It was felt that the enzyme system responsible for this oxidation might leak into the serum similar to such enzymes as prostatic acid phosphatase and β-glucuronidase.

The methods used in this study were essentially the same a those described by WOTIZ and LEMON[1]. Each incubation mixture contained 20 cm³ of KREBS-RINGER phosphate buffer at pH 7·3, 1 cm³ of serum, 2 mg of testosterone and 1 mg DPN. The incubation was carried out for 3 h at 38°C. Recoveries from control experiments obtained by boiling the serum or by incubation at pH 9 are shown in Table I.

Table I

Exp. No.	Inhibition	mg Testosterone added	mg Testosterone recovered	% Recovery
1	Serum boiled . . .	2	1·8	90
2	Serum boiled . . .	1	1·0	100
3	Incubation at pH 9	1	1·0	100
4	Incubation at pH 9	1	1·0	100

In Table II the results obtained from incubation of six sera from different healthy subjects are listed. As can be seen, all of the sera actively metabolized testosterone at approximately the same rate. One half of each total extract was applied to a paper-chromatogram and developed in a ligroin-propylene glycol system[2]. In each case two new ketosteroids were found on staining with the ZIMMERMANN reagent, except for the control experiments, which showed no steroid other than starting material at any time. One of the new products gave a purple color and was identified as androstenedione by mixed paper chromatography and infra-red absorption spectra. The other compound, giving a purple ZIMMERMANN color, occupied a position intermediary to testosterone and androstenedione.

Table II

Exp. No.	mg Testosterone added	mg Testosterone recovered	%Testosterone metabolized
1	2	0·88	56
2	2	0·84	58
3	2	0·89	57
4	2	1·22	39
5	2	1·03	49
6	2	0·90	55

These findings should serve as a caution to clinical and scientific investigators utilizing serum or serum fractions to solubilize steroids. This method is sometimes applied for infusion of steroids into living animals or is used in the study of the metabolism of steroids by perfusion of isolated glands. The possibility that other androgens, as well as corticoids and estrogens, may be similarly affected must be taken into consideration and is now being investigated in this laboratory. Possible qualitative and quantitative changes in this reaction in cases of disease, especially cancer, are also under investigation utilizing 4-$\dot{C}^{14}$-testosterone.

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Departments of Medicine and Biochemistry, Boston University School of Medicine, Boston, Massachusetts, U.S.A., March 9, 1954.

Zusammenfassung

Durch *in-vitro*-Experimente konnte gezeigt werden, dass im menschlichen Serum ein Enzymsystem vorhanden ist, das Testosteron abzubauen vermag. Als Endprodukte wurden Androstendion sowie weitere bisher noch nicht identifizierte Steroide nachgewiesen.

On the Nature of Some Smooth Muscle Active Substances from the Platelets

Several smooth muscle active substances from the platelets have been described in recent years. Evidence that active substances found in serum by several authors[1] came from the platelets had been given first by FREUND[2], STEWART and ZUCKER[3], and lately by REID and coworkers[4].

Recently RAPPORT, GREEN, and PAGE[5] isolated from beef serum, as a creatinine sulfate complex, a crystalline vasoconstrictor substance, serotonine, identified as 5-hydroxytryptamine. REID admitted that the substance extracted from platelets, previously described by him as thrombocytin, can be identified with 5-hydroxytryptamine[6]. Serotonin can also be liberated from the platelets by antigen-antibody reaction *in vitro* as have been shown by HUMPHREY and JACQUES[7].

We showed in a previous paper[8] that horse anti-platelet serum liberated from washed horse platelets, *in vitro*, an active substance, contracting the guinea-pig ileum, inducing hypotension on atropinized rabbits, and not identified with histamine, acetylcholine, kalikrein, substance P, tyramine, adenilic acid, bradycinin, or potassium ions.

The purpose of the present paper was to establish the inter-relationship between the substance liberated from the platelets by the anti-platelet serum and two others: smooth muscle contracting fraction and serotonin, both found in the platelets. The stability of the smooth muscle contracting fraction at alkaline pH indicate that it can not be identified with the substance liberated from the platelets by anti-platelet serum (thrombocytolysine). Differences with serotonin could now be established using three kinds of experiments:

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[2] H. FREUND, Arch. Exp. Pathol. Pharmakol. *86*, 266 (1920).
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[5] M. M. RAPPORT, A. A. GREEN, and I. H. PAGE, J. Biol. Chem. *176*, 1243 (1948).
[6] G. REID and M. RAND, Nature *169*, 801 (1952).
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[8] H. MOUSSATCHÉ and W. O. CRUZ, Arch. int. Pharmac. *91*, 224 (1952).
[9] M. B. ZUCKER, Amer. J. Physiol. *142*, 12 (1944).

