·25 vol. water	0·303
0·25 vol. water + 0·0125 vol. methanol	0·313
0·25 vol. salt soln.	0·370
0·25 vol. salt soln. + 0·0125 vol. methanol	0·365
0·25 vol. salt soln. + 0·0125 vol. methanol + emicymarin	0·468
0·25 vol. salt soln. + 0·0125 vol. methanol + alloemicymarin	0·469
0·25 vol. salt soln. + 0·0125 vol. methanol + scillaren A	0·467
0·25 vol. salt soln. + 0·0125 vol. methanol + hexahydroscillaren A	0·463

SNELLMAN and GELOTTE[2] have reported that in aqueous extracts of acetone heart powder they found what appeared to be an ATP or adenylic deaminase, subject to inhibition by cardio-active glycosides. We have been unable to determine whether such inhibition might also be produced by cardio-inactive glycosides because the actin-containing extracts tested for this purpose were free of such deaminase activity.

Not all actin preparations showed increased polymerization on addition of steroid glycosides. Results such as those presented in the Table were obtained with actin from hearts which had remained in the body for some time after death. When actin was prepared from calf

[1] I. HORVÁTH, C. KIRÁLY, and J. SZERB, Nature *164*, 792 (1949).
[2] O. SNELLMAN and B. GELOTTE, Nature *165*, 604 (1950).
[3] J. B. COWLE and R. H. THORP, Nature *171*, 1067 (1953).
[4] A. WOLLENBERGER, Pharmacol. Reviews *1*, 311 (1949).
[5] G. FEUER, F. MOLNÁR, E. PETTKÓ, and F. B. STRAUB, Hungarica physiol. acta *1*, 150 (1948).

[1] K. BAILEY, Biochem. J. *43*, 27 (1948).
[2] O. SNELLMAN and B. GELOTTE, Nature *165*, 604 (1950).

hearts which had been excised immediately after stunning the animal and had been placed in ice-cold water while still beating vigorously, addition of salt caused a sharp rise in viscosity, comparable in magnitude to the increase costumarily obtained with actin from fresh skeletal muscle. In these instances the steroid glycosides were without effect.

Following the discovery by HORVÁTH *et al.*[1] of the influence of cardiac glycosides on the polymerization of actin a variety of other effects of these drugs on contractile muscle proteins were described in the literature. Whether some of these effects are likewise unspecific in the sense that they can be reproduced by structural analogs devoid of cardiac activity is at present under investigation.

This work was undertaken during tenure of a Life Insurance Medical Research Fellowship. I wish to express my gratitude to Prof. T. REICHSTEIN for a generous gift of emicymarin and alloemicymarin, and to Prof. A. STOLL for a generous gift of scillaren A and hexahydroscillaren A. I am also grateful to Dr. O. SNELLMAN and Dr. B. GELOTTE for their interest and cooperation.

A. WOLLENBERGER[2]

Institute of Biochemistry, University of Uppsala, March 3, 1954.

Zusammenfassung

Die fördernde Wirkung der herzwirksamen Glykoside Emizymarin und Szillaren A auf die Polymerisation von Aktin aus Herzmuskel wird in demselben Masse von den strukturell nahe verwandten, jedoch herzunwirksamen Glykosiden Alloemizymarin und Hexahydroszillaren A ausgeübt.

[1] I. HORVÁTH, C. KIRÁLY, and J. SZERB, Nature *164*, 792 (1949).

[2] Present address: Pharmakologisches Institut der Humboldt-Universität, Berlin NW 7.

