may bring about the division, a fact which was confirmed by J. LOEB.

The analogy between the effect of substances dissolving fat on the development of eggs on the one hand, and on the acceleration of phagocytosis on the other, may be carried on still further, if we bear in mind that a copious action of these substances causes paralysis in the phagocytes (or destruction, as with camphor) and cytolysis in the eggs.

b. It is a well-known fact that narcotics stimulate when applied in small quantities and only paralyse in greater amounts. Viewed in the light of the facts we have observed about phagocytes, the explanation is easy to find.

At first, namely, the lipoid membrane of the cells is weakened, consequently the surface-tension grows less, and the rapidity of motion, the activity becomes greater. As soon as a greater amount of the narcotic has entered, its paralyzing effect on protoplasm becomes manifest.

For some general considerations see p. 998 and foll.

Groningen, January 1911.

Physiology. — "*On the stimulating effect of Chloride of Calcium and of intestinal mucous membrane extract on the action of Trypsin.*" By Mr. E. HEKMA. (Communicated by Prof. H. J. HAMBURGER).

(Communicated of the meeting of February 25, 1911).

The investigation reported on in the following pages found its starting-point in an investigation relating to the question whether chloride of calcium possesses the property of activating trypsinogen. Several investigators, particularly LARGUIER DES BANCELS [1]), DELEZENNE [2]), ZUNZ [3]) and recently also miss AYRTON [4]) have attributed to calcium

[1]) LARGUIER DES BANCELS. C. R. Soc. de Biol. 1895, p. 130.

[2]) DELEZENNE. C. R. Soc. de Biol. 1905, p. 476, 523, 614; 1906, p. 1070; 1907, p. 274.

[3]) ZUNZ. Annal. de la Soc. Roy. des Sc. Méd. et Nat. de Bruxelles. XVI. 1907.

[4]) Miss B. AYRTON. Collected Papers. Inst. of Physiol. University College London. Vol. XV. Edited by E. H. STARLING.

salts, and also to some other salts, the faculty of rendering inactive pancreatic-juice, or pancreatic extract, active with respect to albumen, in other words, capable of activating trypsinogen.

During the last few months I too have made a number of experiments with a view to obtaining, if possible by an independent investigation, a confirmation of the results arrived at by these authors, whose conclusions are for the rest pretty well identical. I became aware that an investigation as to the activating effect of chloride of calcium on trypsinogen (I have not as yet extended my investigation to other salts) though apparently simple, was attended with considerable difficulties. Hence my experiments on the subject have not yet led to any definite result so far as *the activating effect* of chlorid of calcium on *trypsinogen* is concerned. But in the course of this investigation some other facts have come to light which seem to me to be worth publishing, the more so as the difficulties encountered, will be to some extent set forth and explained by them.

In the course of the above-mentioned investigation it was namely discovered that *chloride of calcium* has the property of materially stimulating the action of trypsin, when already active in itself and free from trypsinogen. The same fact was observed about intestinal mucous membrane extracts, but in a much slighter degree.

There is no need to point out that *activating* trypsinogen (i. e. transforming inactive trypsinogen into active trypsin) and stimulating the action of trypsin (i. e. stimulating into greater activity the ferment when free from trypsinogen and already active as it is) are two entirely different notions.

It has already been said that a further investigation relating to this stimulating action was begun on account of observations made in some experiments, which were carried out to study the activating effect of chloride of calcium on trypsinogen. We subjoin some of the experiments made. (Tables I and II). Beforehand it must be mentioned that the pancreatic-juice experimented with, was obtained by pressing out a pig's pancreas, likewise that the extracts from the intestinal mucous membrane were prepared by extracting the scraped-off intestinal mucous membrane, and filtrating it after some time. Further that in all the experiments discussed in this paper, we used for albumen: coagulated white of hens' eggs, according to METT's well tried method, and finally that wherever in this composition chloride of calcium is spoken of, the salt without water is meant.

