[CONTRIBUTION FROM THE LABORATORY OF THE DEPARTMENT OF AGRICULTURAL AND BIOLOGICAL CHEMISTRY, THE PENNSYLVANIA STATE COLLEGE]

The Effect of Certain Salts and Cholesterol on the Activity of Ricinus Lipase[1]

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Ricinus lipase obtained by any method thus far proposed does not catalyze the hydrolysis of fats or oils until it has first been activated. A variety of substances had been used to activate the enzyme before the realization of optimum conditions for its action.[2] The effect of substances other than acids was indicated in the early studies of Nicloux.[3] Sulfates of manganese and calcium were said to have a stimulating action. Manganous sulfate has received especial attention as an activator for Ricinus lipase.[4–9]. Other substances which have been stated to affect the catalysis of fat cleavage are amino acids,[8,10,11] polypeptides and proteins,[12] alkaloids,[13,14] antiseptics,[15,16] pepsin in an acid medium[17] and neutral salts.[6,18,19]

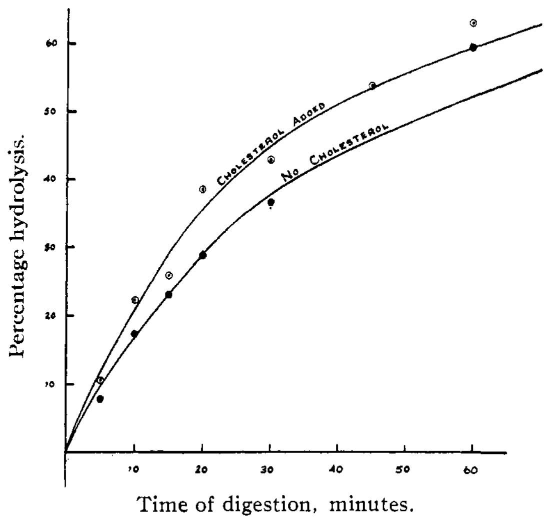

Fig. 1.—The effect of cholesterol on the activity of Ricinus lipase.

Recognizing discrepancies among these reports, the present series of experiments was conducted in an attempt to gain further information on the subject of stimulation or retardation of Ricinus lipase action and with the hope of contributing further evidence as to the nature of the active material in the castor bean.

In connection with the effects of various substances on lipase activity, the opinion apparently prevails that cholesterol retards lipase action. Cholesterol, however, tends to aid the formation of water-in-oil emulsions[20] and it has been observed experimentally[21] that Ricinus lipase appears to be most reactive in an emulsion of this type. Cholesterol should not, *a priori*, affect the lipase as do some materials, *e. g.*, salts as is shown below. The inescapable conclusion was that addition of cholesterol to the digestion mixture should be beneficial to Ricinus lipase action.

Experimental

Ricinus lipase was prepared and its activity estimated by the method of Longenecker and Haley.[21,22] A series of Baker C. P. inorganic salts was added to the digestion mixture and the customary determinations for the extent of hydrolysis of olive oil were made (*cf.* Table I).

The hypothesis regarding the effect of cholesterol was tested by dissolving cholesterol in olive oil and measuring the rate of hydrolysis of the latter in the customary fashion. The data obtained are given graphically in Fig. 1. The concentration of cholesterol (Pfanstiehl C. P.) was 1% with respect to the oil.

Discussion

In no case was there any stimulating affect by the addition of a salt. Salts of cobalt, copper and mercury, even in low concentrations, inhibited lipase action, completely in some cases and to a considerable extent in the others. Salts of calcium, chromium, iron, lead, manganese, magnesium, and zinc likewise were inhibitory but in higher concentrations (when only 25–50 mg. of salt was added, the inhibi-

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(2) Haley and Lyman, THIS JOURNAL, **43**, 2664 (1921).
(3) Nicloux, *Mem. Soc. Biol.*, **56**, 701, 837, 868 (1904).
(4) Tanaka, *8th Intern. Congr. Appl. Chem.*, **11**, 37 (1912).
(5) Falk and Hamlin, THIS JOURNAL, **35**, 210 (1913).
(6) Falk, *ibid.*, **35**, 601 (1913).
(7) Sudborough and Watson, *J. Indian Inst. Sci.*, **5**, 132 (1922).
(8) Takamiya, *J. Agr. Chem. Soc.* (Japan), **5**, 595 (1929).
(9) Keeser, *Arch. exptl. Path. Pharm.*, **160**, 663 (1931).
(10) Hamlin, THIS JOURNAL. **35**, 1897 (1913).
(11) Willstätter and Waldschmidt-Leitz, *Z. physiol. Chem.*, **134**, 161 (1924).
(12) Falk, *J. Biol. Chem.*, **96**, 53 (1932).
(13) Padoa, *Nature*, **129**, 683 (1932).
(14) Padoa and Spada, *Giorn. biol. applicata ind. chim.*, **1**, 153 (1931); *ibid.*, **3**, 121 (1933).
(15) Palmer, THIS JOURNAL, **44**, 1527 (1922).
(16) Kluge, *Z. Untersuch. Lefensm.*, **66**, 412 (1933).
(17) Willstätter, *Chem. Rev.*, **13**, 501 (1933).
(18) Tanaka, *J. Coll. Eng. Imp. Univ. Tokyo*, **5**, 142 (1912).
(19) Falk, *J. Biol. Chem.*, **36**, 601 (1918).
(20) Clayton, Text, P. Blakiston's Son and Co., Philadelphia, Pa., 1935.
(21) Longenecker and Haley, THIS JOURNAL, **57**, 2019 (1935).
(22) Castor beans were furnished by the Baker Castor Oil Co.. Newark, N. J.

