

The  $v$ - and  $w$ -lines from (11) consequently are projected on (9) in a straight line and a  $(C_3)$ .

In connection with § 1 we find from this:

The straight lines, whose projections on a surface of the second degree  $O_2$  (of rank four) have degenerated into a straight line and a  $(C_3)$ , form two congruences; these lines, like the normals of  $O_2$ , are double tangents to the surface of the centres of principal curvature of  $O_2$ .

It is easy to be seen that these two congruences have the same degree and class as the congruence of normals, in which case these numbers are 6 and 2. For example, the number of value systems of  $u, v, w$  which satisfies (11), the ratios of  $x, y, z, t$  being considered as given, indicates the degree of all three congruences.

The two congruences mentioned above are of the sixth degree and the second class.

**Botany.** — On the relation between internal and external medium in *Artemia salina* (L.) var. *principalis* SIMON. By HERBERT WARREN, DONALD KUENEN and L. G. M. BAAS BECKING. (From the Botanical Institute, University of Leyden.)

(Communicated at the meeting of September 24, 1938.)

The unique osmotic regulation of *Artemia* has been commented upon several times, but accurate data on this osmoregulation are still lacking. It seemed, therefore, worth while to investigate the relations between internal- and external milieu in this curious phyllopod. Two of us started to work on this problem at Pacific Grove, California, already in 1929, while the experiments are being continued now at Leyden.

In the earlier experiments living material could be obtained from a near-by salt work, while at Leyden we raise the animals from eggs, collected by one of us in California in 1930. More than 10 % of these eggs are still viable.

The problem was tackled by means of different methods:

1. Direct analysis of the haemocele fluid.
2. Determination of the refractive index of the haemocele fluid.
3. Determination of water endosmosis or exosmosis on transfer from one salt concentration to the other.
4. Volumetry.

1. *Direct analysis of the haemocele fluid.*

One large female may yield as much as 5.9 mm<sup>3</sup> of haemocele fluid, which may be obtained by means of a fine capillary tube. On the average, adult specimens may give 5—6 mm<sup>3</sup>. We worked chiefly with animals grown in a brine of a s.g. 1.075/15° ( $n_D^{25} = 1.3518$ ). The following table gives the data obtained.

Blood of:	1 ♂	15 ♂	3 ♂	8 ♀	6 ♀	10 ♀	Weighted averages
specific gravity:	—	—	—	1.032	1.044	—	1.037
% dry weight:	4.85	4.83	6.33	—	5.15	6.34	5.36
% Na:	.92	.75	—	—	—	.84	.815

The average weight of a male proved to be 7.5 mg and that of a female 9.2 mg. The average dry weight amounted to 7.48 % of the total body weight. If the Na present in the blood were calculated as NaCl, we would obtain 2.65 % NaCl, while the external milieu contained about 9 % NaCl.

From 9 females grown in a brine of s.g. 1.050 the Na- and chloride contents were determined separately. We found Na .67 % and Cl .87 %.

This shows that other chlorides except NaCl, or other Na-compounds except chlorides, should be present as the Cl corresponds to only .34 % Na. The variability, however, seems extremely large as the blood of 15 males, grown in a brine of s.g. 1.050 yielded only .68 % ash, of which ash 70 % consisted of NaCl.

MEDWEDEWA, using BARGER's method, found the haemocele of *Artemia*, grown in a brine of 8° B (s.g.  $\pm 1.060$ ) isotonic with 1.3 % NaCl, while blood from animals from a brine of 4.5° B (s.g.  $\pm 1.033$ ) proved to be isotonic with 1.2 % NaCl.

If we assume the excess sodium to be osmotically less active we obtain (taking the proportion Na : Cl = 67 : 87)

at s.g. 1.075 NaCl 1.75 % of haemocele  
at s.g. 1.050 „ 1.21 % „ „

This corresponds well with the values found by MEDWEDEWA.

