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VI. Studies on Autodigestion. Digestion of Living Tissues by Trypsin.

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Bernard demonstrated that the leg of a living frog can be digested by stomach juice, but not by trypsin solution.[1] Kirchheim's experiments in which he found digestion of frogs' legs with trypsin seem to be objectionable, on the grounds that in all probability his animals were moribund.[2] Subsequent authors have confirmed Bernard's original experiments (lately Dragstedt, Haymond and Ellis[3]*), and have further shown that erythrocytes,[4] the mucosa of the urinary bladder[5] and spermatozoa when exposed to trypsin,[2] and also that organs or tissues when implanted into the duodenum, were not digested (Necheles, Ling and Fernando,[6] and Dragstedt, Haymond and Ellis[3]). Boldyreff[7] was the first to suggest that trypsin may play a rôle in the genesis of peptic ulcer. This idea was received by later authors who attempted to produce gastric ulcer with trypsin,[8] but their evidence is not convincing.[9]

It is common knowledge that a number of proteins are digested only slightly, if at all, by trypsin, but that after a short exposure to pepsin-HCl, complete digestion with trypsin takes place. Based on this principle, it was determined to study the influence of pepsin on tryptic digestion. Living pithed frogs were exposed to pepsin-HCl first for a short period of time, which procedure of itself did not result in any visible digestion, and then immersed in a solution of trypsin, which as has been repeatedly demonstrated is not able to

1 Bernard, C., *Leçons de physiologie Expérimentale*, Paris, 1856, **2**, 408.

2 Kirchheim, L., and Böttner, A., *Arch. exp. Path. u. Pharm.*, 1915, **78**, 99.

3 Dragstedt, L. R., Haymond, H. E., and Ellis, J. C., *Arch. Surg.*, 1934, **28**, 232.

* The pertinent literature on peptic digestion has been reviewed in the previous paper of this series.[10]

4 Matthes, M., *Münch. Med. Woch.*, 1902, **49**, 8.

5 Fermi, C., *Centralblatt f. Bakter.*, 1910, **56**, 55.

6 Necheles, H., Ling, T., and Fernando, F., *Am. J. Physiol.*, 1926, **79**, 1.

7 Boldyreff, W., *Arch. f. d. ges. Physiol.*, 1907-8, **121**, 13.

8 Stuber, B., *Z. f. Exp. Path. und Ther.*, 1914, **16**, 295.

9 Stahnke, E., and Hsieh, T., *Z. f. d. ges. Exp. Med.*, 1927, **55**, 403.

10 Maskin, M. H., Callahan, R., and Necheles, H., *Am. J. Dig. Dis. and Nutr.*, 1936, **3**, 174.

TABLE I.

No. of Series	No. of Frogs	Minutes of Alternating Exposure to: HCl .36% / Peps. 2%	Na_2CO_3 .25% / Tryps. 2%	HCl .36% / Peps. 2%	Na_2CO_3 .25% / Tryps. 2%	HCl .36% / Peps. 2%	Na_2CO_3 .25% / Tryps. 2%
1	6	30	10 S	0	49 M		
2	9	20	18 S	0	120		
3	5	20	19 S	8	12 M		
4	5	8	14	7	15 S	9	10 M
		HCl .18% / Peps. 2%					
5	5	25	11 S				
6	2	26 (22-30)	120 —				
		HCl .36% / Peps. 2%					
7	5	23 (10-33)	120 —				
8	5	30 —	38 S	0 —	85 M		
9	5	20 —	43 S	0 —	120		
10	5	10 —	23	10 —	24 S	8 —	16 M
		HCl .18% / Peps. 2%		HCl .18% / Peps. 2%			
11	5	30 —	27 S	0	70 M (2)*		
					120 (3)*		
12	6	17 —	40 S				
13	5	18 —	35 S (4)*	13 —	24 M		
14	2	45 — (30-60)	60 —				
		HCl .36% / Peps. 2%		HCl .36% / Peps. 2%		HCl .36% / Peps. 2%	
15	6	35 — (30-60)	65 (30-78) —				
16	1	30 —	30 —	5 —	30 —	5 —	25 —
17	3	—	114 (60-162)				

S = Skin Digestion. M = Muscle Digestion. Absence of both S and M denotes no digestion. Numbers without parenthesis denote averages; numbers in parenthesis denote range. * denotes number of frogs.

digest even the skin of a frog. The tests were conducted at a temperature of 38°C. by placing the containers in a constant temperature water bath. One hundred and two successful experiments were performed, but for lack of space only a number are reported below. *Ranae esculentae* with an average weight of 40 gm. were selected. Trypsin 1:300 and pepsin 1:10,000† were used in 2% solutions of HCl and Na_2CO_3 respectively. Of the former, 2 concentrations were used, 0.36% (N/10) and 0.18% (N/20), of the latter only 0.25%. The legs and one-third of the thighs of the frogs were immersed in this solution. The thorax was opened at the end of each experiment for observation of heart activity, and only such experiments in which the heart beat was normal at the termination of the test were accepted.

