THE INFLUENCE OF ALCOHOLS ON THE PROTOPLASMIC MEMBRANE AND COLLOID MODELS

BY G. G. P. SAUBERT

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PROEFSCHRIFT

TER VERKRIJGING VAN DEN GRAAD VAN
DOCTOR IN DE LANDBOUWKUNDE
OP GEZAG VAN DEN RECTOR-MAGNIFICUS
Ir. C. BROEKEMA, HOOGLEERAAR IN DE VEREDELING VAN LANDBOUWGEWASSEN, TE VERDEDIGEN TEN OVERSTAAN VAN DEN SENAAT DER LANDBOUWHOOGESCHOOL OP DONDERDAG 16 DECEMBER 1937 TE 3 UUR

DOOR

G. G. P. SAUBERT.

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Dit proefschrift met stellingen van

GASTON GABRIEL PAUL SAUBERT,

landbouwkundig ingenieur, geboren te Djocjacarta den 27sten Juli 1908, is goedgekeurd door de promotoren Dr. E. Reinders, hoogleeraar in de plantkunde en Dr. H. J. C. Tendeloo, buitengewoon hoogleeraar in de scheikunde.

De Rector Magnificus der Landbouwhoogeschool,
IR. C. BROEKEMA.

Wageningen, 30 November 1937.

STELLINGEN.

I

Vitamine C is niet alleen noodzakelijk voor het dierlijk organisme, maar is ook van invloed op den plantengroei.

Diss. S. VON HAUSEN, Helsingfors 1936.

II

Melk zet de werking van het vitamine D aan.

SUPPLEE, Journal Biol. Chem. 114, 95, 1936.

III

De ervaring in Tonkin en Siam opgedaan met de groene bemesting van rijstvelden met Azolla filiculoïdes, rechtvaardigt het probeeren van andere N-bindende symbiosen dan Leguminosen als groene bemester voor natte rijstvelden in Ned. Indië.

IV

Indien men de symbiose van plant en microorganismen opvat als een machtsverhouding der symbionten, dan kan men met behulp van de veranderlijkheid dier verhouding de meeste verschijnselen, bij symbiose waargenomen, verklaren.

V

Het systematisch onderzoek van den aard der beworteling van grondbedekkers als functie van de bodemgesteldheid (= systeem: gronddeeltjes, bodemoplossing en bodemgassen) is voor de meerjarige cultures gewenscht in verband met de mogelijkheid van vergrooting der wortelruimte voor genoemde cultuurplanten.

VI

Voor het vergelijken van den invloed van stoffen — b.v. ionen — op een levensproces moet men steeds den invloed van verschillende concentraties dezer stoffen bestudeeren.

VII

De physica verkeert ten aanzien van haar object in vergelijkbare moeilijkheid als de physiologie.

VIII

Het verdient aanbeveling, dat de nomenclatuur, welke verband houdt met den kweekerseigendom, volgens internationale normen wordt opgebouwd.

AAN ALLEN
DIE BIJDROEGEN TOT
MIJNE ONTWIKKELING

„Die Philosophie hat (also) keine Axiomen und darf niemals ihre Grundsätze a priori so schlechthin gebieten, sondern muss sich dazu bequemen, ihre Befugnisz wegen derselben durch gründliche Deduction zu rechtfertigen".

IMMANUEL KANT,
Kritik der reinen Vernunft A. 733-734.

Hetgeen KANT voor de philosophie in het algemeen zegt, geldt evenzeer voor de natuurwetenschappen. Niet is daarmede gezegd, dat de natuurwetenschap van geen vooropzetsels mag uitgaan, doch slechts, dat het vooropgezette zich in het betoog mede moet ontwikkelen om in het resultaat van deze ontwikkeling zijne geldigheid te doen blijken.

In dit proefschrift wordt vooropgezet, dat de wetmatigheden, welke in colloïd-systemen gelden zich ook in het protoplasmamembraan — dat opgevat wordt als een gericht systeem van colloïden — zullen doen gelden.

Moge voor den lezer van dit artikel deze praemisse zich in de ontwikkeling van het betoog waar maken.

Van de mij geboden gelegenheid maak ik gaarne gebruik mijne erkentelijkheid jegens de Landbouwhoogeschool te betuigen voor den grondslag voor zelfstandig onderzoek, welke zij in mij legde; een grondslag waarop verder zal kunnen worden gebouwd.

Van het groote voorrecht korteren of langeren tijd onder leiding van verschillende Hoogleeraren in binnen- en buitenland te hebben kunnen werken, ben ik mij ten zeerste bewust. Moge de veelheid van gedachten, waarmede ik in aanraking kwam, in de toekomst bevruchtend blijken te zijn. Tot U Hooggeleerde BUNGENBERG DE JONG richt ik mij in het bijzonder; 2 jaren arbeid onder Uwe leiding hebben van mij, naar ik hoop, Uwen leerling gemaakt. Den Conservator van het Laboratorium voor Medische

Chemie te Leiden Drs. DEKKER, ben ik erkentelijk voor de levendige belangstelling in mijn werk.

U Drs. VAN ZIJP dank ik voor de hulp bij het optische werk. Ik hoop dat Uwe ervaring mij nog vaak van dienst zal zijn. Drs. VAN BUREN ben ik erkentelijk voor de vriendschappelijke hulp bij de metingen van kataphoretische snelheden en het personeel van het Laboratorium, met name de Heeren L. VERSCHRAGEN en H. DEN HOED voor de verdienstelijke en prompte uitvoering van het noodige instrumentarium.

U Heer VELTEMA alle lof voor de verzorging der graphieken.

Voor de hulp bij het vertalen van dit proefschrift wil ik mijn dank betuigen aan Mejuffrouw L. BÖTHLINGK.

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GENERAL INTRODUCTION.

As is well known, the protoplast of living cells does not in the least act as a strictly semi-permeable membrane in the sense that it lets water pass, practically unimpeded, into the vacuole, whereas it does not permit the dissolved substance to pass at all; on the contrary, from solutions of a great number of substances in water, when brought into contact with the cell, the dissolved substance passes more or less quickly through the plasma and enters the vacuole: f.e. glycerol, glycol and several dyes. There are some, however, that do not penetrate, not even in the long run and here a distinction should be made with plant-cells between substances, which, like cane sugar, penetrate into the plasma itself, but do not, or only very slowly, pass through it into the vacuole and those which do not even enter into the plasma itself. In this research, as far as plant-cells are concerned, the former phenomenon, the so-called intrability, will not be discussed; it deals exclusively with the permeability of the plasma, i.e. the penetrability of the plasma up to the vacuole.

Concerning the above mentioned selective permeability of the plasma different explanations were given. These nearly all agree in ascribing to the protoplast a certain boundary layer of special structure and composition and which is supposed to be the bearer of the selective permeability. With HUGO DE VRIES we take, with plant-cells, the tonoplast to be this layer.

The concept of the physico-chemical structure ascribed to this layer, naturally is always very sketchy; it is always projected in such a way as to explain all possible osmotic phenomena of the living cell, known to the projector. So OVERTON (1896) found that of all substances, investigated by him, the most lipoid-soluble pass the protoplasm-membrane easiest. Therefore he supposed this plasm-membrane to be chiefly composed of lipoids. RUHLAND ascertained, that OVERTON's rule did not hold good; he found the size of the molecules to be the deciding factor as to the permeability. The penetrating power of the

dyes, investigated by him, decreased proportionally to their size. This lead him to consider the plasm-membrane a fine filter: the ultra filter theory.