From the experiment detailed in Table I we may draw the following conclusions:

TABLE I.

		Digestion of the egg-white-columns in m.m. after	
		24 hours	2 × 24 hours
1	Pancreatic juice: 3 drops + 2% NaFl-solution: 5 c.c.	8	18
2	„ : 3 „ + extract in 2% NaFl sol.: 5 c.c.	12	22
3	„ : 3 „ + boiled extract in 2% NaFl sol. 5 c.c.	9	19
4	„ : 3 „ + distilled water: 5 c.c.	8	12
5	„ : 3 „ + 0.4% $CaCl_2$ sol.: $2\frac{1}{2}$ c.c. + water: $2\frac{1}{2}$ c.c.	12	25
6	„ : 3 „ + 1% $CaCl_2$ sol.: $2\frac{1}{2}$ c.c. + water: $2\frac{1}{2}$ c.c.	12	26
7	„ : 3 „ + 1% $CaCl_2$ sol.: $2\frac{1}{2}$ c.c. + 2% NaFl sol.: $2\frac{1}{2}$ c.c.	6	8

In the first place it appears from 1) that the pancreatic-juice contained trypsin, for we observe that in a medium of a 2% sol.NaFl a not inconsiderable digestion had taken place. If the pancreatic-juice had been entirely free from trypsin, in other words if it had contained nothing but trypsinogen, then no digestion of albumen would have taken place in a medium of a 2% NaFl-solution. From a comparison between 1) and 2) it appears that in 2) the albumen-digestion is greater than in 1). It followed from this that probably not all trypsinogen had passed into trypsin, because it had to be assumed that in 2) trypsinogen had still been activated by the extract from the intestinal mucous membrane.

Since this pancreatic-juice contained already free trypsin, we evidently could draw no conclusions from it as to trypsinogen being *activated* by chlorid of calcium; from this point of view, therefore, this experiment had to be looked upon as having failed.

On comparing 4) with 1) we see that after after 48 hours the digestion in 4) is considerably less than in 1). This result must be attributed to the fact that in 1) the bacteria-development was impeded by the NaFl-solution, whereas in 4) the bacteria were able to develop themselves freely.

Bacteria (at least some bacteria, I shall, however, not enter into this subject, as it does not bear on the matter under consideration)

have directly opposite effects on trypsin and on trypsinogen, as I gathered from a great many experiments. Whilst on the one hand *trypsinogen* can be *activated* [1]) by the action of bacteria, on the other hand the action of *trypsin* is greatly *impeded* by bacteria (or perhaps by the decomposition-products of albumen at the joint action of trypsin and bacteria. The pancreatic-juice experimented upon containing comparatively little trypsinogen and much trypsin, the antitryptic action of the bacteria prevailed in this case upon its activating influence, which also appears from the fact that in 4) and 1) the albumen-digestion is still the same after 24 hours, whilst after 48 hours (when all the trypsinogen in 4) could be expected to have passed into trypsin) it is considerably less in 4) than in 1).

Obviously this experiment gives support to the opinion that the influence of micro-organisms, in experiments relating to the activation of trypsinogen (and I may add by the way: likewise in experiments on antitryptic factors) should by no means be disregarded.

The condition that the action of bacteria must be effectually obviated in such experiments, e. g. when studying the effect of calcium-salts on the activation of trypsinogen, forms one of the difficulties I alluded to just now. The more so, as it stands to reason that in experiments with Ca-salts no NaFl may be used, which we can also gather for instance from 7) Table I. As we see the digestion in 7) is very inconsiderable. This must be attributed to the Fl. of the NaFl having formed in this case insoluble $CaFl_2$ with the Ca of $CaCl_2$. The precipitate $CaFl_2$ had sunk to the bottom of the test-bottle, carrying part of the albumen of the pancreatic juice with it. Hence the Mett-tubes had got enclosed in a thick precipitate of $CaFl_2$ and pancreatic albumen, in consequence of which the trypsin-action on the Mett's albumen-columns could of course not assert itself so well.