Our results can be grouped into 5 categories:

A. The influence of pepsin-HCl on tryptic digestion time,

B. The influence of HCl alone on tryptic digestion time,

C. A control series using pepsin-HCl followed by 0.25% Na_2CO_3.

D. A second control series using HCl alone followed by 0.25% carbonate, and

E. The negative effect of trypsin alone.

A. Exposure to pepsin-N/10 HCl for 30 minutes followed by trypsin resulted in skin digestion in 10 minutes, and muscle digestion in 49 minutes (Series 1). When the pepsin-HCl exposure was diminished to 20 minutes, tryptic digestion of the skin took place in 18 minutes, and no muscle digestion could be observed after 120 minutes (Series 2). But if an additional exposure of 8 minutes of pepsin-HCl was allowed once skin digestion had commenced, we obtained muscle digestion in the trypsin in 12 minutes, giving a total of 28 minutes in the pepsin and 31 in the trypsin (Series 3). In series 4, three repeated exposures of 8, 7 and 9 minutes in pepsin-HCl alternated with 14, 15 and 10 minutes in trypsin respectively. Skin digestion occurred during the second tryptic exposure, and muscle digestion during the third. Thus, if 24 minutes of pepsin-HCl were distributed over 3 periods, we were able to demonstrate muscle digestion in the trypsin in a total of 39 minutes, whereas it required 59 minutes to achieve the same end following a single initial exposure of 30 minutes to pepsin-HCl (Series 1). When the trypsin was preceded by pepsin-N/20 HCl for 25 minutes, the former digested the skin in 11 minutes (Series 5).

† We are obliged to Dr. D. Klein, Wilson Laboratories, for supply of part of the enzymes.

B. Comparing series 8-13, in which the trypsin was preceded by HCl alone with Series 1-5, it is evident that tryptic digestion of both skin and muscle is much slower than when pepsin is used in conjunction with the HCl. Here too we can demonstrate that repeated alternating exposures are more effectual than an equal single exposure (compare Series 10 with 8, and 13 with 11).

C. In series 6 and 7 frogs were first immersed in pepsin-HCl for 26 and 23 minutes respectively, followed by 0.25% Na_2CO_3 alone for as long as 120 minutes without any trace of digestion.

D. As might be expected, primary exposures to HCl alone (N/10 and N/20) for 30-60 minutes succeeded by Na_2CO_3 0.25%, for as long as 78 minutes failed to induce digestion (Series 14, 15, 16).

E. In the final series (No. 17) frogs exposed to trypsin for more than 2 hours were undigested (confirming Bernard, Dragstedt and others).

Exposure of the legs of living pithed frogs to artificial stomach juice of a physiological concentration of HCl (N/10 and N/20) for such a short time that no digestion could occur, prepares these tissues for digestion by trypsin. This is due partly to a possible destructive effect of HCl *per se,* and partly to the presence of pepsin, as demonstrated by shorter tryptic digestion times when pepsin HCl was employed than when HCl alone was used.

Repeated alternating exposures with short periods of pepsin-HCl and varying periods of trypsin result in more rapid digestion than where the same periods of time are utilized for a single exposure to pepsin-HCl followed by trypsin.

These findings may offer an explanation for the genesis of duodennal and gastro-jejunal ulcer, since these tissues are exposed to successive contacts with acid gastric and alkaline duodenal secretions. This hypothesis must include, however, the existence of an unknown factor which renders the tissues susceptible to digestion. Conditions influencing digestion of living pithed frogs by pepsin-HCl have been discussed in a previous paper.[10]

Summary. The legs of living pithed frogs immersed for a short period of time in HCl or pepsin-HCl without any visible digestion resulting therefrom, can then be digested by trypsin. Primary exposure of the frogs' legs to pepsin-HCl induces a shorter subsequent tryptic digestion time than primary exposure to HCl alone. Therefore, "peptonisation"[11] must play an important rôle in the prepara-

11 Kestner, O., *Chemie der Eiweisskoerper,* 1925, page 91.

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

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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