Later observations caused new difficulties, which were tried to be solved i.a. by ascribing a mosaic-structure to the plasm-membrane, in which certain spots should act as small lipoid-membranes, others as ultra-filters; in the end even more than two kinds of particles were necessary for the mosaic and had to be taken as changeable for the sake of "explaining" schematically the increasing complexity of the growing mass of facts. Moreover the electric charges of the membrane and of the permeating particles and also the adsorption were involved in the explanatory scheme.

Meanwhile from the beginning all the above-mentioned efforts to think out a physico-chemical protoplasm-model showed the same deficiencies, namely a highly insufficient knowledge of the model. When Overton constructed his lipoid-theory f.i. he at first overlooked the fact, that the solvability of all the substances, investigated by him, is in no way the same for solvatated and non-solvatated lipoids. For further progress it was obviously necessary in the first place to get more experience about the behaviour of the substances in question not only in a pure condition, but also — and especially — if brought together in different conditions with water. Then the influence of electrolytes and non-electrolytes has to be examined on these mostly colloid-systems and on account of the fact that permeability is allways connected with the important role played by the interfaces, the attention should very especially be directed towards these interfaces.

In comparison to the complexity of the phenomena of cell-permeability, even now very little is known about the possible physico-chemical "models". Of these systems perhaps the coacervates of lipoids are most thoroughly investigated by BUNGENBERG DE JONG. His investigations are constructed partly with regard to the possible parallelisms as to the permeability of the protoplast. — My own investigations, the results of which are herewith published, were carried out under the stimulation and guidance of Professor BUNGENBERG DE JONG in the laboratory for medical chemistry in Leiden. They comprise two related subjects, the one colloid-chemical, the other biological. The colloid-chemical part deals with the behaviour of oleate- and phosphatid-coacervates towards normal alcohols and the influence of these alcohols on the dielectric (Chapter II);

further (Chapter III) the way, in which coacervates and oriented systems react on changes of temperature and the influence of alcohols on oriented oleate-systems. The biological investigations have been carried out in the laboratory at Helsingfors under the expert guidance of Professor COLLANDER. They deal with the influence of alcohols on the permeability of the protoplast to water/alcohol mixtures (Chapter V). The purpose of both parts was to examine a possible parallelism between the behaviour of the investigated systems in vitro and in vivo.

My experiments lead to a theoretical discussion of the problem, whether there is sufficient reason to assume phosphatids as membrane-components (Chapter IV).

CHAPTER I

The consequences of the assumption of an oriented system as protoplasmic membrane for the pore-theory and the lipoid-solubility-theory.

A. *Necessity for the assumption of a membrane, regulating the permeability to substances.*

§ 1. In comparing the permeation [1]) of substances into living cells and their dependence upon physical and chemical factors with the diffusion of chemical compounds through artificial membranes, the parallelism in behaviour is apparent. From this analogy the assumption has been derived that a differentiated and independant membrane regulates the permeability of the cell for chemical compounds. Further research, however, chiefly on colloids, convinced several workers that the assumption of an independent membrane is unnecessary to account for the phenomena observed in the processes of permeability. These workers account for the changes in permeability for chemical compounds under the influence of various factors, the selective permeability for ions and the validity of DONNAN's law, by means of the colloid-chemical properties, and their variability, of the protoplasm itself.

Both the validity of DONNAN's law and the occurence of selective ion-permeability do not force us to assume a limiting protoplasmic membrane [2]). Only the *separate* occurence of two phases

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[1]) These considerations only deal with the type of permeation in which no energetic processes play a part. The so-called adenoid absorbtion of Overton will not be considered here.

[2]) Compare E. GELLHORN et J. RÉGUIER — La perméabilité en physiologie et en pathologie générale 1937, p. 769—770.

is necessary to satisfy the theoretical requirements. The experiments are in account with this assumption. BIGWOOD showed that gelatin gels absorbed kat- and anions in different amounts dependent upon the charge of the gel. Iso-electric gelatin absorbs equivalent amounts of Ca^{++} and Cl^{-}. At the acid side Cl^{-} is absorbed and at the alkaline side more Ca^{++}. In quantitative respect the partition of the ions between milieu and gel is expressed by the DONNAN law.

The above authors assume therefore that the entire protoplasm is responsible for the permeability.

§ 2. Theoretical considerations and the experimental data do lead to the assumption of a so-called membrane, not to an independent partition wall, separate from the protoplasm, but rather as a protoplasmic interface, with its own relatively specific properties, which properties follow from both the nature and the structure of this interface. The membrane can therefore not be understood as a removable wall but as the function of a boundary in a system, which allows of mathematical interpretation.

Several substances are known to be surface-active, i.e. that they are positively adsorbed at the interface water/air (GIBBS' theorem). According to physical theory this surface-activity is caused by the hydrophilic asymmetry of the molecule and therefore occurs at such places where chemical compounds of asymmetrical [3]) structure occur in phase boundaries.

Such asymmetrical molecules are amassed in the interfaces of phases with different hydrophily and they are directed in such fashion that the respective "poles" penetrate into the phase for which they possess most affinity. In a mixture of substances, e.g. a solution of a surface-active substance in water, this asymmetry causes an uneven distribution of the asymmetrical substance in the other (which may be called relative milieu). The asymmetrical compounds, colloids as well as non-colloids, shall therefore be unevenly distributed in the neighbourhood of every boundary of the protoplasm. The uneven distribution of a substance near a phase-boundary of the protoplasm is a function of its asymmetry (i.e. the number of hydrophilic centra at one

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[3]) With hydrophilic asymmetry, for briefness' sake "asymmetry", of molecules we mean a molecular constitution in which both a hydrophilic and a hydrophobic part may be considered as "poles". Hydrophily and hydrophoby are taken as antipodean properties, because of the fact that our considerations are chiefly concerned with phase-systems in which one of the phases is more or less hydrophilic.

side of the molecule) and of the difference in hydrophilic nature of the neighbouring phases. As protoplasm chiefly consists of water and hydrophilic colloids — which means colloids with one or more hydrated groups — our attention is drawn in the first place towards these colloids and their properties. The hydrophilic colloids with the most pronounced asymmetrical structure will be easily amassed into and directed on the phase boundary. The directive forces in this boundary will be small, if the difference in hydrophily in both phases is small also. But even here the molecules with the most pronounced asymmetrical structure will be comparatively easily amassed on the boundary. Highly asymmetrical hydrophilic colloids may therefore be expected in the phase boundary between protoplasm and milieu. These colloids should be the most hydrophobic under the hydrophilic colloids and as such the lipoids [4]) should be considered in the first place. If proteins are to be expected, cannot be easily said. Proteins may occur in various modifications [5]) dependent upon the milieu, while their capillary activity is very distinct. This activity varies in value from practically zero — e.g. albumen of eggs in water — to the value of the lipoids — e.g. albumen of eggs in dispersed monomolecular layers in the interface water/air. As the modifying forces on an interface of two phases slightly different in hydrophily are small, it cannot be said beforehand if these forces suffice to bring about the modification of the protein. Inasmuch as protoplasm and milieu show comparatively little difference in hydrophily, the alcohol-soluble hydrophobic proteins, in which the hydrophilic centra are distributed over the entire surface of the molecule, will accumulate only with difficulty in the interface, unless they are modified again. The unequal distribution of asymmetrical substances near protoplasmic boundaries determines the specific nature of this boundary. This holds for *every* protoplasmic boundary. Only the specific manner in which the molecules are directed in this boundary and the nature of these molecules, may vary from place to place.