Finally on comparing in Table I the numbers 5) and 6) with 1) and 2), we might at first sight be inclined to assume that under the influence of $CaCl_2$ trypsinogen had been activated, the digestion in 5) and 6) being considerably greater than in 1), and as great, nay even somewhat greater than in 2). On second thoughts I arrived at the conclusion that in this case not merely the *activating* effect of $CaCl_2$ had to be considered, that at any rate there must also be other reasons for the greater digestion in 5) and 6). Indeed it appears from 2) that in this case the amount of trypsinogen could only be

[1]) For further particulars on this subject see an article of mine in the Archiv. für Anat. und Physiologie 1904 p. 343.

small, whilst as regards trypsin, the antitryptic effect of the bacteria would have manifested itself in 5) and 6) as strongly as in 4), if not another factor had been present, which promoted in 5) and 6) the trypsin-action more than it was counteracted by the bacteria.

These considerations suggested the idea to me that chlorid of calcium might perhaps have the property of stimulating the activity of trypsin.

This would, moreover, explain the results of these, and such like experiments, for I repeatedly found similar results.

As a type of the experiments, in which the pancreatic juice used, was free from trypsin, containing nothing but trypsinogen, we may quote the following (Table II).

TABLE II.

	Amount of Pancreatic-juice used	Fluids added	Digestion of two albumen-columns in m.m. after: 24 hrs.	2×24 hrs.
1)	2 drops	+ 2% NaFl-solution: 10 c.c.	0	0
2)	2 „	+ intestinal mucous membrane extract in 2% NaFl-solution: 10 c.c.	5 4	9
3)	2 „	+ boiled intestinal mucous membrane extract in 2% NaFl-solution: 10 c.c.	0	0
4)	2 „	+ 1% chlorid of calcium-solution: 5 c.c. + 2% NaFl-solution: 5 c.c.	0	0
5)	2 „	+ 1% chlorid of calcium-solution: 5 c.c. + water: 5 c.c.	2	6
6)	2 „	+ water: 10 c.c.	1.6	4

In the first place it appears from 1) and 3) of this experiment (Table II) that in this case the pancreatic-juice used, contained only trypsinogen and no trypsin. Regarded therefore as an investigation concerning the activating effect of chlorid of calcium on trypsinogen, the experiment could not be seriously found fault with. Except that in this experiment no sterile water and sterile $CaCl_2$-solution had been used, which evidently should have been done in experiments on the activating effect of $CaCl_2$ on trypsinogen. I intentionally quote an experiment in which no sterile water and no sterile $CaCl_2$ were used, as being more

to the purpose. An experiment like the one, detailed in Table II may even more strongly than the preceding one (Table I) create an impression, when we compare 5) and 6), that trypsin might be activated by chlorid of calcium. In 6) the activation has undoubtedly been effected by the bacteria, which have developed themselves in the non-sterile water (+ pancreatic-juice); in 5) the greater activation might have been caused by the joint action of bacteria and $CaCl_2$. And since in 5) the digestion was greater than in 6) there were plausible reasons for concluding that chlorid of calcium had contributed to the activation of trypsinogen. Still, as my attention had been directed to the possibility of a stimulating action of chlorid of calcium on trypsin, the difference in digestion between 5) and 6), when looked at from this point of view, might find an explanation in this sense. Further discussion of this experiment may be esteemed superfluous; I thought it advisable to insert it here as an additional proof that we have to be very careful about conclusions as to a contingent activating effect of chlorid of calcium on trypsinogen.

Whilst on the ground of the preceding observations it seemed not unlikely that chlorid of calcium might have a stimulating effect on trypsin itself, a closer investigation was begun now in order to test this supposition by means of experiments. For this purpose I made use of some commercial trypsin-preparations, viz. that of GRÜBLER and that of MERCK. As the activity of the preparations in the laboratory was found to be very slight with regard to coagulated albumen I ordered fresh preparations a few times. I informed the firm of GRÜBLER as to this slight activity, upon which this firm sent me, as I was informed, a newly-made preparation.

