some of them have been couverted into a homogenous hyaline mass, with distinct inward and outward boundary. Not that the external portions had become hyaline *bubbles*, their shape seemed little changed, but they were in a way "verquollen" as the German histologists say.

We must refrain from expressing an opinion whether these outer limbs are present in all epitheliumcells or not. It is quite possible that they are much more widely spread than we suspect and that they are frequently destroyed by imperfect fixation. Manipulating correctly and applying the same methods, we were not successful in obtaining a view of them on the surface of the stomach of a small suckling cat.

In studying the literature of the subject, we have found out that at the early date of 1856 a man of KÖLLIKER's importance has seen globules of fat in the epitheliumcells of fresh gastric mucous membranes of young cats, dogs and mice. KÖLLIKER communicated on the 28th of June 1856 to the *Würzburger physikalisch-medicinische Gesellschaft* (VII p. 175) in a small paper entitled: "Einige Bemerkungen über die Resorption des Fettes im Darme, über das Vorkommen einer physiologischen Fettleber bei jungen Säugethieren und über die Function der Milz" that in his opinion he had seen globules of fat and also rather distinct indications of pores. With these pores he meant the openings in the "Porenmembran", the sieve-shaped, pierced, thickened wall of the cell, which we now call "striated border."

As far as we know no attention has been paid to this communication of KÖLLIKER's, except by OGNEW (Biologischer Centralblatt XII, S. 689, 1892) who has also seen the structures of CARLIER. To our mind it is indisputable that thestomach can resorb fat from the food, although it be in small quantities and it is also probable that this excellent naturalist has been able to discern with simple means, what cannot, with the methods of the present time, be effected without difficulty: namely to point out the striated border-shaped outer limbs of the stomach cells.

Physiology. — *"On the liberation of trypsin from trypsin-zymogen."* By Dr. E. HEKMA. (Communicated by Prof H. J. HAMBURGER).

I. *On the influence of acids on the liberation of trypsin from trypsinogen.*

As is well-known, trypsin, the proteolytic digestive ferment of the pancreas, does not appear as such in this gland, but in the form of an inactive precedent stage, which HEIDENHAIN, to whom we owe this discovery [1]) has named "zymogen".

[1]) R. HEIDENHAIN, Beiträge zur Kenntniss des Pankreas. PFLÜGERS Archiv 1875, pag. 557.

As besides trypsin, other enzymes have come to our knowledge which are also secreted when in a preliminary stage, it is preferable, as is frequently done now, not to speak here of "zymogen", but of "trypsinogen" or "protrypsin".

From the very beginning the question arises whether the liberation of the ferment takes place in the gland or in the intestine. According to researches made by CAMUS and GLEY [1]) and DELEZENNE [2]), the latter is usually the case; according to POPIELSKI [3]) always.

Then the second question arises: In what way does *the liberation in the intestine* take place?

Until a few years ago this liberating action was solely ascribed to the acid of the gastric juice.

Influenced by researches made in PAWLOW's laboratory, attention has of late been drawn to the intestinal juice [3]).

As there appeared to be two ways that might effect the liberation of trypsin, it was important to know, what relative value could be ascribed to each of them. I have therefore made the action of acids, amongst others also of hydrochloric acid, a subject of close investigation.

Tracing the communications in literature in respect to the influence of acids on the liberation of trypsin, one is always being directed to the publication of R. HEIDENHAIN, just mentioned. When we examine these writings we find that scarcely a page has been dedicated to this problem. Only the method, by means of which HEIDENHAIN has obtained the result, is shortly referred to, positive experiments are not described however. *He only mentions, that, when he had arrived at the end of his investigations, he found that glycerine-extracts from pancreassubstance operate much more effectively when the gland-substance is mixed with acetic acid, before glycerine is added; an observation which never failed in any of the cases when he applied this method.*

When a man like HEIDENHAIN publishes his observations, we have to take them into account, even although the experiments are not published along with them. In different text- and handbooks and monographs, we find related that acids possess the power to effect

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[1]) CAMUS and GLEY; DELEZENNE. C. R. Soc. de Biol. LIV. (1902).