When an asymmetrical compound is sufficiently accumulated in a boundary, a more or less stable membrane may originate if the directive forces are sufficient. If the packing of the molecules

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4) With lipoids we mean substances
   a. more soluble in hydrocarbons than in water.
   b. actual or potential as esters connected with fatty acids.
   c. with a function in the organism.

5) G. Th. Philippi, On the nature of proteins — Thesis, Leiden 1936.

is close enough, this membrane may even assume a "solid" character. A loosely packed membrane has "liquid" properties. A "directed" system may assume a solid as well as a liquid character, as is shown by the study of *liquid crystals* in their modifications from smectic to nematic. Inasmuch as the membrane originates by the directive forces near a protoplasmic boundary and as these forces are zero at a small distance from the boundary, the membrane as described above, cannot be more than a few molecules thick. If the concentration of the directed molecules in the protoplasm is high, the membrane may become heavier, like a crystal may grow, if the mother-lye contains crystal-substance enough. In judging the stability of a protoplasmic membrane, which question becomes important in cell permeability, one should always keep in mind that such a membrane is situated between two aqueous phases, viz. protoplasm and milieu.

§ 3. These theoretical considerations only indicate a probability. Since Hugo de Vries introduced the tonoplast from "abnormal plasmolysis", the following experimental data (taken from E. GELLHORN et J. RÉGNIER p. 772—779) were obtained and warrant the assumption of a membrane:

a. Microdissection experiments of CHAMBERS and SEIFRIZ.
b. Behaviour of the cell in relation to intra- or extra-cellular ions. (cf. also Eichberger).
c. Physical measurements (conductivity, capacity) [6]).

These experiments prove that what is called the protoplasmic boundary, shows different properties in relation to both the permeation [7]) of substances and to its physical behaviour from the other protoplasm, in other words we may use the term membrane [8]). If this be the membrane that regulates the permeation of substances is not proved; the important question is not whether a membrane exists, but whether the assumption of a membrane, the properties of which should account for the permeability, is necessary. For cells rich in protoplasm, this question may probably be answered using the ideas developed by R. COLLANDER and H. BÄRLUND [9]). The reasoning of these authors started with the idea that the motion

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[6]) Compare R. REMINGTON, Protoplasma V, 1929, p. 338.
[7]) We mean by permeation the movement of the substance from one milieu to the other.
[8]) This membrane is not always thought to be at the boundary between cell and milieu. For cells with a central vacuole the tonoplast may probably function as such.
[9]) Acta Botanica Fennica 11, 1933, p. 25—35.

of a substance from the surrounding cellular milieu into the cell sap has to surmount a certain resistance, and that the nature of this resistance determines the way in which this substance permeates. If the resistance is localized in a thin layer, while the rest of the cell does not offer a measurable resistance, the permeation of the substance should follow the well-known equation $\frac{dm}{dt} = k.q.(C\text{-}c)$, in which dm stands for the amount of substance penetrated in the interval dt, q the active cell-surface, C and c resp. the equilibrium concentration and the *momentary* concentration of this substance in the cellsap, k being a constant. When $m = v \times c$, the equation becomes after integration $k = \frac{v}{qt} \times \ln \frac{C}{C\text{-}c}$. If the resistance, however, be equally large in all parts of the cell, the following equation holds $\frac{dc}{dt} = \frac{D}{r} \frac{d}{dr}(r \frac{dc}{dr})$, in which c, r and D are respectively the concentration of the permeating substance in an arbitrary point of the cell at the time t, the distance from that point to the cell-axis and the diffusion constant. Both equations are derived from the assumption that permeation is a diffusion process and that FICK's law holds. COLLANDER and BÄRLUND distinguished between a diffusion-resistance localized in the protoplasmic boundary and a diffuse resistance over the entire cellular contents. They found that the permeation of various substances follows the former example. The resistance should therefore be localized in the membrane. With the extremely thin cytoplasmic layer of their experimental object (*Chara ceratophylla*) diffusion may progress according to equation I, no matter if there be a membrane or not. *Chara ceratophylla* seems, therefore, not a suitable object, because we cannot distinguish between: a. the resistance of the protoplasmic boundary and b. the resistance of the rest of the protoplasm. In the case of *Chara ceratophylla* we should rather distinguish between the resistance of a membrane and the resistance of a protoplasm *plus* the cell sap. In reality the assumption of permeation as a diffusion process will always lead to results a little different from those expected from the two extreme types; the process may be intermediate with a tendency towards one or the other type.

For if the protoplasm becomes poor in water, the resistance for the motion of water-soluble compounds may increase and the permeation has to follow the equation II, in which case the membrane loses its significance. Considering the protoplasm-membrane as an oriented system, the validity of FICK's law has

to be questioned. The mechanism of permeation may give by first approximation "flux (:) concentration gradient" [10]), but then the proportion-factor needs not indicate a diffusion constant. The space between the molecules of a membrane represents an energy originating from these molecules, and not is concerned here the diffuse thermal motion of permeating particles in a liquid, nor this motion in capillaries, the so-called pores of the pore-theory.

The above considerations warrant, in my opinion, the assumption of a membrane regulating permeability, the more so as we take into account that the observed phenomena may be easily accounted for by means of this assumption. As to the structure and chemical composition of this membrane the opinions vary.

B. *Structure and chemical composition of the membrane.*

§ 1: The observed parellelism of lipoid-solubility and permeation-velocity of series of chemical compounds into animal and vegetable cells has created the concept of the lipoid protoplasmic membrane. Fast-permeating substances should be very soluble in the lipoid membrane, which membrane was thought to consist of undependent lipoid molecules in *free* motion. A substance soluble in such a membrane should permeate, inasmuch as the permeation is caused by the diffuse thermal motion of the dissolved molecules. The membrane was conceived, therefore, to be a liquid, even if this was not specifically stated [11]).

Nowadays many authors supporting the lipoid theory accept a "directed" (or oriented) system as a membrane. Such a system may be described as one in which the free motion of the molecules is restricted so that they are fixed within certain limits. Crystallographically we may distinguish between orientation in one, two or three dimensions. Three-dimensional oriented systems are called crystals and uni- and two-dimensional oriented systems are called "liquid" crystals because of their liquid nature in morphological respect. For our concept of membrane structure these liquid crystals are of importance (see colloid-chemical part). The objection that this assumption would exclude solubility, does not hold, because even mutual solubility of solids has been established. Solution of a substance in a certain medium only describes the tendency of this substance to divide itself equally over this medium.

§ 2. The parallelism between molecular volume and permea-

10) Therefore, the above ideas developed by R. COLLANDER and H. BÄRLUND do hold.