Yet the activity of this preparation was no greater than that of the preceding ones. That is to say as regards albumen, as regards fibrin the action of these preparations left nothing to be desired. By making the concentrations of the trypsin-solutions (suspensions) considerably stronger than prescribed by the firm I could use these commercial preparations for my purpose. The trypsin-solutions (suspensions) were prepared by means of soda-solutions. As the use of Na_2CO_3 seemed liable to some objections, however, owing to the slight solubility of the $CaCO_3$, resulting from $CaCl_2$ being added, I made use of very weak (as a rule 0.1 %) solutions of Na_2CO_3.

That there was no objection to the use of a 0.1 % sol. of Na_2CO_3 is seen from the following experiment, which for the rest served to investigate the stimulating effect of $CaCl_2$ on trypsin-action (Table III).

For this experiment 1 gramme of trypsin-GRÜBLER was dissolved

in 100 cc. Na_2Co_3 of 0.1 %. The trypsin dissolved in it with a slight opalescence, the solution was not filtrated before it was used. To 5 cc. of this solution were added 5 cc. water and 5 cc. $CaCl_2$-solution, respectively.

TABLE III.

	Digestion of two albumencolumns in m.m. after:	
	2×24 hrs.	3×24 hrs.
1) Trypsin sol. 1/100: 5 c.c. + water: 5 c.c.	3	4
2) Trypsin sol. 1/100: 5 c.c. + $CaCl_2$ sol. of 1%: 5 c.c.	9	15
3) Trypsin sol. 1/100: 5 c.c. + $CaCl_2$ sol. of 1%: 2½ c.c. + water: 2½ c.c.	10	15

From this experiment it appears that in 2) and 3) where $CaCl_2$-solutions had been added to the trypsin-solutions, the albumen digestion was considerably greater than in 1), where water had been added. We observe that the trypsin itself (1) showed very little activity as regards coagulated albumen, though as I observed before, 1 gramme of trypsin had been taken upon 100 c.c. Na_2Co_3 of 0.1 % (an addition of an equal volume of water making the concentration 1/200). And further that this power was considerably heightened under the influences of a 1 % and a 0.5 % $CaCl_2$-solution, respectively.

Still from this result it might not be concluded yet that $CaCl_2$ had promoted the action of trypsin. And that, for this reason: the trypsin-preparations might still contain some trypsinogen, which might have been turned into trypsin by $CaCl_2$.

Before continuing our experiments in this direction, we therefore had to settle first whether the trypsin-preparation used, was indeed free from trypsinogen. This question could be solved by adding in a following experiment also intestinal mucous membrane extract to the trypsin-solution and at the same time the boiled extract. [It is generally held, and in my opinion absolutely certain (Cf. 3) Table II) that boiling renders inactive the substance in the intestinal mucous membrane extract which causes trypsinogen-activation].

In order to solve the question whether the trypsin was free from trypsinogen or not, it seemed advisable to make a double experiment

by using in the first place a solution of trypsin in 0.1 % Na_2CO_3-sol. (Table IV A 1—6) and secondly a solution of trypsin in a 2 % Na Fl-solution (Table IV B 1—6).

As was observed already the trypsin-GRÜBLER used for this experiment (Table IV) was the same as that applied in the preceding one (Table III); instead of a trypsin-concentration 1/100, 1/50 was used. Moreover, as appears from this Table, an intestinal mucous membrane extract in water as well as in 2 % NaFl-sol. was taken. It ought to be mentioned that the proper trypsinogen-activating effect of the extracts of the intestinal mucous membrane was in all cases tested by means of pancreatic-juice.

TABLE IV.