As compared with other crustaceans *Artemia* seems to have a similar NaCl percentage in the ash (*Artemia* 68, *Potamobius* 50, *Limulus* 83, see VON FÜRTH), while the NaCl contents seems low (*Artemia* 1.21—1.75 %, *Ecrevissia* 2.94, *Carcinus* 2.70, *Potamobius* 1.73, *Nephrops* 2.77, see FRÉDERICQ).

## 2.

By means of a fine capillary pipette blood may be obtained from carefully cleaned and dry animals from the dorsal side. The refractive index of the blood of a single animal is easily determined by means of an Abbe refractometer at 25° C.

The refractive index of the blood proved to be variable, but markedly influenced by the salinity of the external milieu.

In statistical treatment differences in the fourth decimal place of the refractive index were used as class-differences. The following table summarizes the results.

$N_{25}^D$ external milieu	$N_{25}^D$ Mean internal milieu (haemocele fluid)	Number of variants	$\sigma$ Mean in classes (percentual distribution)
1.3325	1.3366	16	0.93
1.3389	1.3377	31	1.15
1.3470	1.3382	35	1.15
1.3525	1.3387	86	1.00
1.3563	1.3416	20	1.35
1.3620	1.3407	43	2.74
1.3770	1.3518	25	8.55

The results are, moreover, represented on the accompanying graph. Here the abscissa gives the  $N_{25}^D$  of the haemocele fluid, the ordinate shows this constant for the external milieu. The line marked "no exchange" indicates equality between external and internal milieu.

The heavy line gives the smoothed curve of the averages. Extreme variation is indicated by the broken boundaries. The circles between A and B indicate values obtained by ourselves and by MEDWEDEWA for NaCl, showing that while, as found in the previous paragraph, the NaCl seems