[2]) L. POPIELSKI, Ueber die Grundeigenschaften des Pankreassaftes. Centralbl. für Physiol. 9 Mai 1903.

[3]) N. P. SCHEPOWALNIKOW, Diss. Petersburg 1899; PAWLOW, Das Experiment. Wiesbaden 1900, p. 15; WALTHER, Archives Ital. de Biol. 1901.

H. J. HAMBURGER and E. HEKMA, "On intestinal juice of man." Report Royal Academy of Sciences 1902, p. 713.

the transformation of trypsinogen into trypsin, resp. of promoting it [1]).

I have been perfectly able to confirm HEIDENHAIN's *investigations, but systematic researches have shown me, that they are only available for glycerine-extracts from the gland, but in no wise for watery extracts or for the pressed out juice of the pancreas.*

From the great number of experiments which I have made to this end, and which always led to the same results, I shall state here a single series.

First a repetition of HEIDENHAIN's experiment. The method which HEIDENHAIN indicates is as follows:

To every gram of pancreassubstance, which has been cut into small pieces and subjected to pressure, is added 1 c.c. acetic acid of 1%. The mass is again rubbed for 10 minutes, and the thus obtained compound then mixed with 10 grams of glycerine. After 3 days this compound is filtered. I now composed a glycerine-extract according to this prescription and, along with this, other glycerine-extracts whereby instead of 1 cc. acetic acid of 1%, I took respectively 1 c.c. acetic acid of $2^1/_2$ %, 1 c.c. acetic acid of 0.5 % and 1 c.c. water.

The hereby obtained extracts I allowed to act on white of egg without water (Col. II) and also after addition of water (Column III) [2]).

It is seen that where the glycerin-extracts of Col. I are brought to act on white of egg no digestion appears after 3 days (Col. II). This had to be expected. Even if trypsin had been set free, it could not have worked actively in the pure glycerine; for it is well-known that trypsin is not soluble in pure glycerine. Trypsin is liberated however when the glycerine-extracts are diluted with water (Col. III) and *more so* with those extracts which are composed with acetic acid (1, 2, 3) *than in those where ordinary water was used* 4). The acetic acid therefore furthers the liberation of the trypsin in glycerin-mixtures with water. A proportion between the concentration of the acetic acid and the extent of its operative power, does not exist however.

It could now be suggested that the trypsin from Col. III in Table I, which was at first inactive being in an indissoluble condition, now

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[1]) I only mention here: HAMMARSTEN, Lehrbuch der physiol. Chemie, 1899. 4er Druck, pag. 295.

A. GAMGEE, (Deutsche Ausgabe von ASHER und BEYER), Die Physiol. Chemie der Verdauung. 1897, pag. 231.

C. OPPENHEIMER, Die Fermente und ihre Wirkungen, 1900. p. 74 and 116.

[2]) For the quantitative determination of the proteolytic digestion the method of METT was followed. The experiments were only made with pig's pancreas. The temperature of the incubator varied from 37 to 39° C.

TABLE I.

I.	II.	III.
	Millimeters of white of egg consumed.	Millimeters of white of egg consumed after addition of 5 cc. water on 1 cc. extract, after the first 3 days.
	After 3 days.	After the following 3 days.
1). Pancreas substance 1 gram Acetic acid of 1% 1 c.c. Glycerin 10 c.c. } 3cc.	0	4 50 + 4.70 4.70 + 4 80 } 18.70
2). Pancreas substance 1 gram Acetic acid of 2½% 1 c.c. Glycerin 10 c.c. } 3cc.	0	4 40 + 4.60 4 70 + 4.60 } 18.30
3). Pancreas substance 1 gram Acetic acid of 0.5% 1 c.c. Glycerin 10 c.c. } 3cc.	0	4.80 + 5 4 90 + 5 } 19 70
4). Pancreas substance 1 gram Water 1 c.c. Glycerin 10 c.c. } 3cc	0	2.10 + 2 80 2 20 + 2.80 } 8 50

became active because of the addition of water. Table II shows however that in the original glycerin-extract, not diluted with water, *no* trypsin *whatever* was present.