11) OVERTON thinks of a membrane impregnated with lipoids and therefore as a non-liquid. The later authors are usually vague.

tion-velocity of series of substances into various objects leads to the assumption that a protoplasmic membrane should be an ultrafilter. In this theory the membrane was thought to consist of "micellae" in which the inter-micellary spaces formed the pores. These pores should be of molecular dimensions, if they could be able to act as a selective sieve [12]). One even finds in the literature a comparison with the zeoliths.

In radial section this membrane may be thought off as a system of perforating channels. These channels do not need to be directed in a straight line.

Closer inspection of this theory and the corresponding concept of the membrane leads to the following considerations. The theory in its strictest form, in which form it finds no supporters, assumes that the size of the permeating particle is the only factor with the exclusion of all others. In this case the membrane should be perforated with pores, the statistical diameter of which should be larger than the diameter of the largest permeating (spherical) particles. All smaller (spherical) particles [13]) permeate faster, if the diffusion velocity is greater (if diffusion constants are taken as a measure for the molecular volume). Evidently the electric factor will come into play when ions are considered. Moreover, the pores in the membrane should have a statistical diameter independent of the permeating particle. Only in this case a series of substances arranged according to the particle size will permeate as expected from this arrangement. If, however, the permeating substance influences the diameter of the pores, if the diameter is no more constant, the sequence is disturbed and no relation is found between permeation velocity and particle size.

§ 3. At first it seemed that both concepts were antagonistic [14]). When more experimental data became available, it appeared that neither principle was fully satisfied when the sequence of permeation velocity of various substances was determined. The principles are mutually additive. Phenomena that cannot be accounted for by means of the first, may be accounted for by the other and the form in which this is given, is known as the mosaic theory or the lipoid-filter-theory.

The proof of the validity of both principles has changed the

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12) How this image could account for the selective passage of colloidal dyes next to molecular dispers substances, remains obscure to me.

13) For organic compounds with long carbon chains the reasoning becomes more complicated.

14) R. COLLANDER — Koningsberger lecture, 1937.

controversy. We may now ask which parts are played by both lipoid solubility and molecular volume in the permeation of a certain substance.

Adherents of the pore theory still assume the presence of an ultra filter thought to consist, however, of lipoid [15]) micellae in which substances with a so-called affinity for lipoids may accumulate. Moreover, the so-called *diffusion diameter* of these substances is thought to be larger than that of lipophobic substances, for in the first category of substances the attraction sphere of the pore wall is still available. Both because of this accumulation causing an increase of the concentration gradient and because of the larger diffusion diameter, lipophilic substances will permeate faster than can be expected on the basis of their molecular volume. In this reasoning the membrane is always thought to possess pores of a constant statistical diameter in the sense as described above. If a permeating substance should influence the pore diameter markedly, the diffusion diameter will also change and the molecular volume does not remain a factor which primarily determines the permeation. This trend may be followed as long as the membrane is assumed to be constructed of micellae in which the pores are formed by completely perforating intermicellary spaces. The permeating substance may influence the pore wall in that case, but the pore itself does not need to change its diameter markedly inasmuch as the micellae function as a rigid skeleton and prevent any change. Such a membrane should be comparatively heavy.

As soon as we assume lipoids as component parts of the membrane, the formation of a thin "micellar" membrane seems improbable. The hydrophilic centra of the molecules in such a micell are directed outwards and such particles will repel one another. Such a membrane should therefore be comparatively heavy. Measurements, however, of electric conductivity, electric capacity and experiments with lipoids of erythrocytes (GORTER and GRENDEL) warrant the assumption that the membrane should be a few molecules thick. This indicates again that these molecules situated between two aqueous phases (the protoplasm may be considered as such) should be orientated.

§ 4. If the membrane be an oriented system, the spaces between the molecules may hardly be called pores. One could only speak

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15) Certain investigators under which ULLRICH (note p. 720), avoid the word lipoid. They speak of hydrophobic substances without further definition. FRY WYSSLING, 1935, mentions hydrophobic proteins as possible building blocs of the membrane.

about pores in the sense given by adherents of the pore theory, if the following conditions are satisfied:

1. the building blocks of the system should occupy a fixed position in relation to one another. This condition is satisfied in a three-dimensional oriented system; but not in a two-dimensional oriented system. In the latter case the components are directed in relation to one another only more or less parallelly;

2. in a tangential plane (looked on the top of the membrane) the components of the system should be arranged in such a fashion that they surround spaces, and therefore form a porous membrane. This condition is not satisfied by a directed system;

3. on a radial plane the components should be arranged in such a fashion that they surround completely perforating channels. This condition is not satisfied either. If this last condition is not put, any crystal should have pores, for every crystal plane shows cavities.

If all these conditions are satisfied and real "pores" in the sense of the adherents of the pore theory exist [16]), one cannot imagine — assuming large molecules forming the membrane — the existance of pores as defined above, of molecular dimensions. If only the first condition be fulfilled and if still the concept of pores should be maintained (in this case meaning the spaces between the molecules of the membrane), this assumption seems to be warranted, keeping in mind, however, that *our image is intrinsically changed.* We have to introduce into our considerations concepts different from those current in capillary chemistry and applied by biology. For in the membrane which consists of a few layers of oriented molecules — a membrane therefore comparable in structure to a liquid crystal — no capillaries occur and, therefore, concepts derived for capillaries do not apply here.

§ 5. Accepting an oriented system removes all distinction between pore theory and lipoid solubility theory. The pore theory places the ultrafilter as the primary condition for the understanding of the phenomena of permeability, as the frame within there is room for the activity of other factors [17]).

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[16]) Compare H. ULLRICH, Protoplasma 26, 183, 1936: "Dringen nun hydrophobe Moleküle zwischen die Ketten, die die Poren Wandung bilden, ein...." ULLRICH accepts therefore a pore membrane constructed out of carbon chains — condition 2.

[17]) Und dann schiene uns wissenschaftlich etwas gewonnen, wenn die Theorie, wie wir vermuten möchten, gleichsam nur den grossen Grundrahmen abgeben würde, innerhalb diesen noch sehr weiter Raum wäre für das Spiel jener physiologischen Dispositionen und möglicherweise auch

ULLRICH supposes a constant pore diameter, but the affinity for the membrane substance determines the diffusion diameter. For water the available diffusion diameter is smaller than for hydrophobic substances for which the attraction sphere of the membrane components is still available.

Within the size of the pore diameter in this theory not the molecular volume but the undefined "affinity" seems to be the factor that primarily determines permeation. This affinity determines the size of the diffusion diameter and therefore the permeation velocity [18]).

The lipoid solubility theory expresses the same thing. For the solubility of a solute in a solvent is a measure of the affinity of the solute for the solvent. Here again the affinity is the determining factor and this remains valid, if the membrane is considered as an orientated system.

§ 6. Summarizing, we may say that the antithesis between lipoid solubility and pore theory or even the combination of the two has lost most of its meaning, because 1. both theories assume an undefined affinity of a substance for the membrane components as the chief determining factor and 2. because of the fact that in an oriented system as a membrane the name "pore" is given to the space between the membrane molecules. The two expressions have intrinsically the same meaning in the case when permeation of a substance due to its solubility in the membrane is considered — which means the placing of a substance between the membrane molecules — or the permeation of a substance as caused by its passage through pores, which also means the placing of the permeating molecules between the molecules of the membrane.

