300 cc. of 96 % alcohol. After boiling for 6 hours, the alcohol is recovered by distillation and the aniline which still contains a little nitrobenzene is distilled in a current of steam. It is then converted into the hydrochloride to separate it from the admixed nitrobenzene; about 5 grams of aniline hydrochloride are obtained. In the same manner were treated *o-m-* and *p*-nitroanisol, *m*-chloro- and bromonitrobenzene and dichloro- and dibromonitrobenzene 1. 3. 5. from which were readily obtained the corresponding amido-derivatives to the extent of about 70 % of the theoretical quantity.

In the case of *ortho-* and *para*-chloronitrobenzene where the halogen atom is replaced by S_2 a simultaneous reduction takes place to a slight extent with formation of *o-* and *p*-chloroaniline.

Ortho- and *m*-nitrotoluene readily yield *ortho*-and *meta*-toluidine; with *para*-nitrotoluene a secondary reaction occurs, *p*-amidobenzaldehyde being formed as well as *p*-toluidine [1]).

Besides the above mentioned mononitro-compounds a few dinitrocompounds were subjected to a partial reduction. From sym-dinitrotoluene we readily obtain by means of alcoholic Na_2S_2 3-nitro-5-amidotoluene; sym-dinitroanisol yields 3-nitro-5-amidoanisol; from 2-4-dinitroanisol (or phenetol) is obtained 2-amido-4-nitroanisol (or phenetol) whilst sym. trinitrobenzene yields 3-5-dinitraniline. A small quantity of the azo-oxycompounds is generally formed in addition to the amido-derivatives. I will also point out that in the reducion with Na_2S_2 the formation of chlorinated byproducts, which are often generated in the reduction of aromatic nitro-compound with Sn and HCl, is avoided. The fact that sodium disulphide may be weighed also gives it an advantage over ammonium sulphide as a reducing agent.

From the above facts it is obvious that an alcoholic solution of Na_2S_2 may be used as a convenient reducing agent.

Physiology. — *"About the determination of hardness in muscles."* By A. K. M. NOYONS, Assistant in the Physiological Laboratory at Utrecht. (Communicated by Prof. H. ZWAARDEMAKER.)

(Communicated in the meeting of May 30, 1908).

At an inquiry into the causes and qualities of the autotonus it struck me how a muscle seemed to become harder, as its autotonus increased. Hitherto the hardness of a muscle was always estimatively determined by digital touching. The above mentioned fact caused

[1]) Chem. Centr. 1900. I. 1084. I hope to communicate more analogous cases of intramolecular oxidation later on.

me to look for means of expressing such changes in hardness more accurately in measure and number, as an approximate determination would not do here.

A communication by J. von Uexküll [1]) on the 18th of April last "Die Verdichtung der Muskeln", led me to a separate description of my investigations about the determination of hardness in muscles. In this communication he says: "wir besitzen zwar kein geeignetes Instrument, um das Hartwerden der Muskeln zu messen", while he winds up as follows: "Ich habe geglaubt, auf diese wichtige, aber allzusehr vernachlässigte Eigenschaft der Muskeln hinzuweisen, in der Hoffnung, dass sich jemand findet der einen brauchbaren Apparat konstruiert, um die Muskelverdichtung unabhängig von der Muskelverdickung zu registrieren."

For many decades together mineralogists have made determination of the hardness of materials, in which a number of methods were employed, which, however, in that form could not be applied to living objects. The literature only gathers for what is called hardness in general, data, for which I refer to some authors [2]) in behalf of those who wish to become more thoroughly acquainted with the subject.