In the experiments mentioned in Table II, a Na_2CO_3 sol. of 1.2% has namely been added to the glycerin-extracts. If indeed trypsin had been set free, we might here have expected digestion of white of egg. The trypsin operates very effectively in presence of Na_2CO_3 of 1.2 %, whereas the latter entirely prevents the transformation of trypsinogen into trypsin; a fact, already proved by HEIDENHAIN and which I have taken advantage of in all my experiments to prove, whether in certain cases I had to deal with material containing trypsin or trypsinogen.

From these figures we notice that 1 cc. acetic acid in concentrations of resp. 1, 2½ and 0.5% has not the power, just like 1 cc. water, to liberate trypsin from 1 gram of pancreas substance in the

TABLE II

	Millimeters of white of egg consumed.	
	After 3days	After 6days
1) Pancreas substance 1 gram Acetic acid of 1% 1 cc. Glycerin 10 cc. } 3 cc + 12 cc Na_2CO_3 opl. v 1.2%	0	0
2). Pancreas substance 1 gram Acetic acid of 2½ % 1 cc. Glycerin 10 cc } 3 cc. + 12 cc. Na_2CO_3 opl. v. 1.2%	0	0
3). Pancreas substance 1 gram Acetic acid of 0.5 % 1 cc. Glycerin 10 cc. } 3 cc. + 12 cc. Na_2CO_3 opl. v 1.2%	0	0
4). Pancreas substance 1 gram Water 1 cc. Glycerin 10 cc. } 3 cc. + 12 cc. Na_2CO_3 opl. v. 1.2%	0	0

time (mentioned by HEIDENHAIN), during which these liquids had come into contact with the pancreas substance, before glycerin was added [1]) It is however possible, as has been proved from Table I, to liberate trypsin *from the glycerin-extracts* by means of water, after having been brought into contact with it for a lengthened period and this process is aided by acetic acid being present. But the action of acetic acid is only of indirect nature, *it only seems to neutralize in some degree the unfavourable action which glycerin exerts on the liberation of trypsin.*

Then I thought, if this be the case, the favorable action of the acetic acid must fail to be effective in watery extracts and pressed out juice of the pancreas. This proved to be true, as table III and IV will show.

[1]) It should be observed that 1 cc. acetic acid, resp. water and 1 gram pancreas substance only give the relative proportions. In reality 5 cc. liquid on 5 grams pancreas substance was always taken and of course 50 cc. glycerin.

TABLE III.

	Fresh Panc. juice. Two drops.		Directly	After 18 hours.		After 40 hours.	
			Reaction litmus-paper.	Reaction litmus-paper.	Millimeters of white of egg consumed.	Reaction litmus-paper	Millimeters of white of egg consumed
1	fresh P.juice	+5 cc. acetic acid $2^1/_2$ %	acid	acid	0	acid	0
2	»	+5 cc. » 1 %	»	»	0	»	0
3	»	+5 cc. » 0.5 %	»	»	0	»	0
4	»	+5 cc. » 0.1 %	»	»	0	»	0
5	»	+5 cc. » 0.05 %	»	»	0	weak ac.	(1.10+1) (1.10+1.20) 4.40
6	»	+5 cc. water	neutral	neutral	0	weak alk.	(1.40+1.30) (1.20+1.30) 5.20
7	»	+5 cc. Na_2CO^1 sol 0.1%	alkaline	alkaline	0	alkaline	(1.20+1.10) (1.20+1.10) 4.60
8	»	+5 cc. » 0.5%	»	»	0	»	(0.10+0.10) (0+0) 0.20
9	»	+5 cc. » 1 %	»	»	0	»	0
10	»	+5 cc. » 1.5%	»	»	0	»	0
11	»	+5 cc. » 2 %	»	»	0	»	0
12	»	+5 cc. » 3 %	»	»	0	»	0
13	»	+5 cc. Extract from the intestinal mucosa [1]	neutral	weak alk.	(1.90+1.80) (1.81+1.80) 7.30	weak alk	(4+4.10) (4+4) 16.10