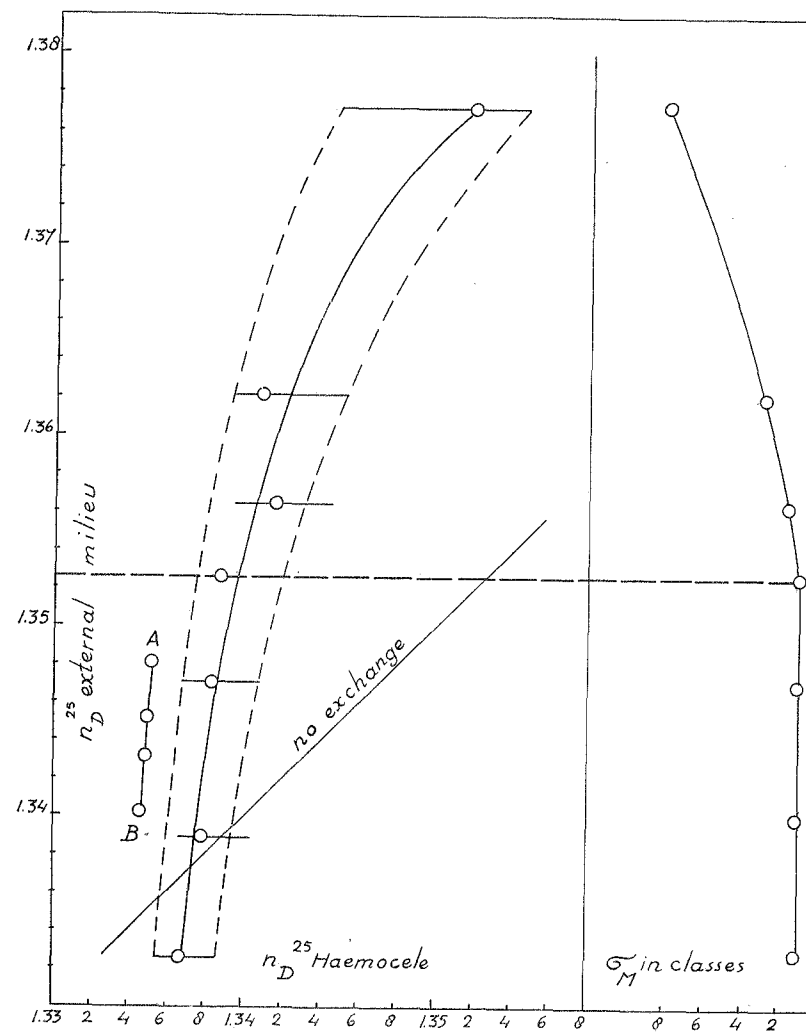


Fig. 1.

to account almost quantitatively for the osmotic pressure, the average refractive index of the haemocele fluid appears to be higher.

The line of the average refractive index seems, at first, very little influenced by the salinity of the environment. Later, above about 2.0 molar

(external environment) the influence appears to be much more marked. The limit is indicated in the graph by a horizontal, broken line.

In the above table the variability of the refractive index values seems to remain almost constant to the same value in the outer environment, while at higher salinities it increases. This is depicted on the right-hand side of the graph. Both results seem to indicate that active regulation is most efficient up to about 2.0 mol. NaCl in the outer fluid, after which the blood seems increasingly influenced by an increase in salinity in the outer environment.

It should be stated that, assuming the index of refraction to be a linear function of the concentration, the concentration of the "non-osmotic components" remains constant in an outer environment of 0—2.0 mol NaCl.

## 3.

From the graph it appears that, if the percentage of solid matter in the body fluid be proportional to its refractive index minus 1.3325 transfer from low to high concentration will cause a considerable loss of fluid from the body. At about 2 molar NaCl in the external environment we know the blood to contain about 5 % of dry matter.

As the NaCl contents at 1 molar NaCl in the external milieu is only 80 % of that at 2 molar NaCl we may assume the dry matter in the blood at 1 molar NaCl in the external milieu to amount to 4 %.

From the assumed linear proportionality of ( $N_{25}^D - 1.3325$ ) and solid matter in the blood, we might conclude that the body fluid of animals kept in 5 molar NaCl should contain about  $4 \times 4 = 16$  % solid matter.

As one animal might contain about  $6 \text{ mm}^3$  of available haemocoel fluid the animals in 1 molar NaCl contain,  $96 \times 6 = 5.76 \text{ mm}^3$  water. If the animals be transferred to 5 molar NaCl, where they should contain  $\pm 16$  % solid matter and if the amount of water left in the body be called  $x \text{ mm}^3$  the relation  $\frac{4 \times 6 \cdot x}{x} = 16$  should hold. Accordingly  $x = 1.60$ . Therefore  $5.76 - 1.6$  or  $\pm 4 \text{ mm}^3$  of water should be excreted.

If one animal be transferred from 1 molar NaCl into 1 cc of 5 molar NaCl the molarity should be lowered by 0.04 corresponding to a lowering of 2.64 units in the fourth decimal place of the refractometer. It seemed feasible, therefore, to study exchange in this way.

For practical reasons not less than one cc of liquid should be used as the animals, even in aerated brine, do not seem to survive.

As salt, given off by the animals might obscure the result, *Artemiae* were transferred from brine into distilled water. On the average, (27 animals studies in 6 series) one animal increased the refractive index of 1 cc of water by 0.25 in the fourth decimal place.

Transfer of 36 animals in 11 series from 1 molar to 5 molar brine yielded an average decrease per animal per cc of 1.15 units in the fourth decimal place of  $N_{25}^D$ . To this value should be added the correction 0.25,

as this correction runs in the opposite direction from our base-value. Therefore the decrease of  $N_{25}^D$ /animal/cc amounted to 1.4 units in the fourth decimal place. As 0.91 units correspond to a decrease in molarity of 0.01, 1.4 units signify a decrease in molarity of 1.54, corresponding to an secretion of  $x \text{ mm}^3$  of water.