Hardness is a collective idea, including and typifying an amount of qualities: cohesion, elasticity, plasticity, gliding, splitting and fracture. It is on the value which in a concrete case is assigned more especially to one of the qualities mentioned, that depends the general definition which shall be given of hardness. For living objects gliding, splitting and fracture need not be taken into account. I desist from a more detailed separate description of the three remaining qualities: cohesion, elasticity and plasticity. But if these three qualities are paid attention to, Auerbach's [3]).definition of hardness will no doubt be agreed to: "Harte ist eine Art von Festigkeit, nämlich der Widerstand gegen die Bildung von Unstetigkeiten oder dauernden Deformationen beim Drucke zweier sphärischer Oberflächen gegen einander, und kann Eindringungsfestigkeit genannt werden ... Sie ist quantitativ durch den Grenzeinheitsdruck im Mittelpunkte der Druckfläche bestimmt."

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[1]) J. v. Uexküll. Die Verdichtung der Muskeln. Originalmitteilung. Zentralblatt für Physiologie. Bd. XXII N°. 2.

[2]) H. Rosenbusch und E. A. Wülfing. Physiographie Allgemeiner Teil. Stuttgart 1904.

G. Tschermak. Lehrbuch der Mineralogie. Wien 1905.

Egon Müller. Ueber Härtebestimmung Inaug. Dissert. Jena 1906.

[3]) F. Auerbach. Kanon der Physik pag. 119. Leipzig 1899.

The determination of hardness may give absolute and relative values. Among the methods of relative determination that of THOULET[1]) appeared to be useful also to determine the relative hardness of living objects.

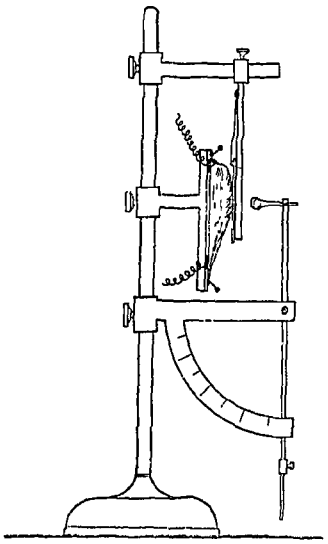
THOULET examined the elasticity of rocks and found points of comparison for this in the number of reflections and in the angle of reflection of a swinging ball suspended in the air. Indeed, if we drop a hard, elastic object upon an other, it will among others depend on the hardness of the surface that is hit, how often and how far the reflection will take place. Now, if this principle is put into practice with a much weaker object like a muscle, these reflections will, though in a smaller degree, yet take place in the same manner, which is corroborated by experience.

The angle of reflection of a falling globule resp. the number of its perceptible taps or reflections depends:

1. on the cohesion, elasticity and plasticity of the falling globule.
2. on the cohesion, elasticity and plasticity of the object hit, in this case the muscle.

Now as in case of comparing determinations sub 1 remains constant, sub 2 must be the only changeable, determinative factor.

The investigation takes place as follows with an apparatus that I call physiological sclerometer.



Physiological Sclerometer. Schematic drawing.
Fig. 1.

[1]) M. J. THOULET. Recherches sur l'élasticité des mineraux et des roches. Comptes rendues de l'Academie des sciences. Paris. Tome 96. 1883.

A small pendulum with a fixed turning-point, of which the short beam points upwards to a height of 6 cM., bears on the head of that short arm a handled glass-tear, whilst the other longest arm, 15 cM. long, is provided with a small, movable weight, in consequence of which the moment of that lever-beam is variable. By this way the force with which the head of the glass-tear hits the object, can be made variable. In order to enlarge the living force of the falling object, the pendulum may be given different initial amplitudes. On a scale along which the longest lever moves, this height of falling is expressed in degrees.

The muscle to be examined is by means of its two tendons attached to a somewhat rough surface, here a hard cork-plate, to prevent removal of the muscle by the falling, tapping object. It is advisable in this way to determine the hardness of a muscle under isometric conditions, for, when the muscle is examined under isotonic conditions, the data are getting far less trustworthy, as: 1. in shortening the muscle, the point that is to be touched, by not shortening changes its place and can only be found back by marking it beforehand with colouring matter; 2. the weight necessary for the stretching seems to make the differences in hardness smaller.

