Physiology. — *The Significance of the concentration of calcium-ions for the movements of the stomach caused by stimulation of the N. Vagus*". By R. Brinkman and Miss E. van Dam. (Communicated by Prof. H. J. Hamburger).

(Communicated at the meeting of December 18, 1920).

The great significance of the calcium-ion as an antagonist of the Na- and K-ions has been set forth by numerous researches [1]) since the fundamental experiments by Ringer and Loeb. The physico-chemical explanation of the action of calcium-ions must be sought in the balancing effect that this ion has towards the monovalent Na- and K-ions, as is very clearly illustrated, for instance, by the researches of Neuschlosz [2]), published but latily, about the influence of salt-equilibration on the surface-tension of lecithine-soles in water. From the table below one can form an idea of this action. In this list it is stated how the strongly-increasing influence which definite (physiological) NaCl-concentrations exercise on the surface-tension

TABEL I.

Total concentration of the mixture.	1 NaCl.	1 NaCl/ 1 $CaCl_2$	1 NaCl/ $^1/_5$ $CaCl_2$	1 NaCl/ $^1/_{10}$ $CaCl_2$	1 NaCl/ $^1/_{20}$ $CaCl_2$	1 NaCl/ $^1/_{30}$ $CaCl_2$	1 NaCl/ $^1/_{50}$ $CaCl_2$	1 NaCl/ $^1/_{100}$ $CaCl_2$
1 mol.	90.3	89.4	83.8	80.3	**76.3**	77.5	82.7	88.8
$^1/_2$ mol.	90.7	90.3	84.4	80.8	**76.6**	78.4	83.6	90.6
$^1/_4$ mol.	92.9	90.8	85.6	81.7	**76.8**	79.4	84.4	91.5
$^1/_8$ mol.	94.5	91.7	86.1	82.1	**76.4**	80.0	85.2	92.4
$^1/_{16}$ mol.	92.9	89.7	84.4	81.2	**76.0**	79.8	84.9	91.0
$^1/_{32}$ mol.	87.6	89.0	84	80.5	**75.9**	79.6	83.3	90.3
$^1/_{64}$ mol.	83.6	88.3	83.6	80.1	**75.7**	78.5	82.5	89.4
$^1/_{128}$ mol.	80.1	87.5	82.7	78.5	**75.9**	77.5	81.9	88.1

[1]) Summary in Höber: Physikalische Chemie der Zelle und Gewebe, Kap. VIII (1914); v. Tschermak: Allgemeine Physiologie, p. 120 (1916); Bayliss: Principles of General Physiology, p. 215 (1915); Höber: Pflüger's Archiv. **166**, 531, 1917.

[2]) Neuschlozs: Pflüger's Archiv. **181**, 17, 1920.

of lecithine-soles, is almost entirely neutralized by a definite concentration of $Ca^{\cdot\cdot}$-ions.

The surface-tension of a pure 1 °/₀ lecithine-sole amounted to **75.9.** [1])

Consequently it appears from this table that the influence of a definite concentration of an unbalanced NaCl-solution on the surface-tension of a lecithine-sole, may be neutralized almost entirely by the addition of Ca-ions, but it appears at the same time that only one definite $[Ca^{\cdot\cdot}]$ can do this and that this balancing effect can be produced neither by a too large $[Ca^{\cdot\cdot}]$ nor by a too small one. The degree of this balancing $[Ca^{\cdot\cdot}]$ depends on the ion-system present.

How we should explain this balancing is not known with certainty; it seems that Loeb [2]) and others have modified the theory of the electro-chemical ion-proteid-compound in favour of an ousting from the surface. In a biological respect examples have of late come to our knowledge from which it appears that also with the physiological ion-balancing the degree of $[Ca^{\cdot\cdot}]$ is decisive, and that very slight fluctuations of these $[Ca^{\cdot\cdot}]$ may have an important physiological consequence. [3])