Table III shows us, that when a few drops of fresh, pressed out pancreas juice, which according to fig. 9, 10, 11 and 12 contained no trypsin, are mixed with acetic acid of $2^1/_2$, 1, 0.5 and 0.1 %, there is no digestion of white of egg. But when the acetic acid is used more diluted, viz. 0.05, then after a long time, formation of trypsin takes place, but not to a greater extent than when water is taken instead of acetic acid.

It could now be supposed that the trypsin would, under the influence of the acetic acid be liberated, but could not operate actively in the present acid reaction. Table IV shows that this is partly the case. For when an old pancreas is taken, in which according to 9,

[1] Extract from the intestinal mucosa may be used for the liberation of trypsin instead of the natural intestinal juice. In a following communication we expect to treat this subject more fully.

TABLE IV.

	Juice of a pancreas which has been exposed for 24 hours to room-temperature. Two drops.	Directly.	After 18 hours.		After 40 hours.	
		Reaction litmus-paper.	Reaction litmus-paper.	Millimeters of white of egg consumed.	Reaction litmus-paper.	Millimeters of white of egg consumed.
1	old P juice + 5 cc. acetic acid $2^1/_2$ %	acid	acid	0	acid	0
2	» + 5 cc. » 1 %	»	»	0	»	0
3	» + 5 cc. » 0.5 %	»	»	0	»	0
4	» + 5 cc. » 0.1 %	»	»	(1.30+1.30) (1.40+1.40) 5.40	»	(3+3 / 3+3) 12
5	» + 5 cc. » 0.05 %	»	»	(1.70+1.70) (1.70+1.70) 6.80	weak ac.	(3.40+3.30) (3.30+3.20) 13.20
6	» + water	neutral	weak alk.	(2+2.10) (2+1.80) 7.90	weak alk.	(3.70+3.70) (3.80+3.80) 15
7	» + 5 cc. Na_2Co_3 sol. 0.1 %	alkaline	alkaline	(1.50+1.60) (1.50+1.60) 6.20	alkaline	(3.20+3.10) (2.90+2.90) 12.10
8	» + 5 cc. » 0.5 %	»	»	(1.50+1.50) (1.40+1.50) 5.90	»	(2.90+2.80) (2.80+2.70) 11.20
9	» + 5 cc. » 1 %	»	»	(1.20+1.30) (1.30+1.30) 5.10	»	(2.60+2.70) (2.60+2.70) 10.60
10	» + 5 cc. » 1.5 %	»	»	(1.20+1.30) (1.30+1.30) 5.10	»	(2.40+2.40) (2.50+2.60) 9.90
11	» + 5 cc. » 2 %	»	»	(0.90+0.90) (1+1) 3.80	»	(1.90+2) (1.80+1.70) 7.40
12	» + 5 cc. » 3 %	»	»	(0.50+0.50) (0.50+0.50) 2	»	(1+1.20) (1.10+1.20) 4.50
13	» + 5 cc. Extract from the intestinal mucosa	neutral	weak alk.	(2.40+2.60) (2.60+2.50) 10.10	weak alk.	(4.40+4.30) (4.20+4.20) 17.10

10, 11 and 12 free trypsin is found and according to Table III, acetic acid has been added of $2^1/_2$, 1 and 0.5 %, there is no action whatever. The acid in these concentrations prevents the trypsin from acting. When however acetic acid of 0.1 % is used, then the action of the trypsin is not neutralized as is shown in Table 4, fig. 4. Therefore in fig. 4, Table III, *the liberation of trypsin must have been prevented by acetic acid of* 0.1 %.

Moreover Table III teaches us that in no single case digestion of white of egg was obtained with fresh pancreasjuice after 18 hours, except in fig. 13.