The number of times that the glass-tear is reflected by the muscle before it is at rest, is determined either acoustically or by means of photography. The photographic registration has this advantage that at the same time the width of the reflections can be followed.

The photographic registration takes place as follows: the light of an arc-lamp of 220 volt and 10 ampère is by a condenser more or less pressed together into a cone of rays having its focus in a diaphragm. This focus in its turn serves as a source of light and procures by means of a biconvex lens the parallel bundle of rays emitted. This bundle reaches the removable slit of a small box which in its opposite side is provided with a cylinder-lens of GARTEN. The light that has entered through the slit, is by the cylinder-lens, which is graduated, nipped together to an horizontal line of light, which falls through another slit into a second larger box on a drum that is in rotatory motion and to which sensitive bromide-paper of Dr. SCHÄEFELEN is fixed. The box containing the drum is impenetrable to light by means of light-free axes. This drum is moved on by a clock, as it is used in the telegraphic Morse-apparatus. Between cylinder-lens and the larger box is placed the long beam of the lever of the sclerometer which during its movements removes a silhouette on the sensitive silver-paper.

The following experiment was made: M. sartorius of Rana temporaria is alternately passed through by an electric current, arising

by a potential difference of 1.4 volt. For this purpose two brass plates serving as electrodes for the current, had been sunk in the cork sub-stratum of the muscle, whilst by means of a commutator the direction of the current can be changed. At the beginning of the experiment the anode is found at the distal tendon; afterwards the current is turned and ends in its original direction. In the subjoined table occur the widths of the first 4 reflections in mM.

Reflection	Anode at the distal tendon		Kathode at the distal tendon				Anode at the distal tendon	
I	52.5mM.	52mM	54mM.	53 mM.	54mM.	53mM.	50 mM.	50 mM.
II	29.5	29	31	30	31	31	29 5	29
III	21	21	23	22.5	22	22	20.5	21
IV	16	16	18	17	17	17	16	15.5

The following table gives the difference between the M. sartorius of Rana temporaria through which a galvanic current has passed and another through which it has not passed.

Reflection	Muscle through which no current is passing			Muscle through which a current is passing Anode at the distal tendon			
I	40 mM.	40.5mM.	40.5mM.	45 mM.	43 mM.	43 mM.	43 mM.
II	24.5	25	25	28	26.5	26	27
III	16.5	17	17	18	17.5	17	17.5
IV	11	11	11	12	11.5	11.5	12

That abundant moistening of a muscle with mutually equimolecular salt-solutions, the effect of which on the autotonus is antagonistic, can alter the hardness, appears from what follows, also holding good for the M. sartorius of Rana temporaria.

Reflection	Moistening with kaliumchloride				Moistening with natriumchloride					Moistening with kaliumchloride			
	mM.	mM.	mM.	mM.	mM.	mM.	mM.	mM.	mM.	mM.	mM.	mM.	mM.
I	48	42.5	41	40.5	40	37	33	38	38	38	38	38	39
II	33	25.5	25.5	26.5	25.5	24.5	22.5	24	23.5	23	22	22	23
III	24	17	16.5	17	16.5	17.5	16.5	16	15	15	14	14.5	14.5
IV	17.5	11	10.5	11.5	12	12.5	12	10	10	9.5	9	9	9.5
V	12.5	8.5	8	8	9	9	9	7.5	8	8	8	8	8
VI	9	7	7.5	7.5	7.5	8	8	7	7	7	7	7	7

For a plain muscle whose fibres are parallel as in the M. sartorius of Rana, the above method is a rather fit one, though not in all respects. For the shortening of the muscle is accompanied by a thickening, in consequence of which the distance between muscle and glass-tear is somewhat altered. This is not of much importance for the thin M. sartorius, but if the experiment is made with muscles like M. gastrocnemius, this difference becomes more considerable, so that it ought to be taken into account. Besides the peculiar rounding of the surface of the muscle may somewhat alter the place of tapping, and in the end the glass-tear sometimes slightly sticks to the muscle, when we are tapping with a small load on the longer beam of the lever.