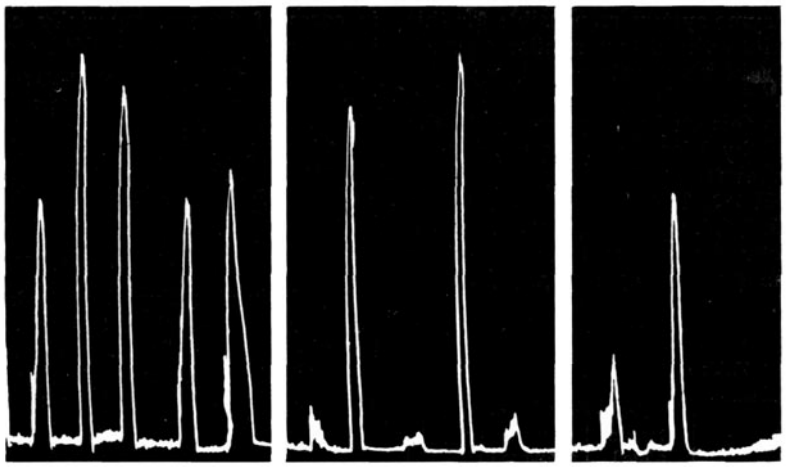

Fig. 1.—Guinea-pig ileum in 10 ml Tyrode. *S* 0·3 ml serotonin creatine sulfate 2×10^{-5}; *H* 0·5 ml histamine dihydrochloride 10^{-6}; *Trb* 0·3 ml Thrombocytolysine 10^{-3}; *Trpt* 0·8 ml Tryptamine hydrochloride 10^{-3}. When tryptamine was added to the bath, serotonin was specifically inhibited; contractility recovered 40 min after.

(1) GADDUM[1] showed that large dose of tryptamine applied to perfused guinea-pig ileum desensitizes the gut to 5-hydroxytryptamine, although the organ still responds normally to other drugs.

In our experiments (Fig. 1), moderate contractions of the guinea-pig ileum could be induced by 5-hydroxytryptamine[2] (*S*), histamine (*H*), thrombocytolysine (*Trb*) and tryptamine (*Trpt*)[3]. When a high dose of tryptamine is added to the bath, a good contraction occurred, relaxation being complete some minutes after, and then the preparation becomes desensitized to 5-hydroxytryptamine for more than 20 min, while contractions by histamine and thrombocytolysine are normal. 40 min after contractility the serotonin has been restored.

Using serotonin ROCHA and SILVA and coworkers[4] showed that a high dose of this substance inhibited the guinea-pig ileum for the smaller one. Adding a high dose of serotonin to the bath perfusing the gut, we could inhibit the contractions previously obtained with smaller doses of serotonin, while returning those obtained with thrombocytolysine and histamine.

(2) SHEPHERD, WEST, and ERSPAMER[1] showed that serotonin and tryptamine give a golden-yellow and bright-yellow fluorescence respectively under ultraviolet light when treated with solutions containing nine parts of 0·1 % of potassium dichromate and one part of formaldehyde solution (37–41 %).

This test has been applied to thrombocytolysine, no fluorescence being evidenced by 100 μg, using "spot test" on filter paper, while it appeared clearly using a few μg of serotonin and tryptamine. Hopkins-Cole reaction has also been assayed on thrombocytolysine with negative results.

(3) A third type of evidence against the serotonin nature of thrombocytolysine has been obtained using the specific inhibitory action of yohimbine and ergotamine on serotonin. It has been shown by several authors that yohimbine and ergotamine can inhibit some of the pharmacological actions of serotonin. Recently WOOLEY

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[2] I am indebted to Dr. R. K. RICHARDS, from the Abbotts Laboratories, for the sample of serotonin creatinine sulfate supplied by Hoffmann-La Roche S.A.

[3] Tryptamin hydrochloride was kindly supplied by "Hoffmann-La Roche" S.A.

[4] M. ROCHA, SILVA, J. R. VALLE, and Z. PICARELLI, IV Annual Meeting Brasilian Soc. Advanc. Sci., Résumé in Ciencia e Cultura *4*, 130 (1952).

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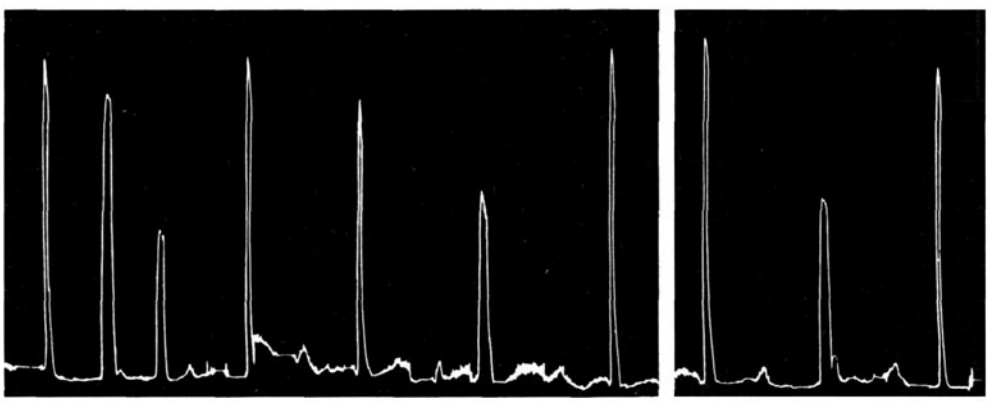

Fig. 2.—Influence of ergotamine on serotonin and thrombocytolysine contraction. Guinea-pig ileum, in 10 ml Tyrode. *H* 0·05 ml histamine dihydrocl. 10^{-6}; *S* 0·2 μg/ml serotonin creatine sulfate; *Trb.* 0·3 ml thrombocytolysine 10^{-3}; *E.* 0·25 μg/ml, ergotamine tartarate.

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

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