TABLE I

EFFECT OF INORGANIC SALTS ON RICINUS LIPASE ACTION

In each experiment, 1.00 g. olive oil, 0.050 g. lipase preparation (L. U. = 910) and 0.6 ml. of 0.1 N acetic acid containing 100 mg. of the salts indicated were shaken together for three minutes at the start, then let stand at 37–38°.

Salt added	Time of digestion, min. 20	25	40
None (check)	38.8	43.6	53.0
Mercuric acetate	1.2	2.0	2.1
Mercuric chloride	2.6	2.7	2.6
Cupric acetate	4.0	4.1	4.1
Cobalt acetate	10.4	12.0	13.0
Ferric chloride	9.9	10.3	15.5
Zinc chloride	9.6	20.0	23.2
Lead acetate	18.0	20.7	28.0
Lead chloride	21.0	24.0	31.4
Mercurous chloride	22.9	28.1	35.0
Chromium chloride	26.2	27.5	28.5
Nickel chloride	26.9	30.4	38.1
Calcium acetate	28.6	33.9	42.8
Manganese acetate	27.9	33.4	44.8
Manganese chloride	28.5	32.0	40.0
Ferric acetate	32.0	37.6	53.4
Chromium acetate	35.9	42.8	53.0

tion was not so marked in these cases). It is interesting to note that the preparations at this Laboratory invariably contain 7.15–7.25% ash which on qualitative analysis has been shown to contain manganese, magnesium, calcium and iron.[23] These naturally occurring salts have not shown any marked effect on the lipase action except when added in fairly high concentration. These observations support the findings of Willstätter and Waldschmidt-Leitz[11] that manganous salts had no stimulating effect under optimum conditions of activity.

(23) We are indebted to Mr. W. H. Stahl for these analyses.

While optimum conditions were maintained in the experiments with cholesterol, a preparation with a lower lipase unitage (L. U. = 285) was used.[24] In this way, cholesterol was shown to have a definite stimulating effect on the rate of Ricinus lipase action. The effect is the more pronounced in the early stages of the hydrolysis, complicating factors[24] probably accounting for the same extent of hydrolysis being reached in each of the three series. It is suggested that this stimulation was due to the formation of a desirable emulsion, water-in-oil. In a separate series of unreported experiments, the effects of other substances chiefly gums known to produce definite types of emulsions were studied. Abnormally low figures were obtained for the percentage hydrolysis in these cases. It is quite possible that these results were due to the emulsifying agents. This problem is receiving further study.

Summary

The effect of certain salts, chiefly chlorides and acetates, on the activity of Ricinus lipase has been studied. Each added salt retarded or inhibited the normal action of the enzyme. Salts of cobalt, copper and mercury showed the greatest inhibiting action. Iron, lead, nickel and zinc salts definitely retarded the action, as to a less extent did the salts of calcium, chromium, and manganese.

Cholesterol has been shown to accelerate the action of Ricinus lipase. This observation is taken to indicate further that a water-in-oil emulsion is desirable for Ricinus lipase action.

(24) Longenecker and Haley, THIS JOURNAL, 59, 2156 (1937).

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[CONTRIBUTION FROM THE CHEMICAL LABORATORY OF NORTHWESTERN UNIVERSITY]

The Rearrangement of Acetylenes into Allenes at High Temperature

BY CHARLES D. HURD AND ROBERT E. CHRIST

This paper deals especially with the pyrolysis of 1-hexyne and 1-heptyne by the flow method. The moles of gas produced per mole of hexyne decomposed at 500 and 600° were 0.34 and 1.03, respectively. These ratios for heptyne were 0.83 and 2.0. The chief gaseous products in all experiments were propylene, methane and ethylene. Lesser quantities of ethane and hydrogen were observed but no more than a trace of acetylene was ever found among the products.

The liquid products were distilled through a very sensitive fractionating column and the various fractions were studied. The important reaction products found were 1,2-hexadiene (propylallene) from 1-hexyne, and 1,2-heptadiene (butylallene) from 1-heptyne. Traces of 2-hexyne or 2-heptyne were evident in the experiments at 600°, but not otherwise. Conjugated hydrocarbons were absent and no evidence could be obtained for benzene or toluene among the products. The liquids from heptyne yielded small quantities of pentenes and hexenes.

To prove the presence of the allenes the fraction in question was freed from its 1-alkyne content by precipitation as the silver salt. The remaining liquid was subjected to ozonolysis. From 1,2-hexadiene there was obtained a mixture of butyric and formic acids, and from 1,2-heptadiene a mix-