When we put the question in its provisional and general way, the permeation of a substance depends upon the possibility for

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noch mancher physikalischen und chemischen Besonderheit des Plasmas sowohl wie der Stoffe. — W. RUHLAND und C. HOFFMANN, Archiv für Wiss. Botanik, Bd. 1, Heft 1, 1925, p. 80.

18) If, in order to prove the validity of the pore theory, we divide the permeating substances into hydrophilic and hydrophobic compounds, classifying the hydrophobic substances according to their hydrophobic nature (see ULLRICH, l.c. p. 720), following this classification by an arrangement in every class after molecular volume, we only prove that properties other than the molecular volume (to wit relative hydrophoby as measured by relative lipoid solubility) are of primary importance for the permeation of substances. We prove the opposite of what we wanted to prove. For other properties besides the molecular volume determine the size of the diffusion diameter of the membrane pores for the substance in question, the molecular volume remaining as a secondary factor.

the permeating molecules to shove themselves between the membrane components. In this case all properties of both permeating molecules and membrane molecules as following from their molecular structure, have to be taken into account. Inasmuch as we are almost ignorant of the structure of the membrane components, we have to direct our attention chiefly to the structure of the permeating molecules. According to my opinion we might obtain a clearer picture of the membrane, if theories are put aside and the problem be stated as follows:

given a membrane of probable lipoid nature (see Chapter IV) and substances of a certain structure which should be investigated on their permeation velocity through this membrane.

Within physiological tolerance [19]) homologous series and compounds in which groups may be substituted or into which groups may be added, are studied in order to learn the influence of important chemical radicals such as CH_2, $C = O$, $H—C = O$, COOH, —O—, OH, NH_2, etc. and the influence of the position of these groups in the molecule. In pharmacology or better in pharmacodynamy [20]) this method is already used. Overton has initiated this approach which he summarized in his so-called "rules".

This method seems more fertile than the controversy between lipoid- and pore theory with the concomitant consequence of a *selection* of the substances investigated. For research of the last decade was chiefly concerned with large lipoid soluble molecules and small lipoid *in*soluble molecules for the sole reason to settle the validity of one of the two theories. A systematic study of cell permeability as suggested above, may teach us something about the nature of the membrane components. The permeation of lipoidal compounds point to lipoids as probable membrane components. The influence of various compounds upon the permeation of water may suggest something about the *structure* of the membrane.

For if we take a membrane composed of micellae of hydrophobic substances, water will diffuse through the intermicellary spaces. These spaces remaining practically of the same diameter, an influence of substances upon the permeability should be accounted for by means of adsorption of particles on the pore

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19) Not only the resistivety of the cell limits the study of permeation, but also the so-called free diffusion into the cellsap. For if this diffusion equals or is smaller than the permeation velocity, it becomes impossible to measure this last magnitude. Substances with great lipoid solubility cannot be used for this reason.

20) Eléments de Pharmacodynamie speciale et générale par Edg. Zunz.

wall, by which adsorption the diffusion diameter available for the water molecules decreases. The consequence of the theory may be tested by experiment.

If, on the contrary, we depict the membrane as an oriented system, the consequences become of an other nature as will be shown in the following chapters.

CHAPTER II

The influence of alcohols on oriented colloid-systems.

A. *Introduction.*

Assuming the protoplasmic membrane to be an oriented system, the permeability of this membrane for chemical compounds is determined by the way in which the components are related to one another. If the system is compact, in other words if the lipoid components are densely packed, such a membrane will offer a greater resistance to the passage of lipophobic compounds, e.g. water. If the packing is loose, if the membrane components are far, the resistance against the permeation by water cannot be as great as in the first case.

Therefore, it is important for the problem of permeability to study the changes in the protoplasmic membrane under the influence of all sorts of factors.

This, however, only pertains indirectly to the protoplasmic membrane itself, for the changes in this membrane are always deduced from analyses of cell-sap and milieu, from changes in cellular volume, etc.

The explanation of possible changes cannot be found by studying the living object. For that purpose objects have to be taken, the composition of which is known and on which changes can be measured directly.

In my opinion, such an object is found in the so-called condensed systems. In a sol of a bio-colloid the particles have the tendency to distribute themselves equally over the milieu; they are, as it were, in gaseous state.

Under certain circumstances, e.g. by addition of a suitable salt, the particles may be brought into a condition in which they attract one another; it seems as if they condense and separation takes place in a phase relatively rich in colloid and a phase relatively poor in colloid. In that case the phase rich in colloid is the condensed system which in morphological sense is distinguishable in coacervates (homogeneous), flocks (hetero-

geneous) and oriented systems. The phase poor in colloid is called equilibrium liquid.

For the adherent of the phase-theory every component of the phases is equally important and in a description of the phases, he will make no distinction between important and less important components, nor will he occupy himself with the question how the phases originate.

The colloid-chemist, however, chiefly pays attention to the colloid-components of a system and occupies himself with the changes in this system [21]). This selective point of view of colloid-chemistry, maintained for systems where demixing takes place — like flocculations [22]), oriented systems, coacervates — will be adopted in this work.

The advantage of such a concept is that coacervates and oriented systems, which in morphological respect differ widely, may theoretically be traeted in the same way.

We are concerned with the way in which the "essential" components — those components which are important for the colloid-chemist — are related to one another.

The laws governing one system are to be deduced from those governing the other system.

The purpose of this research is to investigate the relations existing in coacervates and oriented systems of those colloids which may be assumed to be protoplasmic membrane components (see Chapter IV). At the same time the change of these relations at the transition of the system from the amorphous condensed state (coacervate) into the directed condensed state is taken into consideration.

When these relations and their modifications while changing their connecting system are known, some facts may be predicted concerning possible changes in the protoplasmic membrane under the influence of e.g. permeating substances.

The expectations based upon the knowledge of the relations existing in the systems mentioned (Chapter II and III), were tested on the living object (Chapter V).

Chapter IV deals with the arguments for assuming phosphatids to be integral components of the protoplasmic membrane.

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21) H. G. Bungenberg de Jong, Kolloid-Zeitschrift 79, 223, 1937 and 80, 221, 1937.

22) In reality, flocculations shcuId be called „aggregates of a system". They are heterogeneous. A coacervate poor in water may form e.g. by a very slow confluence of the droplets, an aggregate which is, in morphological sense, a flock.

In order to elucidate the trend of this work, a discussion of the influence of various alcohols on oleate-coacervates will follow.

B. *Influence of alcohols on Oleate-coacervates.*

§ 1. *Connection of constitution and action, of alcohols.*

Under certain conditions, the volume of the phase rich in colloid may be measured, if it occurs as coacervate. Experiments with K, Na-oleate coacervates, in which we measured the coacervate-volume as a function of the concentration of added non-electrolytes, gave, briefly, the following results [23]):

the action of these non-electrolytes on condensed systems depends on:

a. the constitution of the non-electrolyte
b. the constitution of the essential component of the condensed system (here the oleate)
c. the condition of the system:
   I amorphous or oriented
   II condition of the charge, dependent on electrolyte-components
   III presence and nature of the sensitizers.