To meet these and similar drawbacks the following alterations were made. Between muscle and tapping glass-tear is inserted a thin glass plate, which intercepts the taps and transfers them to the muscle. In these circumstances the angle of reflection resp. the number of collisions depends on:

1. the cohesion, elasticity, plasticity of the tapping glass-tear;
2. the cohesion, elasticity, plasticity of the inserted glass plate;
3. the cohesion, elasticity, plasticity of the object to be examined.

Sub 1 and 2 remaining constant, only sub 3 is variable.

In order to come to a determination, the following technical precautions ought to be taken into consideration. The glass plate, a covering glass, is hanging, slightly movable, on a couple of rather stiff horse-hairs. Now the muscle presses this glass plate against an immovable metal fork, so that the glass plate can only make movements in one direction, viz. in the direction of the muscle, as soon as the glass plate is hit by the tapping glass-tear. At every touch of the covering glass the glass globule produces a clearly audible tap. The number of taps is easy to count and is a pretty accurate measure for the number of real movements of the glass, without agreeing with it in number. In proportion as the covering glass is pressed more against the fork by a harder mass of muscles, the oscillations of the little lever will retain a longer and wider amplitude and will also occur more frequently.

The height of falling is of great importance for the effect that is to be reached, in the first place with respect to the number and amplitude of the oscillations.

When at different heights of falling the number of corresponding audible taps is counted for the same muscle, the latter may be represented by a curve, in which the ordinate renders the number of audible taps and the abscis the height of falling in degrees. The curve thus got shows a peculiar course.

N°. of the experiment and culture time	Components of culture medium in grams.	Inoculation-material	Produced calcium-carbonate in grams	Volatile acid in grams	Totally disappeared limesalt in grams	Nitrogen found after KJELDAHL in milligrams	Nitrogen fixed per gram of decomposed calciumsalt	Observations
1 24 Febr.—10 April	4 gr. of Calc. Mal. 0,05 K^2 H PO^4, 200 cM^3. tapwater.	1st re-inoculation of crude culture in canalwater in same liquid	1 57	0.403	3.3	8.1	2.5	
2 26 Febr.—4 April	2 gr.of Calc.Mal.0,05 K^2 H PO^4, 200 cM^3. tapwater.	2d transferring of the preceding.			2	5.2	2.6	
3 13 March—4 April	2 gr of Calc.Mal.0,05 K^2 H PO^4, 100 cM^3. tapwater.	3d transferring of the preceding.	0.872					
4 26 March—29 April	12gr.ofCalc.Mal.0.05 K^2 H PO^4, 200 cM^3. tapwater.	4th transferring of the preceding.	5.7	0.632	10.7	18.1	1.8	
5 6 April—23 April	4 gr.of Calc.Mal 0,05 K^2 H PO^4, 100 cM^3. tapwater.	Pure culture of *Azotobacter*	0.552	0.112	1.1	1.6	1.5	Microscop visible infection
6 4 April—29 April	4 gr.of Calc.Mal.0,05 K^2 H PO^4, 100 cM^3. tapwater.	Pure culture of *Azotobacter*			2.2	5.9	1.7	Microscop. visible infection
7 7 April—2 May	4 gr.of Calc.Mal.0,05 K^2 H PO^4, 100 cM^3. tapwater	Pure culture of *Azotobacter*	0.494	0.042	0.89	1.5	1.7	No microsc. visible infection
8 13 May—29 May	2gr.of Calc,Mal.0.05 K^2 K PO^4, 100 cM^3 tapwater.	Pure culture of *Azotobacter*	0 8103	0.036	1.39	2.49	1.8	Microcosp. and bacteriologically pure
9 6 April—23 April	4gr. of Calc. Lactate 0,05 K^2 H PO^4, 100 cM^3. tapwater, 1 gr. of chalk.	Crude culture from 1.		0.101		1.8	1.8	
10 16 March—29 April	1 gr.of Calc.Acetate, 0,05 K^2 H PO^4, 100 cM^3. tapwater.	Crude culture from 1.	0.576		1	2.8	2.8	
11 16 March—8 May	1 gr. of Calc.Propionate, 0 05 K^2HPO^4, 100 cM^3 tapwater.	Crude culture.	0.494		1	4.7	4.7	

