Spectrophotometric Method for Estimating Enzymatic Synthesis of Butyl Oleate

RAMAKRISHNAN and NEVGI[1] prepared an acetone powder from castor seed which affected 39% synthesis of butyl oleate in five days. HOFSTEE[2] has recently worked out an assay system to estimate esterase spectrophotometrically using Salycilate ester as substrate. The author has worked out a similar assay system to estimate the synthesis of butyl oleate spectrophotometrically.

Experimental. Acetone-dried lipase powder was prepared from castor seed according to RAMAKRISHNAN and NEVGI's method[1] and used for the experiments. N-butyl alcohol, oleic acid and petroleum ether (B.P. 40–60°) were used. The absorption was studied in a BECKMAN spectrophotometer. The results of the various experiments are given in the following Tables.

Table I

The reaction mixture consisted of castor lipase: 0·1 g; N. Butyl alcohol: 0·005 M; oleic acid: 0·005 M; petr. ether: to make up the vol. to 3 cm³; incubation for 2 h at 37°C

Set No.	Wave lengths mμ	Absorbance
1	390	0·075
2	395	0·085
3	400	*0·092*
4	405	*0·092*
5	410	0·090
6	415	0·070
7	420	0·065
8	430	0·062
9	440	0·055
10	450	0·045
11	500	0·014
12	540	0·009

In all the experiments, petroleum ether, alcohol and enzyme blanks had negligible absorptions. The absorbance was proportional to the concentration of ester.

Table II

The reaction mixture consisted of castor lipase 0·2 g, different amounts of alcohol and acid and petroleum ether to make up the volume to 3 cm³, incubated for 2 h at 37°C and read at 400 mμ against blank containing all except the enzyme

Set No.	Concentration of alcohol (M)	Concentration of oleic acid (M)	Absorption	% Synthesis
1	0·001	0·005	0·037	2·4
2	0·002	0·005	0·073	4·8
3	*0·005*	*0·005*	0·184	*12·0*
4	0·008	0·005	0·184	12·0
5	0·005	0·001	0·038	2·4
6	0·005	0·002	0·070	4·6
7	0·005	0·008	0·182	11·9

From the above results, it can be seen that butyl oleate shows an absorbance at 400–405 and it is proportional to the concentration of ester. Mixture consisted of 0·005 M acid and alcohol, 0·2 g of lipase and petroleum ether to make up the volume to 3 cm³ and incubated for 2 h at 37°C gives 12% butyl oleate synthesis. This method seems to be a very simple and handy one and similar assay systems can be constructed for estimating the synthesis of different esters.

Table III

The reaction mixture consisted of 0·005 M alcohol and acid, and different amounts of enzyme and petroleum ether added to make up the volume to 3 cm³ incubated for 2 h at 37°C and read against blank at 400 mμ

Set No	Concentration of enzyme	Absorption	% Synthesis
1	0·1	0·092	6·0
2	0·2	0·184	12·0
3	0·3	0·180	11·7

Table IV

The reaction mixture consisted of 0·2 g of castor lipase, 0·005 M each of alcohol and acid, and petroleum ether added to make up the volume to 3 cm³, incubated for different amounts of time and read against blank at 400 mμ

Set No.	Incubation time h	Absorption	% Synthesis
1	½	0·100	6·5
2	1	0·152	9·9
3	1–½	0·178	11·6
4	2	0·184	12·0
5	2–½	0·184	12·0
6	3	0·182	11·9

Acknowledgement. The author wishes to thank Dr. D. M. BOSE, the Director and Dr. J. K. CHOWDHARY, the Head of the Chemistry Department, for the keen interest they have shown throughout the progress of the problem.

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Bose Institute, Calcutta, India, November 20, 1953.

Zusammenfassung

Es wird eine einfache spektrophotometrische Methode für die Bestimmung der enzymatischen Synthese von Butyloleat beschrieben.

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[1] C. V. RAMAKRISHNAN and G. V. NEVGI, J. Ind. Chem. Soc. *27*, 6, 260–261 (1950).

[2] B. H. J. HOFSTEE, J. biol. Chem. *1952*, 357.

Enzymatic Conversion of Testosterone to Androstenedione by Human Serum

Recent studies on the *in vitro* metabolism of testosterone by human tissues have shown that active metabolism of this steroid is carried on by a variety of normal and malignant human tissues[1]. These tissues include target-organs (prostate) as well as non-target-organs.

[1] H. M. LEMON, H. H. WOTIZ, and T. ROBITSCHER, J. clin. Endocrinol. Metabolism *13*, 948 (1953). – H. H. WOTIZ, H. M. LEMON, and A. VOULGAROPOULOS, J. biol. Chem. (in press).

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–C¹⁴-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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