The action of these non-electrolytes is related to the:

a. condensing action of hydrocarbon chains and strongly polarisive atoms on the system. These atoms and atom-groups are so to say cohesive media which attract the carbon chains of the oleate molecules; or, more specifically, make the oleate carbon chains less mobile in respect to one another.
b. opening up action of the polar groups (which, owing to their hydratation, bring solvate between the oleate molecules and cause a dispersion of these molecules) on the system. These non-electrolytes chiefly exert an effect on the interaction of the oleate carbon chains.

This condensing resp. opening up action of the non-electrolyte is measurable by the change in volume of the oleate coacervate. The closer the oleate molecules draw together, the less room is left for solvate between these molecules and the smaller is the volume of the coacervate. Inversely, if the molecular distance increases, solvate is assimilated and the volume of the coacervate formed is larger.

Starting from an already existing coacervate and adding to

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23) H. G. Bungenberg de Jong, G. G. P. Saubert: Protoplasma 28, 498, 1937 and H. G. Bungenberg de Jong, H. L. Booy, G. G. P. Saubert, 5 consecutive articles yet to be published in "Protoplasma".

this a condensing substance, an excretion of solvate into vacuoles will occur, owing to the condensation of the system. The solvate expelling action of a condensing compound [24]) may be clearly perceived in this way.

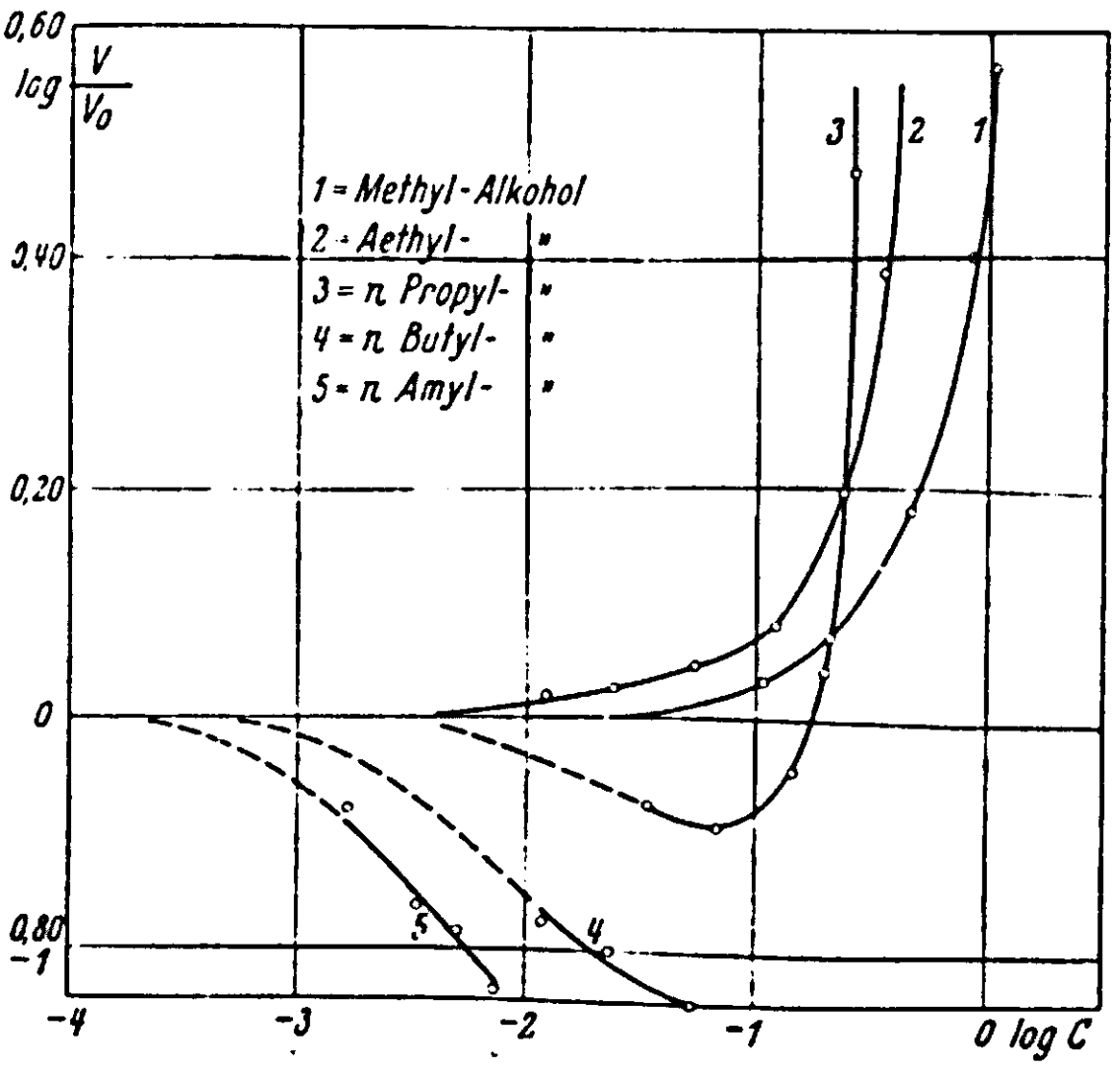
The transformations of the volume cannot be explained by an increase resp. decrease of the absolute quantity of oleate in the layer of coacervate, as analyses of the percentage of soap in the coacervate showed that this percentage decreases with increase of the volume of the coacervate and increases with decrease of this volume.

The richer resp. the poorer the "equilibrium liquid" is in colloid, the richer resp. the poorer the coacervate is in solvate.

§ 2. *Discussion of the graphs of oleate-coacervates.*

Plotting the volume of an oleate coacervate against the concentration of an alcohol, the influence of an alcohol in different concentration on the volume of the coacervate may be seen in the accompanying curves. In order to show clearly the action in low concentration, the logarithms of the alcohol concentrations are always plotted on the abscissa of the graphs, while the ordinate shows the log. of the relative (relative to the

Graph I.