It may be understood therefore that these $[Ca^{\cdot\cdot}]$ should be kept constant in the blood-plasm, as well as, e.g. the $[H^{\cdot}]$. The buffer-system by which this is principally effected has been indicated by Rona and Takahashi [4]). According to these authors there is for the free calcium-ion-concentration in the blood the equation: $[Ca^{\cdot\cdot}] = K . \frac{[H^{\cdot}]}{[HCO']}$ (K being about 350), a relation we could entirely confirm by direct measurement of the $[Ca^{\cdot\cdot}]$. [5]) As the $[H^{\cdot}]$ practically varies very little in physiological and also in pathological cases, the $[Ca^{\cdot\cdot}]$ will consequently be controlled chiefly by the concentration of the bicarbonate-ions. An increase of the $[Ca^{\cdot\cdot}]$ will depend in the first place on a decrease of the $[HCO'_3]$, in other words of an acidosis.

The main object of this communication is what influence the $[Ca^{\cdot\cdot}]$ and its fluctuations have on the irritability of the N. vagus. As a

[1]) Measured with the stalagmometer of Traube: Handbuch der Biochemische Arbeitsmethoden V, Bd. 2. 1912.

[2]) Loeb: Journal of General Physiology, Vol. I en II.

[3]) Hamburger en Brinkman: Biochemische Zeitschrift **88**, 97, 1918; Brinkman: Biochem. Zeitschr. **95**, 101, 1919.

[4]) Rona en Takahashi: Biochemische Zeitschrift **49**, 370, 1913.

[5]) Brinkman and miss van Dam, Verslagen Kon. Akad. v. Wetenschappen, meeting of 25 Oct. 1919.

test-organ we selected the perfused, surviving frog's stomach, on which we can easily study the influence of the N. vagus on the motility.

The general significance of the Ca-ions for the irritability of the cerebro-spinal and the autonomic, central and peripheric nervous system has been known for some time.

LOCKE[1]) demonstrated that the Ca-ion is necessary for conducting the stimulus from a nerve to a voluntary muscle. OVERTON[2]) proved that it was equally indispensable for preserving the synapsis between nerve-ending and ganglion-cell.

BUSQUET and PACHON[3]) showed that the irritability of the N. vagus, which soon disappears on perfusing the heart with a pure NaCl-solution (HOWELL)[4]), returns by adding small quantities of Ca. They further found, as did also SABBATANI[5]) by testing many calcium-salts of widely differing degree of dissociation, that we are definitely concerned with an ion-influence and that undissociated Ca-salts were of no importance for the balancing effect.

It is the concurring opinion of all investigators that the explanation of this Ca-ion effect must be sought again in the influence on the synapsis-colloids which is antagonistic to Na and K. From the above-mentioned experiments of NEUSCHLOSZ[6]) as well as from said physiological experiments [7]) it appeared also that *this* $[Ca^{\cdot\cdot}]$ *must have a very special constant value,* and that slight variations of the physiological $[Ca^{\cdot\cdot}]$ may be of great influence. A total absence of $Ca^{\cdot\cdot}$-ions will never occur in vivo, but especially these slight fluctuations of $[Ca^{\cdot\cdot}]$ are important under physiological conditions.

It is true that in the literature of the subject there are indications to be found that a too large quantity of Ca is as detrimental as a

[1]) LOCKE: Zentralblatt f. Physiologie **8**, 166, 1894. See farther CUSHING: American Journal of Physiology, **6**, 77, 1902; MINES: Journal of Physiology, **42**, 251, 1911.

[2]) OVERTON: Pflüger's Archiv. **105**, 261 and 280, 1904.

[3]) BUSQUET et PACHON: Journal de Physiologie et de Pathologie Gén. **11**, 807 and 851, 1909.
MINES l.c.; LOEWI: Archiv. f. Exp. Pathol. **70**, **343**, 1912.
HAGGAN and ORMOND: Amerc. Journ. o. Physiol. **30**, 105, 1912.
CAZZOLA: Archivio di Fisiol. **11**, 88, 1913.

[4]) HOWELL: Americ. Journ. o. Physiol. **15**, 280, 1906.

[5]) SABBATANI: C. r. Soc. Biol. **54**, 716, 1902.

[6]) NEUSCHLOSZ: l.c.

