tion of these tissues for tryptic digestion. The above experiments demonstrate that tryptic digestion of living tissues can take place under appropriate conditions.

8789 C

In Vitro Hydrolysis of Fats by Lipase and Bile Salts.

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The chemical literature apparently reveals no methods for the *in vitro* hydrolysis of animal fats by lipase and bile salts for the special purpose of quantitatively measuring their digestibility. Since it is sometimes desirable to test the relative digestibility of fats which are either untreated or have been treated with a preservative, it occurs to me that an artificial digestion test of this kind might prove useful. I have devised such a test in which I attempt to simulate body conditions of temperature, fat emulsification, gut motility, H ion reaction, and enzyme action. The test was devised for the digestion of lard, although butter and tristearine have also been used as substrate in a few tests.

Method. To 30 gm. lard in a 125 cc. Erlenmeyer flask, add 20 cc. of 0.5% aqueous bile salt solution. Incubate at body temperature for 20 minutes and shake flask to form emulsion. Add enzyme (0.5 gm. Wilson's lipase is about optimum). Place flasks in a wheel, which will rotate 10 times per minute, within an incubator kept at 37°C. Allow fat to digest in this manner for 3, 6, 12 or optional number of hours. At conclusion of digestion period, add 80 cc. benzene to the flasks, set them back in incubator and rotate for 20 minutes more. Remove flasks and place them in ice box for 20 minutes. Pipette off 10 cc. of supernatant benzene, add 3 drops 1% phenolphthalein solution, and titrate with 0.1 N alcoholic sodium hydroxide to a faint pink color. Compute amount of base required to neutralize the fatty acids in the whole benzene phase. A correction of 0.2 cc. should be subtracted, as representing the base required to titrate the benzene phase after a control test in which the substrate is excluded. The resulting acid number is a substantial index to the extent of the hydrolysis which has occurred.

The quantity of substrate to be tested may be reduced (*e. g.*, to

5 or 10 gm.) provided the quantity of bile salt solution and lipase are reduced proportionally.

Calculation:

$$D = \frac{N}{10} \times \frac{M}{1{,}000} \times \frac{f}{f.a.} \times \frac{1}{W} \times 100$$

Where: D = percentage of the fat which has been hydrolized.
N = cc. of 0.1 N NaOH used to titrate whole benzene phase.
M = average molecular weight of the constituent fatty acids in the fat.
$\frac{f}{f.a.}$ = ratio, weight of fat molecule to weight of acids contained therein.
W = weight neutral fat in sample before hydrolysis.

I used a commercial bile salt compound (Wilson) as emulsifying agent. Various concentrations in aqueous solution were tried and a 0.5% solution of the compound proved most effective. The optimum ratio of fat to water for the formation of a stable emulsion was determined by visual inspection and by hydrolysis tests (Table I). The emulsion selected as best promoting hydrolysis is one formed by 3 parts of fat and 2 parts of water; and is an emulsion of water-in-fat.

TABLE I.
Digestibility of Lard in Various Emulsions by a Constant Weight of Lipase (0.5 gm. for 30 gm. lard).

Emulsion gm. lard : cc. 0.5% bile salts in H_2O	% Digestion in 12 hr.
3:2	21.2
1:1	18.9
1:2	12.8
1:4	12.1
1:9	11.8

0.5 gm. of lipase (Wilson) is employed when 30 gm. of lard is to be tested. The initial pH of the bile salt solution before hydrolysis is about 7.3. Its alkali content is not sufficient to saponify an appreciable quantity of fatty acid. Twenty cc. of the 0.5% bile salts solution containing 3 drops of phenolphthalein becomes pink with the addition of 0.05 cc. of 0.1 N sodium hydroxide. The same amount of base is sufficient to titrate to a pink color 20 cc. of our distilled water.

Fig. 1 shows the relationship of time to the hydrolysis of lard. After 24 hours of digestion, an equilibrium point, apparently, has been reached. Dietz[1] showed in an amyl butyrate-water-lipase sys-

[1] Dietz, W., *Z. physiol. Chem.*, 1907, **52**, 279.