The following relation should hold:

$$1.00 x = \frac{5.0000}{4.9846} \text{ or } x = 3.1.$$

As a more sensitive dip-refractometer could not be used there seems to be a fair agreement between the value of  $4 \text{ mm}^3$  calculated and  $3.1 \text{ mm}^3$  found in the experiment. If other substances besides pure water were excreted the discrepancy would be even less.

## 4.

As osmotic swelling and shrinkage of the animals was repeatedly observed in transfers of the animals from brines of higher to those of lower density and vice versa, attempts were made to determine the shrinkage and the swelling of the animals. To this end a simple dilatometer was designed by Mr. E. HANSON, which we intend to describe elsewhere.

The following results were obtained.

Numbers of animals	Transfer from	To	Average % swelling	Calculated %	Average % shrinkage
9	1 molar	3 molar	—	(80)	19
8	1 "	2 "	—	(24)	12
8	2 "	1 "	11	(19)	—
5	2½ "	½ "	11	(42)	—
5	1½ "	½ "	7	(20)	—
5	½ "	1½ "	—	(28)	3
2	3 "	1 "	10	(41)	—
5	2 "	0 "	10	(37)	—
4	2½ "	1½ "	7	(28)	—

The numbers in the column "% swelling calculated" were obtained in the following way.

If, after uptake or excretion of water, the percentage in dry weight changes from  $a_1$  to  $a_2$ , the water-content will change from  $(100 - a_1)$  to  $(100 - a_2)$  %. The procentual swelling or shrinkage will amount to,

$$(100 - a_1) \frac{a_2}{a_1} - 100 \text{ resp. } 100 \left( \frac{a_2}{a_1} - 1 \right) - a_2.$$

From the table it follows that the observed swelling and shrinkage is much less than predicted by the above calculation (up to  $\frac{1}{4}$ th of the calculated value). It is very well possible that part of the water excreted by the body fluid remains in the gut, or inversely, the animal takes up water out of the gut, which would make no difference in the total volume. As the intestinal tract occupies a large percentage of the body-volume, the above assumption might well account for this discrepancy.

CONCLUSIONS.

1. The osmoregulation of *Artemia salina* seems very efficient up to concentrations isotonic with 2.0 mol. NaCl.
2. The NaCl-content of the blood may account quantitatively for its osmotic properties.
3. There are indications that regulation is effected by means of excretion or uptake of water.
4. Swelling and shrinkage of the animals as a response to osmotic gradient is only about  $\frac{1}{4}$ th of the predicted values. Exchange between haemocoel and gut may account for this fact.

LITERATURE.

FRÉDÉRICQ, L., Influence du milieu ambiant sur la composition du sang des animaux aquatiques. Arch. Zool., 2me Sér., 3 (1885).  
 FÜRTH, K. v., Vergleichende chemische Physiologie der niederen Tiere. Jena, G. Fischer (1903).  
 MEDWEDEWA, N. B., Ueber den osmotischen Druck der Haemolymph von *Artemia salina*. Z. vergl. Phys., 5, 547—554 (1927).

Mathematics. — Beiträge zur Theorie der WHITTAKERSchen Funktionen. (Dritte Mitteilung)<sup>51)</sup>. Von C. S. MEIJER. (Communicated by Prof. J. G. VAN DER CORPUT.)

(Communicated at the meeting of September 24, 1938.)