[7]) HAMBURGER and BRINKMAN: l.c.

too small one[1]), but a careful study, showing the relation between the $[Ca^{\cdot\cdot}]$ degree and the vagus-irritability, has not come to our knowledge. For this reason we have tried to find this relation as it was also done with the surviving frog's kidney [2]) and the haemolysis [3]).

The perfusions were done as follows:

The abdominal wall, thorax wall, and clavicula of the frog (♂) are carefully cut away, also the extremities are removed and the test object is nailed to a board. For a better survey the intestines may also be removed as far as the duodenum provided the mesogastrium is not injured. The canula is inserted into the a. coeliaca; the a. mesenterica is tied tight. In this way stomach + liver and gall-bladder (art. hepatica) are perfused. The pressure may be regulated by the level of the liquid-reservoir and the orifice of the canula.

Should it be desired to perfuse the whole of the intestines + portal circulation, than the a. mesenterica is not tied; liver (arterial and venous), wall of gall-bladder, stomach and intestines are then perfused. The proximal part of the v. abdominalis must be tied.

The n. vagus is stimulated by inserting electrodes in the tubae Eustachii; this is done most easily, by hammering 2 copper nails through the tubae into the board.

With this method of stimulation we always see (by very constant coils-distance) the vagus-effects on heart and stomach-intestines.

The duration of each experiment was about $1^1/_2$ hours.

We have now observed the influence of the Ca-ion-concentration in about 75 perfusions. Beforehand the irritability and the motility of the stomach-wall of the newly-killed not-perfused frog was determined, which existed as much as possible under physiological conditions.

Afterwards the perfusions took place with the following solutions:

1. NaCl 0,6 %.
2. NaCl 0,6 %, then NaCl 0,5 %, $NaHCO_3$ 0,20 %, $CaCl_2$.6 aq. 0,040 %, KCl 0,020 % $P_H = \pm 8,6$ [4]).
3. NaCl 0,6 % + KCl 0,02 %.
4. NaCl 0,6 % + KCl 0,02 % + $CaCl_2$. 6 aq. 0,005 %, 0,010 %, 0,012 %, 0,014 % etc., 0,020 %, 0,025 % etc.
5. NaCl 0,5 %, $NaHCO_3$ 0,28 %, $CaCl_2$.6 aq. 0,040 %, KCl 0,02 %, P_H varying considerably: from 8,6 to 7,2.
6. NaCl 0,6 %, $CaCl_2$.6 aq 0,040 %, KCl 0,02, $P_H = 8,6$, $NaHCO_3$ 0,05 %, 0,010 %, 0,0015 % etc.

[1]) JOSEPH E. MELTZER: Americ. Journ. o. Physiol. **29**, 1, 1911.
BENDA: Zeitschr. f. Biol. **63**, 11, 1914.

[2]) HAMBURGER u. BRINKMAN, l.c.

[3]) BRINKMAN: l.c.

[4]) Colorimetrical according to SÖRENSEN.

1. *The influence of a pure NaCl-solution on the motility and the irritability of the vagus of the muscular-stomachwall.*

When observing the stomach of a newly-killed frog, one often notices spontaneous local contractions or peristaltic waves in both directions. Stimulation of the vagus, brought about in the way described above, causes strong peristaltic movements, especially in the pyloric part; at the same time one can observe a frequent lengthwise contraction. The stimulation has a rather long after-effect (5 minutes). It was constantly found that the minimum degree of effective stimulation was with a coils-distance of 7 to 8 c.m.

If the stomach is perfused with a 0.6 °/₀ NaCl solution $P_H = 8.6$), we see that the spontaneous peristalsis has disappeared after 5 to 10 minutes and that the stomach has become quite limp; the mechanical irritability has completely disappeared.

The vagus-irritability is as follows: before the perfusion a vagus-effect is observed with a coil-distance of 7 to 8 c.m.; after a 5 minutes perfusion a distance of 5 c.m.; after 10 minutes a distance of 4 to 3 cm.; after 15 to 20 minutes even the strongest stimulation of the vagus takes no effect.