A			Digestion of the two albumen columns in m.m., after:		
	Trypsin-GRÜBLER 1 gramme to 50 c c. Na_2CO_3 sol. of 0.1 %	Fluids added	24 hrs	48 hrs	72 hrs
1)	3 c.c.	water: 2 c.c.	2	4	5
2)	3 c c.	$CaCl_2$ sol. (1 %): 2 c.c.	8	16	20
3)	3 c.c.	intest. mucous membrane-extract in 2 % NaFl-sol. 2 c.c.	4	8	12
4)	3 c.c.	intest. muc. membr.-extr in water: 2 c.c.	3.60	7	10
5)	3 c.c.	Boiled int. mucous membrane-extract in 2 % NaFl-sol.: 2 c.c.	4	8.50	13
6)	3 c.c.	Boiled int. mucous membrane extract in water: 2 c.c.	8	8	11
B	Trypsin-GRÜBLER 1 gramme to 50 c c. of a 2 % NaFl-sol.				
1)	3 c.c.	Water: 2 c.c.	3	6	8
2)	3 c.c.	$CaCl_2$-sol. (1 %): 2 c.c.	0	0	0
3)	3 c.c.	intest. mucous membrane-extract in 2 % NaFl-sol. 2 c.c.	4	8	12
4)	3 c.c.	intest. muc. membr.-extr. in water: 2c.c.	4.40	8.50	12
5)	3 c.c.	Boiled int. mucous membrane-extract in 2 % NaFl sol.: 2 c c.	4	9	13
6)	3 c.c.	Boiled int. mucous membrane-extract in water: 2 c.c.	4	8 50	12

From the preceding experiment (Table IV A and B) we may conclude what follows. Comparison between A 3—6 and A, and likewise between B 3—6 and B teaches that also in this experiment the intestinal mucous membrane extracts have had a favourable effect on the trypsin-action. From the result that the albumen-digestion in A 3, 4, 5, and 6, and likewise in B 3, 4, 5, and 6 differed little or nothing, that consequently the *boiled* extracts of the intestinal mucous membrane were found to have the same favourable effect as the unboiled ones, we may conclude that in the trypsin-preparation used, no *trypsinogen* was present. The fact that in spite of this the albumen-digestion in A 3—6 was found to be considerably greater than in A_1, whilst this was also the case with B 3—6 and B_1 respectively, could in my opinion be explained only by assuming *that in the extracts of the intestinal mucous membrane, in other words, in the intestinal mucous membrane, a substance is found which can promote the trypsin-action, a substance which (contrary to the substance activating trypsinogen) could not be rendered inactive by being boiled.*

From the result that the trypsin used, was found to contain no trypsinogen, it also follows that the favourable effect of chlorid of calcium, observed in the preceding experiment (Table III) must be attributed to *the heightened action of trypsin, occasioned by chlorid of calcium.* The intensifying action of chlorid of calcium, as regards trypsin, likewise manifests itself, and that very obviously, in the last experiment (Table IV A and B). For in A_2 the albumen digestion is seen to be much greater than in A_1. That in B_2 no digestion took place at all, must undoubtedly be attributed to a precipitate of $CaFl_2$ being formed here, which had sunk to the bottom of the test-bottle, and surrounded the Mett's tubes, so that the trypsin-action could not manifest itself. Comparison between A 3—6 and A_2 shows, moreover, that the stimulating effect of the intestinal mucous membrane extract is a slighter one than that caused by chlorid of calcium, at least in the concentration used [1]).

Further it may be concluded from A 3 and 5 and B 3 and 5 (Table IV) that *the substance deriving its origin from the intestinal mucous membrane, and promoting the trypsin-action, is in all probability no calcium-salt.* For it may be assumed that a calcium-salt, if it were present in the extracts used, would have been precipitated by NaFl as insoluble $CaFl_2$. This last observation likewise holds good,

[1]) An investigation as to the influence of the concentration of the chlorid of calcium-solution, and also as to the effect of some other soluble calcium-salts on trypsin, is in progess, but has not yet been completed.

of course, for other salts which may be found in extracts of the intestinal mucous membrane, in other words in the intestinal mucous membrane itself, so far as they form insoluble compounds with NaFl.