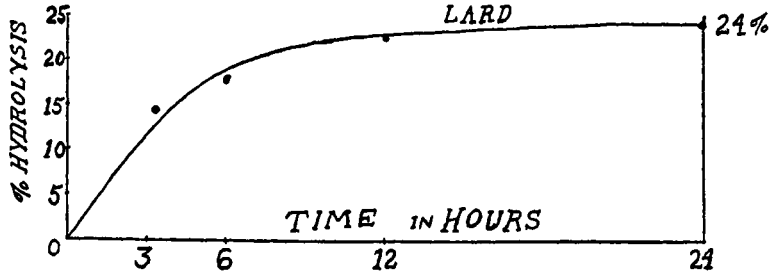

FIG. 1.

tem that a chemical equilibrium comes to exist in which the reverse reaction of ester formation proceeds at exactly the same rate as the hydrolysis. Lipase reactions *in vitro* are probably always reversible.

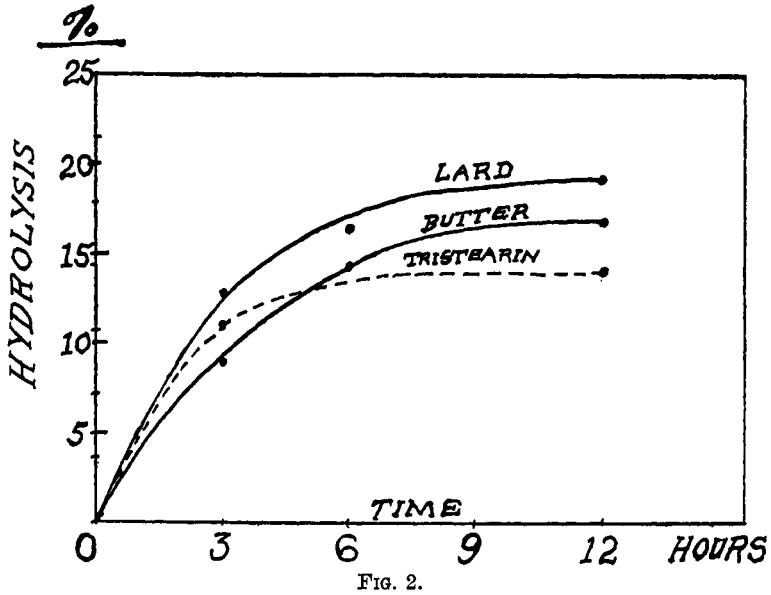

FIG. 2.

Fig. II shows the results of hydrolysis of lard, clarified butter, and tristearine over periods of 3, 6, and 12 hours. Stearine will not form a stable emulsion with the quantity of water specified for lard in this test.

According to Mathews,[2] tripalmitin and tristearine constitute the bulk of the fat of mammals. I have therefore used the figure 270 as the average molecular weight of the fatty acids contained in lard. For the average molecular weight of the fatty acids of butter

[2] Mathews, A. P., *Physiological Chemistry*, 1925, 4th Ed., 70, Wm. Wood & Co.

I have used the figure 238, which is based upon an analysis by Dean and Hilditch.[3]

Altogether, I have performed about 200 digestions of various durations on lard. In any one digestion test, the percentages of hydrolysis in the separate samples do not differ by more than 0.5% and are usually much closer.

Bile salts alone will not hydrolyse fats at all, but that they are a definite aid to lipase action is shown in a 6-hour digestion test in which lipase alone produced 14.5% hydrolysis of lard, whereas lipase with bile salts produced 16.1% hydrolysis.

Summary. A method is presented for the *in vitro* hydrolysis of fats by bile salts and lipase. In this method an attempt is made to simulate body conditions of temperature, enzyme action, gut motility, and emulsification. The hydrolysis reaches an equilibrium point after about 24 hours, when about 24% of the fat has been broken down.

8790 C

Insulin Absorption from Application to the Skin.

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Soon after the discovery of insulin there were several reports in the literature of attempts to introduce insulin into the blood stream by methods other than those of intravenous and subcutaneous administration. Telfer[1] reported that insulin in an inunction when rubbed on the skin of rabbits produced a fall in blood sugar. Harrison[2] repeated this work and found that the inunction of insulin under these conditions had no effect even when introduced in very large amounts. More recently Hermann[3] and Hermann and Kassowitz[4] have reported a marked fall of blood sugar in both animals and patients following the application of an insulin salve on the skin.

3 Dean, H. K., and Hilditch, T. P., *Biochem. J.*, 1933, **27**, 889.

1 Telfer, S. V., *Brit. M. J.*, 1923, **1**, 715.

2 Harrison, G. A., *Quart. J. Med.*, 1926-27, **20**, 187.

3 Hermann, Siegwart, *Arch. f. exp. Path. u. Pharm.*, 1935, **179**, 529.

4 Hermann, Siegwart, und Kassowitz, Heinz, *Arch. f. exp. Path. u. Pharm.*, 1935, **179**, 524.