Sull'ossidazione dell'acido lattico ed acido piruvico e di alcuni componenti del ciclo citrico in omogenati di epitelio corneale

Le ricerche sulla partecipazione del ciclo citrico di KREBS nell'ossidazione dell'acido piruvico da parte di tessuti oculari sono scarse. In due mie note[1] ho riferito sull'attività deidrogenasica generale della cornea, cristallino e retina mediante il Trifenil-tetrazolio, e sulla localizzazione istochimica della succinodeidrogenasi col Blu Tetrazolio. I miei dati hanno permesso di poter localizzare l'attività deidrogenasica elettivamente nell'epitelio corneale ed in minima parte nell'endotelio. Nella presente nota riferisco i risultati delle mie ricerche sulle deidrogenasi del ciclo citrico di KREBS in omogenati di epitelio corneale bovino mediante il Trifenil Tetrazolio.

Per le mie ricerche ho adoperato la tecnica consigliata da NORDMAN e NORDMAN[2]. L'epitelio di cornee bovine veniva scarificato e si preparava un omogenato al 20% nell'omogenizzatore in vetro di POTTER[3]; per la determinazione dell'attività deidrogenasica la miscela d'incubazione era preparata nelle seguenti proporzioni: In tubi da centrifuga si aggiungevano 1 ml di omogenato al 20% (200 mg), 0,5 ml Puffer Fosfati (0,1 M) pH 7,4, 0,5 ml di substrato (0,2 M) (acido lattico, piruvico) (acido citrico, α-chetoglutarico, succinico, malico) 1 ml di Trifenil Tetrazolio (0,1%) si completava a 4 ml con acqua distillata previa aggiunta di 0,3 ml di ATP (0,01 *M*).

I tubi da saggio si lasciavano incubare a 37°C per 30′ agitando due tre volte durante l'incubazione, si aggiungevano successivamente 10 ml di acetone, si agitava e si centrifugava.

Il liquido soprastante veniva decantato e si leggeva l'estinzione in colorimetro KLETT con filtro 420. In ogni prova si praticavano due controlli uno senza substrato ed il secondo anche senza substrato ma con aggiunta di una determinata quantità di Tetrazolio. Nella Tabella riporto la media dei dati di più esperimenti, l'attività enzimatica è riferita in μg di Tetrazolio ridotto in 30′ da 1 mg di tessuto (peso fresco).

Deidrogenazione dell'Acido Lattico, Piruvico e di alcuni componenti del ciclo citrico in omogenati di epitelio corneale

Substrati	N. Esp.	μg Tetrazolio rid. da 1 mg di tess. 30′	Errore Standard
Acido lattico	12	0,10	±0,04
Acido piruvico	9	0,22	± 0,05
Acido citrico	15	0,63	± 0,12
Acido chetoglutarico	10	0,24	± 0,08
Acido succinico	8	0,78	± 0,10
Acido malico	14	0,12	± 0,03

L'esame della tabella mostra che in presenza di tutti gli acidi del ciclo citrico saggiati è stato possibile mettere in evidenza nell'epitelio corneale una notevole attività deidrogenasica, e pertanto si può ammettere che anche nel tessuto corneale come in tutti i tessuti il ciclo tricarbossilico abbia un'importanza fondamentale nei processi ossidativi.

Addendum. Mentre la presente nota era in corso di pubblicazione, ho potuto prendere visione di alcuni lavori di JAEGER[1] sullo stesso argomento. L'Autore adoperando lo stesso metodo, ha ottenuto risultati che concordano con i miei dati.

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Summary

Dehydrogenase Activity of some components of citric cycle in corneal Epithelium homogenates is determined by means of 2-3-5 Triphenyltetrazoliumchloride.

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Cortical Representation of the Cortico-Motoneuronal System in Monkeys

Bunctional evidence has recently been presented by BERNHARD, BOHM, and PETERSÉN[1] and by BERNHARD and BOHM[2] for a direct activation of the spinal motoneurones by descending volleys in corticospinal fibres in the monkey. That fraction of the corticospinal system, the descending volley in which activates the spinal motoneurones directly, i.e. monosynaptically, we refer to as the cortico-motoneuronal system or the *CM system*. We have now attacked the problem concerning the topography of the cortical representation of the CM system for different muscles.

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