The results described in connection with the tables inserted, were confirmed by other experiments made with the same view, which, as was observed already, were carried out with different trypsin-preparations of GRÜBLER and of MERCK. We subjoin one more experiment, made with trypsin-MERCK. (Table V).

TABLE V.

		Digestion of the two albumen-columns in m.m. after		
Trypsin-MERCK 1 gramme to 50 c.c. Na_2CO_3-sol. of 0.1 %	Fluids added	24 hrs	48 hrs	72 hrs
3 c.c.	Water: 2 c.c.	4	10	11
3 c.c.	$CaCl_2$-sol. of 1%: 2 c.c.	8	20	empty
3 c.c.	Int. muc. membr. extr. in 2% NaFl: 2c.c.	4.40	16	empty
3 c.c.	Int. muc. membr. extr. in water: 2 c.c.	5.50	15	empty
3 c.c.	Boiled int. muc. membr. extr. in 2% NaFl: 2 c.c.	5.60	15	empty
3 c.c.	Boiled int. muc. membr. extr. in water: 2 c.c.	6	16	empty

It is seen that the result of this experiment (Table V) is analogous to that of the preceding one. Further remarks are not suggested by Table V, except that comparison with Table IV, shows that the trypsin-MERCK was somewhat more active than the trypsin-GRÜBLER, a fact which could invariably be established in the experiments.

SUMMARY.

1. The experiments described above have shown that chlorid of calcium can increase to a considerable extent the activity of trypsin which contains no trypsinogen.

2. This promotive effect of chlorid of calcium on trypsin should not be confounded with the activating effect of chlorid of calcium on trypsinogen, which latter property is ascribed to this salt by several authors.

3. The extracts of the intestinal mucous membrane were also found to possess the property of being able to increase the action of trypsin, to a smaller extent, however, than chlorid of calcium.

4. The substance originating in the intestinal mucous membrane, which brings about this action, is not destroyed by being boiled, and is in all probability no calcium.

5. Besides a substance which, as we know, possesses the faculty of being able to activate trypsinogen, which substance is rendered inactive by being boiled, the intestinal mucous membrane contains, therefore, also another substance which has the power of stimulating active trypsin, a substance which is not rendered inactive by being boiled.

Groningen, January 22nd 1911. *Physiological Laboratory.*

Physics. — "*Isotherms of monatomic substances and of their binary mixtures. IX. The behaviour of argon with regard to the law of corresponding states.*" By Prof. H. KAMERLINGH ONNES and C. A. CROMMELIN. Comm. N°. 120*a* from the Physical Laboratory at Leiden.

(Communicated in the meeting of February 25, 1911).

§ 1. *The mean reduced surface of state for monatomic substances.* A difficulty which is by no means small is introduced into theoretical investigations dealing with the equation of state by the fact that, for every substance, and, in particular, for substances of simple molecular construction, the region that has been experimentally investigated extends over a small range of reduced pressure and of reduced temperature. If the law of corresponding states were strictly obeyed, this difficulty could be obviated by reducing and then combining with each other the regions investigated for the various substances. In this way the mean reduced equation of state has been synthesized: in the form VII. 1 [1]). It has been obtained from AMAGAT's observations on hydrogen, oxygen, and nitrogen, YOUNG's on isopentane and AMAGAT's and RAMSAY and YOUNG's on ether. In this way the equation of state has been obtained for an imaginary substance which, if further amplified by the disturbance function [2]) for the neighbourhood of the critical point, is suitable for all calculations in which the validity of the law of corresponding states is assumed. And this equation is of particular use in tracing deviations from the law of corresponding states, for it affords a suitable means of easily comparing

[1]) Suppl. N°. 19 (May 1908).

[2]) Proc. Febr. 1908. Comm. N°. 104.