This disappearance of the vagus-irritability is reversible. If, after half an hour's perfusion with the pure NaCl solution, the liquid is replaced by a well-equilibrated salt-solution (NaCl 0.5 °/₀, $NaHCO_3$ 0.28 °/₀, $CaCl_2$ 6 aq 0.040 °/₀, KCl 0.020 °/₀, $P_H = 8.6$) spontaneous contractions are again observed after five minutes; after 10 minutes vagus-effect occurs at 10 cm. coil-distance, after 25 minutes vagus-effect can be observed clearly at a coil-distance of 7 cm.

So it is clear that after half an hour's perfusion with a pure NaCl solution, the harmful action is still perfectly reversible.

2. *The influence of NaCl + KCl.*

Now we have tried to find out which ions of the physiological solution in this respect caused the balancing effect. It soon appeared that the addition of K-ions, to which one has to assign such an important effect in heart-perfusion, have no effect of importance here. A concentration of K-ions which can cause the return of the vagus-irritability cannot be found.

3. *The influence of NaCl + KCl + $CaCl_2$. 6 aq.*

The vagus-influence may be re-established by the addition of a certain calcium-concentration to the (in itself insufficient) system of

NaCl 0.6 °/₀ + KCl 0.02 °/₀ ($P_H = 8.6$). The following experiments give a brief survey of it:

a. unperfused stomach, vagus-effect at a distance of 8 cm., afterwards NaCl 0.6 °/₀ + KCl 0.02 °/₀; after 10 minutes the vagus is un-irritable, the stomach is limp. Then NaCl 0.6 °/₀, KCl 0.02 °/₀, $CaCl_2$. 6 aq 0.002 °/₀; vagus-effect still fails to appear, stomach remains limp, though somewhat less so than when it is perfused with a pure NaCl solution;

b. unperfused stomach, vagus-effect at 7.5 cm., then NaCl 0.6 °/₀, KCl 0.02 °/₀; after 15 minutes the stomach is limp, stimulation of vagus has no effect. Then NaCl 0.6 °/₀, KCl 0.02 °/₀, $CaCl_2$. 6 aq 0.004 °/₀. Whereas the effect of this Ca-concentration on the perfused heart is clearly visible, there is no effect whatever on the stomach, except a slight tonic contraction.

c. Nor could a return of the vagus-irritability be established in numerous perfusions, when to the NaCl 0.6 °/₀ + KCl 0.02 °/₀ was added respectively $CaCl_2$. 6 aq 0.006 °/₀, 0.008 °/₀, 0.010 °/₀, etc. But

d. the addition of 0.015 °/₀ *$CaCl_2$. 6 aq to NaCl* 0.6 °/₀ + *KCl* 0.02 °/₀ *caused the vagus-irritability to return completely.* We must, however, stress the fact, that, to obtain this result, one should take special precautions. As namely the liquid does not possess at all a buffer-system against H-ions, fluctuations of $[H^{\cdot}]$ occur very easily. It is necessary that the P_H of this perfusion-liquid should be 8.6 and remain constant during the experiment. The use of a rubber tube is very dangerous in this experiment, as it nearly always makes the liquid too acid.

These precautions being taken, one can always demonstrate that a concentration of 0.015 °/₀ $CaCl_2$. 6 aq (and also 0.016 °/₀) is able to balance the concentration of alkali-ions; this concentration corresponds to a free $[Ca^{\cdot\cdot}]$ of about 9 milligrammes per litre.

It is an interesting fact that exactly the same concentration of Ca-ions proved necessary for the preservation of the impermeability of the glomerulal membrane for physiological quantities of glucose.[1])

e. a concentration of $CaCl_2$. 6 aq of 0.020 °/₀ and higher concentrations are unable to preserve or recall the vagus-irritability; then tonic contractions of the stomach-wall too disappear again completely iu this case.

4. *The influence of a concentration of hydrogen-ions.*

By choosing the total quantity of Ca of the perfusion-liquid in

[1]) Hamburger and Brinkmann, l.c.

such a manner that a high free [$Ca^{\cdot\cdot}$] cannot arise, it is possible to investigate the influence of the [$H^{\cdot}$] separately.

It appeared already in the above-mentioned perfusions with NaCl 0,6 %, KCl 0,02 %, $CaCl_2$.6aq 0,015 %, that the [$H^{\cdot}$] must be kept within rather narrow limits.

