It is perhaps also possible to explain the phenomena in an other way. It might be assumed, that only one knot of similar fibres connects the heart with the bulb. If the heart were exposed to no other influences, the stimulation of these fibres would always have the same effect, i.e. a decrease of the frequency of the heart beats or a standstill of the heart. But we have to take into consideration, that as a matter of fact in normal conditions there are still other influences which affect the heart, e. g. those, which are brought about by the accelerator nerves. And the variations in the excitatory state of these nerves might perhaps produce all the differences in the effects of vagus stimulation.

This explanation, which in our opinion is a less probable one, can be tested, if our experiments are repeated with this difference, that previously the nn. accelerantes are sectioned.

The detailed account of our investigation will be published elsewhere.

Microbiology. — *"Thermo-tolerant lipase"*. By Dr. N. L. SÖHNGEN. (Communicated by Prof. S. HOOGEWERFF).

Thermo-tolerant lipase means in this communication a fat-splitting enzyme able to resist a temperature of 100° C. during 5 minutes without being decomposed.

Thermo-tolerant lipase is secreted by the microbes of the group of *B. fluorescens liquefaciëns*, to which in this relation are also reckoned *B. punctatum*, *B. pyocyneum* and *B. liquefaciëns albus*. The enzyme is not formed by the group of *B. lipolyticum*, *B. stutzeri*, *B. fluorescens non-liquefaciëns*, neither by *Oidium lactis*, *Aspergillus niger*, *Penicillium glaucum*, and *Cladosporium butyri*, all of which secrete a fat-splitting enzyme which decomposes already at 80° C.

The most convenient medium for the above melting bacteria is broth with 3 % peptone, the cultivation being successfully effected in ERLENMEYER flasks under aërobic conditions at ± 23° C.

When such a medium is inoculated with one of the said melting bacteria the microbes will after six days' cultivation have secreted a considerable quantity of lipase so that the culture liquid in a fatted tube at 30° C. shows the lipase reaction already after 1 hour.

The following investigations are made with cultures aged 6—10 days.

Experiments on the decomposition of the microbic lipase by influences

of temperature proved that the fat-splitting enzyme of the group of the melting fluorescents is able to resist very high temperatures.

The quantity of lipase which is still active after heating at a given temperature and for a fixed time can be quantitatively estimated by titration of the fatty acids splitted off from the fat by this lipase.

By means of fatted tubes the quantity of lipase still active in the culture can be computed by comparison with more or less heated preparations as the degree of decoloration of the fat in the tubes corresponds with the vigour of the lipase preparations, provided their alkalinity be the same.

The first experiments on the decomposition of the lipase by the action of high temperatures were made at 75° C. and were conducted as follows.

In a water-bath of 75° C. sterile test-tubes were placed filled with 10 cM^3 of a culture containing lipase. The niveau in the tubes was about 3 cM. below that of the water-bath.

After 2 minutes the liquid in the tubes has adopted the temperature of the water-bath, so that from that moment the heating is reckoned to begin. The heating of the culture liquid in the series of test-tubes lasted respectively 10, 20, 30, 40, 50 and 60 minutes; the contents were then, after quickly cooling, poured over into a fatted tube which was subsequently kept at 30° C. for 24 hours.

When comparing the intensity of the decoloration of the fat in the tubes we could state that the lipase was only slightly decomposed. Even heating of an hour at 75° C. caused but a slight decrease of activity of the lipase. Analogous experiments at 84°—85° C. showed that after an hour's heating a considerable portion of the lipase had still remained active; after 40 minutes, however, a distinct decrease of the action of the enzyme could be stated.

These facts show already that the lipase of the group of *B. fluorescens liquefaciëns* resists higher temperatures than other enzymes, such as diastase, catalase, urease, trypsine, etc., all of which are decomposed after being heated during 30 minutes at 75° C.

The following experiments will prove that the enzyme of the melting fluorescents resists still considerably higher temperatures.

Heating of the lipase from 96°— 97°.

On 13 May 1911 a series of tubes with culture liquid were placed in a water-bath at 96°—97°. After a fixed time they were quickly cooled and the liquid was poured into fatted tubes. After 24 hours at 30° the action on the fat was stated.

	Decoloration of the fatted tubes on 14 May:
N 1 after 10 minutes	white; a little less than 00.
„ 2 „ 20 „	white; less than 1.
„ 3 „ 30 „	white; much less than 1.
„ 4 „ 40 „	white; slightly attacked.
„ 5 „ 60 „	hardly perceptibly attacked.
„ 6 „ 60 „	unchanged.
„ 00 was not heated	white
„ 0 boiled 1 minute.	white like 00.

Analogous experiments made on 15, 20, and 24 May gave similar results.

It is clear that the enzyme resists heating from 96°—97° during 10 minutes, just as short boiling, without being perceptibly decomposed.

It is remarkable that the action of the enzyme of the boiled culture is often more intense than that of the non-boiled; this must probably be ascribed to the fact that after ebullition the diffusion of the lipase from the dead bacterial bodies proceeds more readily, which is in accordance with the observation that the precipitate of boiled cultures splits more vigorously than the clear liquid above it.