The above experiment was made with a dead muscle, to avoid as much as possible all variable factors of the living object. These come into operation, as appeared from experiments, in which first a curve was produced by observations of a living muscle, and the next day a second curve could be formed from observations of the now dead muscle, which under a glass cover with saturated vapour of water and thymol-vapour was preserved resp. from desiccating and rotting. The values denoted by the curve are averages got from at least five observations each time, which did not materially differ from each other.

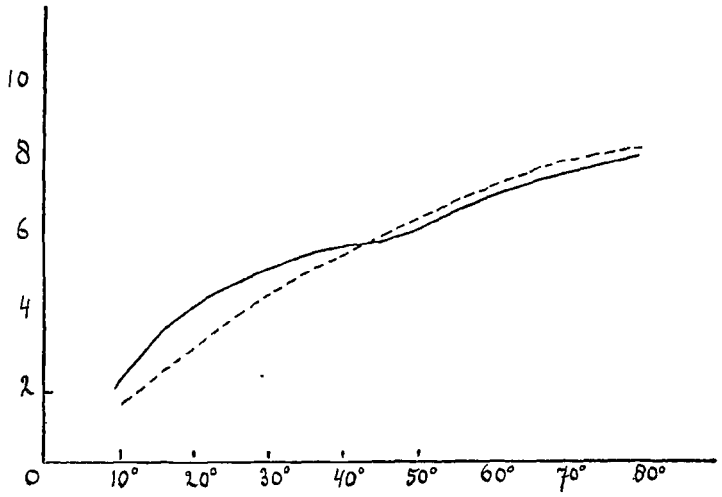


Fig. 2.
Hardness with regard to different heights of falling by a muscle in its dead and living situation.
——— = living muscle. ------ = dead muscle.

The ordinate gives the number of audible taps and the abscis the initial height of falling in degrees.

In different ways the hardness of a muscle can be made to undergo changes, which are either permanent, or which exist long enough for the determining investigation:

1. by making a galvanic current pass through a muscle;
2. by abundant moistening with equimolecular salt-solutions;
3. by faradaic excitement, either direct or indirect, so that the muscle is in tetanus;
4. by heating, resp. cooling.

An example of the two first mentioned manners was given before; one of the two other manners is as follows: a muscle is by indirect excitement with a faradaic current alternately brought to tetanus. At corresponding moments the determinations of hardness take place.

The subjoined table contains the width of the first 8 reflections which were reproduced photographically; at the same time the duration of these 8 reflections was calculated.

M. gastrocnemius of Rana temporaria.

	Normal muscle	Excited to tetanus	Not excited	Excited to tetanus	Not excited	Excited after rest
I	56 mM.	61 mM.	56 mM.	57 mM.	56 mM.	59 mM.
II	38	45	39	43	40	44.5
III	29	35	29	33	31	33
IV	22	28	22.5	26	23.5	26
V	17	22.5	17.5	21	18	21.5
VI	13	18.5	13	16.5	14	17
VII	11	15.5	11.5	13.5	12	13.5
VIII	9.5	12.5	10	11.5	10	11.5
	Duration of the first eight reflections.					
	4.4 sec.	4 sec.	4.3 sec.	4 sec.	4.3 sec.	4 sec.