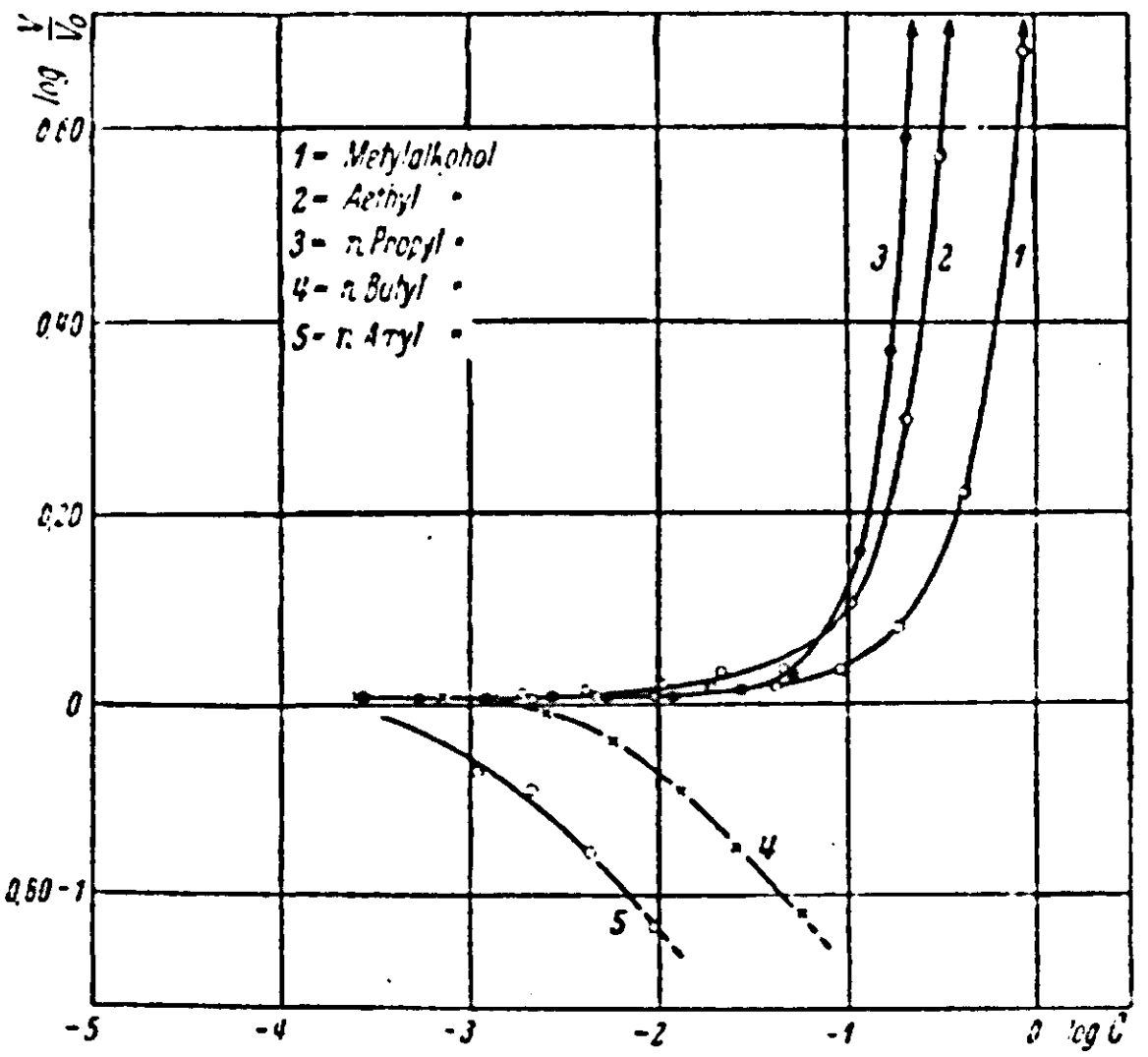
[24]) H. G. Bungenberg de Jong, G. G. P. Saubert — Protoplasma 28, 498, 1937.

coacervate-volume without alcohol) changes in volume. This has the advantage that small relative changes are clearly shown.

Graph I shows the action of the normal alcohols on a coacervate not containing oleic acid. From the graph may be seen:

a. that methyl- and ethyl alcohol at an increasing concentration tend to increase the volume, while n. butyl- and n. amyl alcohol tend to decrease this volume.
b. that n. propyl alcohol in low concentration decreases, and in higher concentration increases, the volume.
c. that with an increasing number of atoms of the alcohol molecule the action is noticeable in lower concentrations of the alcohol. If we compare the alcohol concentration necessary for obtaining an equal relative volume, TRAUBE's rule might be applied.
d. that a reversal in the mode of action appears at the 3rd term of the homologous series of the alcohols.

Graph II.



Graph II shows the action of the normal alcohols on a coacervate sensitized [25]) by oleic acid [26]). The oleic acid with its 18 C atoms — opposed only by one double bound and a very faintly-

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[25]) With sensitizers we mean non-electrolytes which exert a condensing action on the system.

[26]) The graphs I—III are derived from Protoplasma 28, 543, 1937.

dissociated carboxyl group as hydratation centra — has a very condensing action. The variable part of the VAN DER WAALS forces in the complex of factors determining the internal condition of the coacervate increases by the presence of oleic acid. The presence of a very small quantity of oleic acid (originated by CO_2) in the original solution of the oleate may cause a smaller volume of the coacervate as compared with an original solution without oleic acid. Therefore, when we want to compare the action of a certain substance on coacervate-volumes, it is not warranted to compare the absolute volumes, unless they have been prepared from the same stock solution under the same conditions.

From the graph may be seen:

a. that methyl-, ethyl- and n. propyl alcohol at increasing concentration tend to increase the volume, while n. butyl- and n. amyl alcohol tend to decrease the volume.
b. a reversal in the mode of action between the 3rd and 4th term of the homologous series.

On comparing both graphs a drift may be observed of both action and direction of the action of the alcohols under the influence of a sensitizer.

a. Methyl- and ethyl alcohol act in a non-sensitized coacervate at a higher concentration than in a sensitized coacervate. The curve (graph I) for a non-sensitized coacervate is situated to the right of the curve representing a sensitized coacervate.

b. The curves of n. butyl- and n. amyl alcohol for a non-sensitized coacervate are shifted to the left with regard to the curves representing a sensitized coacervate.

c. The descending branch of the curve of n. propyl alcohol of a non-sensitized coacervate cannot be traced for a sensitized coacervate — which is in accordance with the preceding shifting. The ascending branch for a non-sensitized coacervate is situated to the right of the curve representing a sensitized one.