The following experiments were made with boiled lipase on 1 May.

Four ERLENMEYER flasks of 100 cM^3 capacity were each provided with 1 gram of fat, 10 cM^3 $^1/_5$ N natrium carbonate solution and 0.6 cM^3 calcium chlorid, just strong enough to convert all the natrium carbonate. By boiling the contents of the flasks, then quickly cooling it while shaking, the fat is very finely dispersed through the liquid; the calcium carbonate is for the greater part enclosed in the particles of fat.

Now 60 cM^3 of a non-heated culture were added to flasks N. 0 and N. 1; to N. 2 60 cM^3 of a culture boiled during one minute, and to N. 3 the same quantity of a culture boiled 5 minutes.

N. 0 used 41 cM^3 $^1/_{10}$ N acid for the neutralisation.

N. 1, 2, and 3, placed for 24 hours at 37° were titrated on 2 May and gave the results expressed in the following figures.

Number of cM^3 $^1/_{10}$ N acid for the neutralisation of the cultures.	Splitted fat.
N. 0. On 1 May 41	
„ 1 „ 2 May 30	± $^1/_3$ gram
„ 2 „ „ „ 29	± $^1/_3$ „
„ 3 „ „ „ 34	± $^1/_4$ „

Consequently the lipase is not decomposed when boiled one minute; this is the case after 5 minutes' boiling.

It is noteworthy that the quantity of fat splitted by non-heated and by boiled cultures is the same, from which fact it may be inferred that the microbes of the group of melting fluorescents produce only one lipase, namely thermo-tolerant lipase.

Determination of the velocity of the decomposition of lipase by heating at 99°– 100° C.

These experiments were effected just in the same way as those with boiled lipase on 1 May.

7 June 1911	Quantity of fatty acid formed, expressed in cM³. $^1/_{10}$ N.	Decoloration of the fatted tubes
N. 00. Non-heated lipase	25	white
„ 0. Heated 1 minute	24	white
„ 1. „ 5 minutes	22	white
„ 2. „ 10 „	19	less white than 1
„ 3. „ 15 „	12	much less white than 1
„ 4. „ 20 „	3	still distinctly decoloured
„ 5. „ 25 „	0	perceptibly attacked
„ 6. „ 30 „	0	unchanged.

Consequently thermo-tolerant lipase resists heating at 99°—100° C. during 5 minutes without losing a considerable part of its activity. If the heating is prolonged the velocity of the decomposition increases very much so that after 25 minutes' heating the enzyme is quite destroyed.

Influence of acids on the fat-splitting by thermo-tolerant lipase.

From a series of investigations conducted in the same way as those with ordinary lipase described in a previous communication, followed that thermo-tolerant lipase is still more sensible to acids than the former. Already in a medium of $^1/_{100}$ N acid the enzyme splits hardly any fat, whereas ordinary lipase splits still feebly in a $^1/_{50}$ N acid liquid.

Neutralisation of an acidified thermo-tolerant lipase culture renders the enzyme again active; but when the thermo-tolerant lipase is boiled in a very feebly acid medium the enzyme is quite destroyed.

It is very peculiar that finely divided non-volatile acids, e. g. stearic acid, render the lipase already inactive in a culture liquid. If thus we add finely divided stearic acid to a boiled fluorescens liquefaciëns culture it splits no more fat. When we now filtrate the fatty acid, the filtrate contains very little or no lipase, but it is bound to the fatty acid on the filter and can again become active by neutralisation of the acid.

Lipase can thus be almost quantitatively removed from a liquid by means of finely divided fatty acid.

The lipase is not, however, bound to the fatty acid if the liquid which contains it reacts feebly acid. If then the fatty acid is filtrated off, the filtrate contains the lipase, which after neutralisation of the former again becomes active.

From this investigation follows that the group of the melting fluorescents secrete a fat-splitting enzyme which tolerates heating from 99°—100° C. for 5 minutes, or a minute's boiling without being perceptibly decomposed. This enzyme is called *thermo-tolerant* lipase.

It is by this property distinguished from the enzymes known till now and likewise from the lipase formed by the group of *B. lipolyticum, B. stutzeri, B. fluorescens non liquefaciëns,* and from that of the moulds: *Oïdium lactis, Penicillium glaucum, Aspergillus niger* and *Cladosporium butyri.*

The properties of the lipase of the latter micro-organisms and those of the thermo-tolerant lipase exhibit for the rest a great similarity as is shown by the way of diffusion through agar and gelatin media, and by the behaviour of both enzymes towards soluble acids and non-volatile fatty acids.

Chemistry. — "*On retrogressive melting-point lines.*" (Second Communication) By Prof. A. SMITS. (Communicated by Prof. J. D. VAN DER WAALS.)

(Communicated in the meeting of May 27, 1911).

Introduction.

In my first paper on this subject[1]) I started the discussion of retrogressive melting-point lines in the system H_2O—Na_2SO_4, which discussion will now be completed, and improved in a single point.

To reach a stricter accuracy in my reasoning I will now derive

[1]) These Proc. Sept. (1909) p. 227.