From this it appears that in comparing the first 8 reflections not only the amplitude changes, but that also the time in which these oscillations take place, varies with the greater or smaller degree of hardness of the muscle. As the experiment progresses, it may be observed in the table that the heights of the reflections are getting larger also at the moments when the muscle is not excited. This must be connected with the changes in the constant state of contraction (autotonus), which arise in every fatigued muscle. That the muscle becomes really tired, is proved by the fact: 1. that the muscle visibly contracts less, 2. that changes in duration and height of the reflections diminish after repeated excitement, 3. that after the rest the effect of excitement agrees again with what was observed in the beginning of the experiment.

We add a tabulated statement of an experiment in which the muscle at the end of the experiment had become entirely inexcitable, as appeared from the absence of visible contractions, both for indirect and direct faradaic excitement, though still slight alterations in hardness appeared to be perceptible.

M. gastrocnemius of Rana temporaria
becoming inexcitable according to every-day parlance.

	Normal muscle	Excited	Not excited	Excited	Not excited	Excited
I	53 mM.	60 mM.	48 mM.	52 mM.	48 mM.	50 mM.
II	40	50	35	38	35	36
III	28	36	24.5	26	24.5	26.5
IV	21	27	17.5	18	17.5	19
V	15	21.5	13	14	13	15
VI	12.5	17	12	12.5	12	12.5
VII	10.5	13.5	11	11	11	11.5
VIII	10	11.5	10	10	10	10
	Total duration of the first 8 reflections.					
	5.4 sec.	5.2 sec	5.2 sec.	5 sec.	4.4 sec.	4.8 sec.

M. gastrocnemius of Rana temporaria.

	Temperature in temperator			
	12.5° Celsius		56° Celsius	
I	49 mM.	49 mM.	50 mM.	50 mM.
II	38	39	41	41
III	27	27.5	31	30.5
IV	19.5	20	25	24.5
V	16	16	21	21
VI	13.5	14	18	18
VII	13	13	16	16
VIII	12	12.5	14.5	14 5
	Audible taps			
	7	7	10	10
	Total duration of the first 8 reflections in abscis-length			
	3.6 cM.	3.8 cM.	3.2 cM.	3 cM.

If a striated muscle is heated, it shortens: this is accompanied, as appears from the experiments, by changes of hardness. In order to trace this, the muscle in the sclerometer, instead of to a corkplate, was fixed to the thin copper bottom of a temperator, now serving as resting-surface. Through this temperator, as THUNBERG pointed out for the examination of the cold- and heat-points of the skin, alternately cold and hot water could be made to circulate. The copper bottom communicates the heat to the muscle; the temperature in the temperator and that which the muscle gets, will not soon be the same, but still is always in close connection with it.

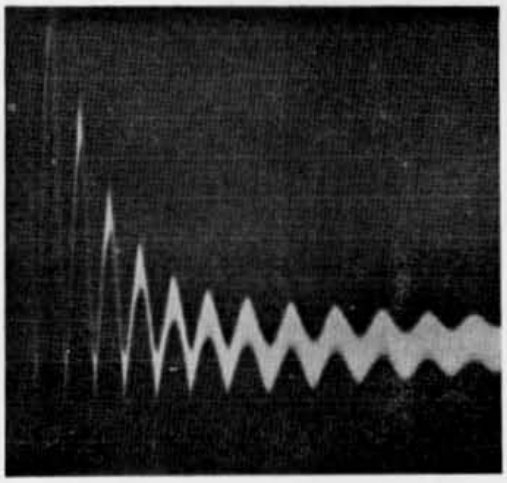
As a demonstration I give here a couple of photographic reproductions of the oscillations of the beam of the sclerometer, as they were made, and from which among others the above table was partly derived. Fig. 3 gives the sclerometric reproduction of hardness of a muscle at a temperature of 12.5° C. in the temperator, whilst fig. 4 shows the reproduction when the same muscle is heated to 56° C. (See figs 3 and 4).

If a muscle is heated to not too high a temperature, a decrease of hardness manifests itself again after cooling, even though the muscle does not quite reach its original degree of hardness.