Hereby is clearly shown that water and acetic acid of 0.05 % are

far behind intestinal mucosa, resp. intestinal juice, with regard to their influence of liberating trypsin from trypsinogen.

Equal results as with acetic acid were obtained with *hydrochloric acid*, *lactic acid* and *butyric acid*. For hydrochloric acid this may appear from the following summary.

TABLE V.

Fresh pancreasjuice, two drops.	Millimeters of white of egg consumed.		After having been allowed to stand for 41 hours in the incubator, so much of a Na_2CO_3 sol. was added to 6 and 7, until the proportion of the Na_2CO_3 amounted to about 1 %.
	After 17 hours.	After 41 hours.	Digest. of white egg after once more 2×24 hours in 6 and 7.
1) pancreasjuice + 3 cc. water	0	1.80+1.70 / 1.70+1.90 } 7.10	
2) » + 3 cc. Na_2CO_3 opl. 1 %.	0	0	
3) » + 3 cc. extr. from the intestinal mucosa.	1.60+1.50 / 1.50+1.50 } 6.10	4+4.20 / 4+4.10 } 16.30	
4) » + 3 cc. HCl 0.02½ %.	0	1.70+1.80 / 1.70+1.70 } 6.90	
5) » + 3 cc. HCl 0.05 %.	0	1.70+1.60 / 1.60+1.60 } 6.50	
6) » + 3 cc. HCl 0.1 %.	0	0	0
7) » + 3 cc. HCl 0.5 %.	0	0	0

These figures show that hydrochloric acid in extremely weak concentrations (0.02½ and 0.05 %) does not hinder the trypsin from being set free. The effect is not favourable however. Somewhat stronger concentrations of hydrochloric acid (0.1 %, 0.5 %) prevent the liberation of trypsin entirely. That no trypsin has been set free in 6 and 7, the action of which may have been prevented by the hydrochloric acid, has been proved from the fact that no digestion of white of egg had occurred, even after 2×24 hours, when after 41 hours a solution of Na_2CO_3 had been added to the liquids named in 6 and 7, until the proportion of Na_2CO_3 amounted to circa 1 %.

From these researches we may with certainty draw the following conclusions.

1) HEIDENHAIN's opinion, which has been current since 1875 and

widely accepted, as if acids could have the power of liberating trypsin from trypsinogen is not correct; on the contrary, they prevent this liberation.

2) That HEIDENHAIN came to this conclusion must be ascribed to the accidental occurrence, that instead of using the pressed out juice or watery extracts of the pancreas, he had taken glycerin-extracts from the gland. The favorable action caused by the presence of acetic acid in his experiments and which I have been able to confirm, is to be ascribed to the fact that acetic acid decreases the injurious action of the glycerin on the liberation.

3) As it has now been proved that the gastric juice does in no wise further the liberation of trypsin, but rather opposes it, we may therefore draw the conclusion, *that in this process of liberation all the work falls to the intestinal juice; a fact still increasing in importance where the investigations of* POPIELSKI *have proved, that no free trypsin whatever appears in the pancreassecreta, but that it is only there in the shape of trypsinogen.*

Having arrived at the end of my communication, I beg Prof. HAMBURGER to accept my warm thanks for the opportunity afforded to me to make these researches and also for the useful hints kindly given to me.

Physiological laboratory of the State University at Groningen. May 1903.

Physics. — *"Some remarks on the reversibility of molecular motions."* By Dr. A. PANNEKOEK. (Communicated by Prof. H. A. LORENTZ).

1. The following considerations deal chiefly with the question whether a mechanical explanation of nature is possible. Mechanics treat the motion of discrete particles or of continuous masses; now the question may be raised, whether all natural phenomena can be explained by means of such a motion. In other words, it is the question, whether or no we know particular properties of these phenomena, which exclude the possibility of a mechanical explanation of general application. A particular property which seems to do so, is the irreversibility of the natural phenomena, the change in a definite direction. When investigating whether this is really the case, we need only consider the simplest form in which the irreversibility of natural phenomena occurs: the second law of the mechanical theory of heat.