When a buffer-system exists ($NaHCO_3 + CO_2$), the [$H^{\cdot}$] may vary within the limits of this system, as appears from the following experiments:

a. Perfusion with NaCl 0,5%, $NaHCO_3$ 0,28%, KCl 0,020%, $CaCl_2$. 6aq 0,015%, $P_H = 8,6$; there are strong spontaneous peristaltic movements; vagus-irritability at a coil-distance of 7.5 cm. Then the same liquid but now with CO_2 passed through until $P_H = 7,1$; the stomach becomes limp in 10 minutes and can no longer be influenced by vagus-irritation.

b. Perfusion with NaCl 0,5%, $NaHCO_3$ 0,28%, KCl 0,02%, $CaCl_2$. 6 aq 0,015%, $P_H = 8,6$; irritability at a distance of 8 cm., spontaneous contractions. Then $P_H = 8,3$, constant irritability at 10 cm.'s distance; spontaneous contractions of stomach. Then $P_H = 7,7$; irritability at 14 cm., spontaneous rapid peristalsis. Then $P_H = 7,3$; irritability at 14 cm., stomach contracted spastically. Then $P_H = 7,1$; stomach not irritable, spontaneous movements have disappeared. Then $P_H = 8,6$; after 10 minutes' vagus-stimulation at 8 cm. spontaneous movements of stomach.

This last survey is an example of many similar experiments, from which it appears that the slight [$H^{\cdot}$] fluctuations do not let the vagus-irritability disappear but certainly influence it.

The actions of the $H^{\cdot}$ and the $Ca^{\cdot\cdot}$ cannot be separated here, because their quantities are directly dependent on each other and because in general the colloid-action of the $Ca^{\cdot\cdot}$-ions depends on the H-ion-concentration which is present. The balancing effect of $Ca^{\cdot\cdot}$-ions can be indicated only with one definite $H^{\cdot}$-ion-concentration.

The fact that an alteration of the $Ca^{\cdot\cdot}$-ion-concentration in itself induces a variation of the vagus-irritability, is shown by the last series of experiments which correspond for the most part to conditions as they occur physiologically and pathologically.

5. *The influence of the $NaHCO_3$-concentration.*

When the $NaHCO_3$-degree of a liquid is modified systematically, the H-ion-concentration remaining constant, one obtains likewise a

modification of the Ca-ion-concentration, because the [HCO'_3] and [$Ca^{\cdot\cdot}$] are inversely proportional to each other, the influence of this modification is great, as appears from the following examples:

a. Perfusion with NaCl 0,6 %, KCl 0,02 %, $CaCl_2$.6 aq. 0,04%, $NaHCO_3$ 0,05 %. $P_H = 8,6$. After 5 minutes the spontaneous contractions have disappeared and the stomach is contracted spastically: no effect of vagus-irritability is visible.

b. Perfusion with NaCl 0,6%, KCl 0,02 %, $CaCl_2$. 6 aq 0,04%, $NaHCO_3$ 0,10%, $P_H = 8,6$. After 10 minutes the stomach is contracting with intense spasms, especially the pyloric part of it. The vagus is extremely irritable, at 15 cm's coil-distance deep waves arise in the stomach-wall which last very long and are displaced very slightly; finally we have a very spastically contracted stomach (pylorospasmus).

c. Perfusion with NaCl 0,5 %, KCl 0,02 %, $CaCl_2$. 6 aq 0,04 %, $NaHCO_3$ 0,15 %, $P_H = 8,6$. After 10 minutes the stomach shows very slight peristalsis with intense spastic contractions in the pyloric part. Vagus-irritability at 12 cm, tonic contractions lasting a very long time.

d. Perfusion with NaCl 0,5 %, KCl 0,02 %, $CaCl_2$. 6 aq. 0,04 %, $NaHCO_3$ 0,20%, $P_H = 8,6$. With this liquid the spontaneous peristaltic movements appear again; the vagus is irritable at a coil-distance of 9 cm. and produces a series of peristaltic movements; the spastic contractions are still present in a slight degree.

e. Perfusion with NaCl 0,5 %, KCl 0,02 %, $CaCl_2$. 6 aq. 0,04 %, $NaHCO_3$ 0,28 %, $P_H = 8,6$. With this liquid the vagus is irritable at a coil-distance of 7 cm.; there are normal peristaltic movements and no spastic contractions. The conditions are completely like dose of the unperfused stomach.