Beweis von Satz 4. Wegen (7) hat man

$$L_{k,m}(ze^{-\pi i}) = e^{-(2m+1)\pi i} L_{k,m}(z) \dots \dots \dots (95)$$

Sind die Bedingungen (14), (15), (19) und (20) mit  $-k$  statt  $k$  erfüllt, so folgt also aus (18), mit  $-k$  statt  $k$ ,  $z = \zeta e^{\frac{1}{2}\pi i}$  ( $-\frac{1}{4}\pi < \arg \zeta < \frac{1}{4}\pi$ ),  $\tau = \frac{1}{2}\pi - \arg z = -\arg \zeta$  und  $u = ve^{-\pi i}$  ( $\arg v = \pi + \arg u = \pi + \tau = \pi - \arg \zeta$ ) angewendet,

$$e^{k\pi i} T_{-k,m}(\zeta e^{\frac{1}{2}\pi i}) = \frac{4ie^{-\frac{1}{2}(m+3k+2\alpha+\frac{1}{2})\pi i} \zeta^{m+k+2\alpha+\frac{3}{2}}}{\Gamma(\frac{1}{2}+k+m)} \left. \begin{aligned} & \times \int_{\infty e^{i(\pi-\arg \zeta)}}^0 L_{-m-\alpha,k+\alpha}(v) K_{m-k-2\alpha-\frac{1}{2}}(2\zeta v e^{-\frac{1}{2}\pi i}) v^{m+k-\frac{1}{2}} dv \end{aligned} \right\} (96)$$

Aus (18), mit  $-k$  statt  $k$ ,  $z = \zeta e^{-\frac{1}{2}\pi i}$  ( $-\frac{1}{4}\pi < \arg \zeta < \frac{1}{4}\pi$ ),  $\tau = -\frac{1}{2}\pi - \arg z = -\arg \zeta$  und  $u = v$  angewendet, ergibt sich auch

$$-e^{-k\pi i} T_{-k,m}(\zeta e^{-\frac{1}{2}\pi i}) = \frac{4ie^{-\frac{1}{2}(m+3k+2\alpha+\frac{1}{2})\pi i} \zeta^{m+k+2\alpha+\frac{3}{2}}}{\Gamma(\frac{1}{2}+k+m)} \left. \begin{aligned} & \times \int_0^{\infty e^{-i\arg \zeta}} L_{-m-\alpha,k+\alpha}(v) K_{m-k-2\alpha-\frac{1}{2}}(2\zeta v e^{-\frac{1}{2}\pi i}) v^{m+k-\frac{1}{2}} dv \end{aligned} \right\} (97)$$

Durch Addition von (96) und (97) erhält man mit Rücksicht auf (70)<sup>52)</sup>

$$T_{k,m}(\zeta) = \frac{2e^{-\frac{1}{2}(m+3k+2\alpha+\frac{1}{2})\pi i} \zeta^{m+k+2\alpha+\frac{3}{2}} e^{\frac{1}{2}\pi i} \Gamma(\frac{1}{2}+k-m)}{\pi} \left. \begin{aligned} & \times \int_{\infty e^{i(\pi-\arg \zeta)}}^{\infty e^{-i\arg \zeta}} L_{-m-\alpha,k+\alpha}(v) K_{m-k-2\alpha-\frac{1}{2}}(2\zeta v e^{-\frac{1}{2}\pi i}) v^{m+k-\frac{1}{2}} dv \end{aligned} \right\} (98)$$

(bei dieser Integration wird der Punkt  $v=0$  vermieden durch einen oberhalb dieses Punktes liegenden Halbkreis).

Nun folgt aus (79), (73) und (74)<sup>53)</sup>, dass das auf der rechten Seite von

<sup>51)</sup> Erste und zweite Mitteilung: Proc. Kon. Ned. Akad. v. Wetensch., Amsterdam, 41, 624—633 und 744—755 (1938).

<sup>52)</sup> Ich nehme an, dass  $m-k \neq \frac{1}{2}, \frac{3}{2}, \frac{5}{2}, \dots$  ist.

<sup>53)</sup> Das Verhalten von  $L_{k,m}(z)$  für grosse Werte von  $|z|$  mit  $\frac{3}{4}\pi < \arg z < \frac{5}{4}\pi$  folgt mittels (95) aus dem Verhalten dieser Funktion für  $-\frac{1}{4}\pi < \arg z < \frac{1}{4}\pi$ .