The subjoined table makes this clear.

M. gastrocnemius of Rana temporaria.

	Temperature in temperator						
	13° Celsius		61° Celsius			11° Celsius	
I	49 mM.	49 mM.	50 mM.	50 mM.	50 mM.	49 mM.	49 mM.
II	37	38	42	41	41	38	39
II	28	30	34	33	33	30	30
V	24	24.5	29	28	28	25	25
V	20	20	25	25	24	21.5	22
VI	17	17.5	22	22	21.5	18.5	19
VII	14	15	20	19.5	19	16.5	17
VIII	12.5	12.5	18	17.5	17.5	14.5	15
	Audible taps						
	10	10	15	16	16	13	13



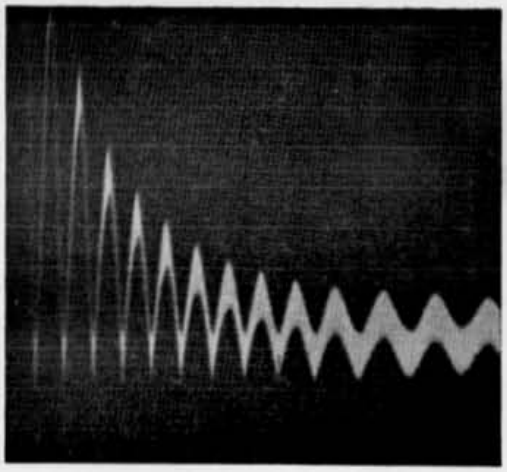


Fig. 3. Fig 4.

Sclerometric reproduction of hardness:

at 12,5° Celsius. at 56° Celsius.

Such warming and cooling can be repeated a couple of times, whilst in proportion to this the number of reflections continues varying, provided the muscle be not for too long a time exposed to too high a temperature, as in this case a clearly perceptible permanent hardness will show itself.

Physiology. — "*On the structure of the ganglion-cells in the central nervous system of Branchiostoma lanc.*" (Second communic) By Dr. J. BOEKE. (Communicated by Prof. G. C. J. VOSMAER).

(Communicated in the Meeting of May 31, 1908).

a. *The infundibular organ.*

The cells of the differentiated part of the ventral cerebral wall of Branchiostoma, which I described some years ago in these Proceedings [1]), and which was then called the *infundibular organ* on account of its place and the homology that could be drawn from that, are quite different in their structure from the other cells, of which I gave a description in my former paper [2]).

Among the authors, who in recent years have published researches on the central nervous system of amphioxus, KUPFFER [3]) gives the same description of the cells as I gave in my paper in 1902, and only mentions the organ as consisting of long cylindrical cells with curved cilia and a clear hyaline protoplasm. KUPFFER homologises the differentiated epithelium with the tuberculum posterius of the craniote embryos. JOSEPH [4]) only mentions the organ without adding anything to the description. EDINGER [5]) who examined preparations stained after the method of BIELSCHOWSKY, calls it "das aüs grossen Flimmer- und Sinneszellen bestehende Infundibularorgan", without mentioning on what is founded the opinion, that there are two kinds of cells to be found. In the drawings reproduced in his paper nothing is to be seen but a faint striation of the ventral wall of the brain at the place of the infundibular organ. According to WOLFF [6]) there is a striking resemblance between the differentiated epithelium of the infundibular organ and the gelatinous tissue that we find in the

1) Proc. Roy. Acad of Sc. of Amsterdam, Math. Phys. Cl. Meeting of April '07 p. 86.

2) Proc. Roy. Acad. of Sc. of Amsterdam, Math Phys. Cl. Meeting of April '02 p. 695.

3) Handbuch der Entwickelingslehre (HERTWIG), Vol. 2, 3d part.

4) Verhandl. d. Anat. Gesellsch. 18. Vers. 1904.

5) Anat. Anzeiger, Bd. 28, 1906

6) Biol. Centralblatt. Bd. 27, 1907.