From these experiments appears clearly the great influence which a change in bicarbonate-concentration has on the irritability of the n. vagus and on the spontaneous rhythmical movements of the stomach-wall. The latter effect is probably also due to the influence on the autonomous plexus of AUERBACH. It cannot be decided with certainty whether we have to think here especially of a direct influence of the HCO_3-ions [1]) or only of the influence of the latter on the [$Ca^{\cdot\cdot}$], but, in connection with the experiments with pure NaCl + $CaCl_2$ solutions, the primary significance of the Ca-ions seems to us by far the more probable.

We attach some significance to the fact that a decrease of [HCO_3'],

[1]) RONA and NEUKIRCH: Pflüger's Archiv. **148**, 285, 1912.

so an acidose, can cause spastic concentration of the stomach and an increased irritability of the n. vagus (vagotony). Whether a decreased [Ca¨] can cause similar phenomena, has not yet been investigated by us.

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Paleontology. — *"On the Significance of the Large Cranial Capacity of Homo Neandertalensis"*. By Prof. EUG. DUBOIS.

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Before the discovery of the fossil man of La Chapelle-aux-Saints our knowledge of the most important character of *Homo neandertalensis*, the cranial capacity, rested only on estimation, especially from the capacity of the calvaria. SCHAAFFHAUSEN, HUXLEY and SCHWALBE started from the supposition that the capacity of the calvaria of the Neandertal Man, which is human as regards its size, was in the same ratio to that of the whole skull as in Man of the present type. It is not surprising, that their results are pretty well concordant [1]).

First SCHAAFFHAUSEN [2]) measured the capacity of the Neandertal calvaria with water, on a level with the orbital plate of the frontal bone, with the deepest notch in the squamous margin of the parietal, and with the superior semicircular ridges of the occipital. He found for it 1033 cm.^{3}, and estimating the capacity of the missing part at 215 cm.^{3} from other skulls, he found 1248 cm.^{3} for the total capacity of the skull. Later, anew measuring the calvaria with water, "mit ihrem oberen Rande horizontal gestellt", he found 930 cm.^{3} for its capacity, and now for the whole capacity, through comparison with the corresponding part and the whole of a "roh gebildeten Schädel" of 1305 cm.^{3} capacity and of a negro skull, only 1093, resp. 1099 cm.^{3}). Accepting the first calvaria measurement by SCHAAFFHAUSEN, HUXLEY [3]) estimated the capacity of the entire skull at about 75 cubic inches (= 1229 cm.^{3}). SCHWALBE [4]) measured the capacity of the Neandertal calvaria with peas up to the transversal

[1]) M. BOULE, Sur la capacité cranienne des Hommes fossiles du type de Néanderthal. Comptes rendus. Académie des Sciences. Tome 148, p. 1352. Paris 1909.

[2]) SCHAAFFHAUSEN, Zur Kenntniss der ältesten Rassenschädel. Archiv für Anatomie, Physiologie und wissenschaftliche Medicin (Johannes Müller). Jahrgang 1858. Berlin, p. 455 and p. 464.

H. SCHAAFFHAUSEN, Der Neanderthaler Fund, p. 43. Bonn 1888.

[3]) T. H. HUXLEY, Evidence as to Man's Place in Nature, p. 156 – 157. London 1863.

[4]) G. SCHWALBE, Der Neanderthalschädel. Bonner Jahrbücher, Heft 106, p. 50—52. Bonn 1901. SCHWALBE erroneously rejects SCHAAFFHAUSEN's second determination, „weil sie durch Wasserfüllung ermittelt ist", which would, indeed, also be applicable to the first determination. In this procedure errors *could* be avoided. It is not clear what caused SCHAAFFHAUSEN to arrive at so much lower capacity