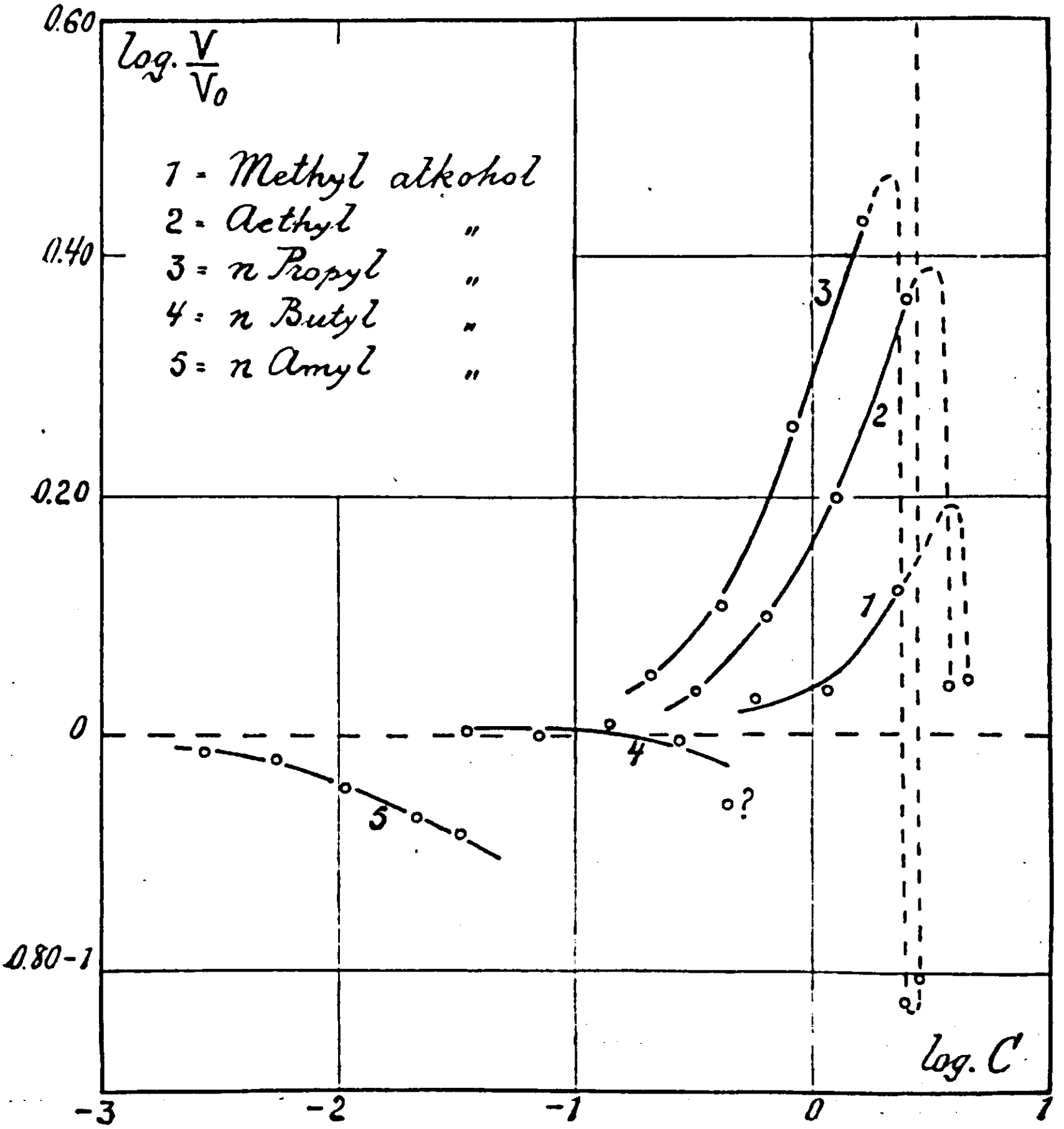
This shifting means that for a coacervate already containing a condensing substance, the condensing action of an added substance relatively decreases, which is shown in the descending branch of the curve by a shifting to the right (always plotting $\log \frac{V}{V_o}$ against log concentration).

Inversely, this relative decrease in the condensing action of a substance is demonstrated, for the ascending branch of a curve, by a relative increase of the opening up action with a concomittant shift of this branch of the curve to the left.

Where the descending branch of the n. propyl alcohol-curve for a non-sensitized coacervate shows a slight decline, it will disappear completely, on shifting to the right.

This shifting of curves may be expected to be the more pronounced, if the sensitizer is more strongly active and the coacervate contains a higher percentage of this sensitizer. In this way, benzene exerts a condensing action on an oleate coacervate of pH = ± 11, but an opening up action on a birefringent system of "oleate : oleic acid" (pH = ± 7.8).

Graph III.



C. *Influence of alcohols on phosphatid-coacervates.*

§ 1. *Discussion of the curves presented by graph III.*

If, instead of oleate-coacervates, phosphatid-coacervates are taken, the action of alcohols on the C-chains of the fatty-acid radicals of the phosphatid molecule, will show the same picture.

However, the phosphatid molecule also has an electric part: the phosphate and choline resp. colamine group. The influence of changes of this electric part upon the system will be discussed later. This influence may be made as small as possible by working as far as possible from the zero charge. In this way the alcoholic action on the C-chains of the phosphatids may be watched. Unfortunately a pure phosphatid does not coacervate, so that we always have to experiment with a smaller or greater number of sensitizers.

The difficulty lies in the heterogenety of the materials purchased. Every jar of a certain trade-product bearing a different serial number is of different composition and requires different handling in order to obtain an usable sol, i.e. a sol, coacervable with suitable salts. By varying the mode of preparation every time, the sol also obtains other properties.

All the experiments with phosphatids described here have been performed with a common trade-product of RIEDEL-DE HAËN, Planticine 90—95% alcohol-soluble, from small jars with one and the same serial number. By this precaution the same mode of preparation of the sols could be followed. The chemical composition of such a sol is not known, but the results are reproducible.

For the experiments used in graph III [27]), a 4% emulsion of phosphatid was used in aq. dist. (without preceding purification; the sensitizers being quantitatively included). On the basis of this stock emulsion the coacervates were prepared at 50° C and at a final concentration of 12 m. eq. $CaCl_2$. The curves again show:

a. for methyl-, ethyl- and n. propyl alcohol an ascending and for n. amyl alcohol a descending branch which may be accounted for resp. by the volume-enlarging action of the polar group of the alcohol and the volume-reducing action of the carbon chain of the alcohol. The n. butyl alcohol hardly acts at all. In the highest concentration the curve drops, which means a shrinking of the coacervate.

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27) H. G. BUNGENBERG DE JONG, H. L. BOOY, G. G. P. SAUBERT — Protoplasma 28, 543, 1937.

b. the methyl-, ethyl- and n. propyl alcohol curves show, beyond a certain alcohol concentration, a sharp drop which is changed again into a rise for n. propyl alcohol.

The minimum coincides with the zero charge. For by addition of alcohol, a coacervate reaches its zero charge at lower concentrations of a salt. It appears that at 12 m. eq. $CaCl_2$ in a milieu of a certain n. propyl alcohol concentration, the phosphatid coacervate concerned reaches its zero charge. The descending branch of the curves of the three first terms of the homologous series of the alcohols may be accounted for by a decrease of the negative charge of the particles with increasing alcohol concentration and the concomittant decrease in mutual electrical repulsion of these particles.

Here the electric factor (to which we will refer later on) makes itself felt. Only the ascending branches of the curves of the first three alcohols and the descending curves for n. butyl- and n. amyl alcohol will be considered at this place.

c. the inverse action only takes place at the fourth term of the homologous series. The influence of the sensitizers may be followed also for the phosphatids as a hampering of the shrinking action of alcohols which is obvious when we realize that the sensitizers themselves already cause a shrinking action which, as a rule, is stronger than that of the alcohols.

Now it may be objected that phosphatids can not be compared with oleates. Considering, however, that stearates [28]) show the same behaviour as oleates and that phosphatids are composed of glycerol, esterized with saturated and unsaturated fatty acids, it may be assumed that also the phosphatid molecules will show the same behaviour (taking into consideration the possible complication of the elctrical part of the phosphatid molecules).

The method followed for the preparation of the phosphatid coacervates did not yield satisfactory results, however, because

a. At the temperature of 50° C, whereby is experimented and during the time (14—16 hours) the coacervate is exposed to this temperature, the phosphatids decompose, which may be observed by the change of the coacervate's volume as a function of time.

b. The nature of the chemical substances used remains unknown.

§ 2. *Preparation of phosphatid sol.*

It was, therefore, tried to prepare a sol of purified phospha-

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[28]) Unpublished result of S. ROSENTHAL from the Laboratory for Medical Chemistry, Leiden.

tids, to which, in order to obtain condensation by means of suitable salts, chemically as well as quantitatively well-defined sensitizers were added, such as triolein, cholesterol, soaps, a.o.

This attempt was not succesful. The produced coacervates were flocky, so that a measurement of the layers was out of the question.

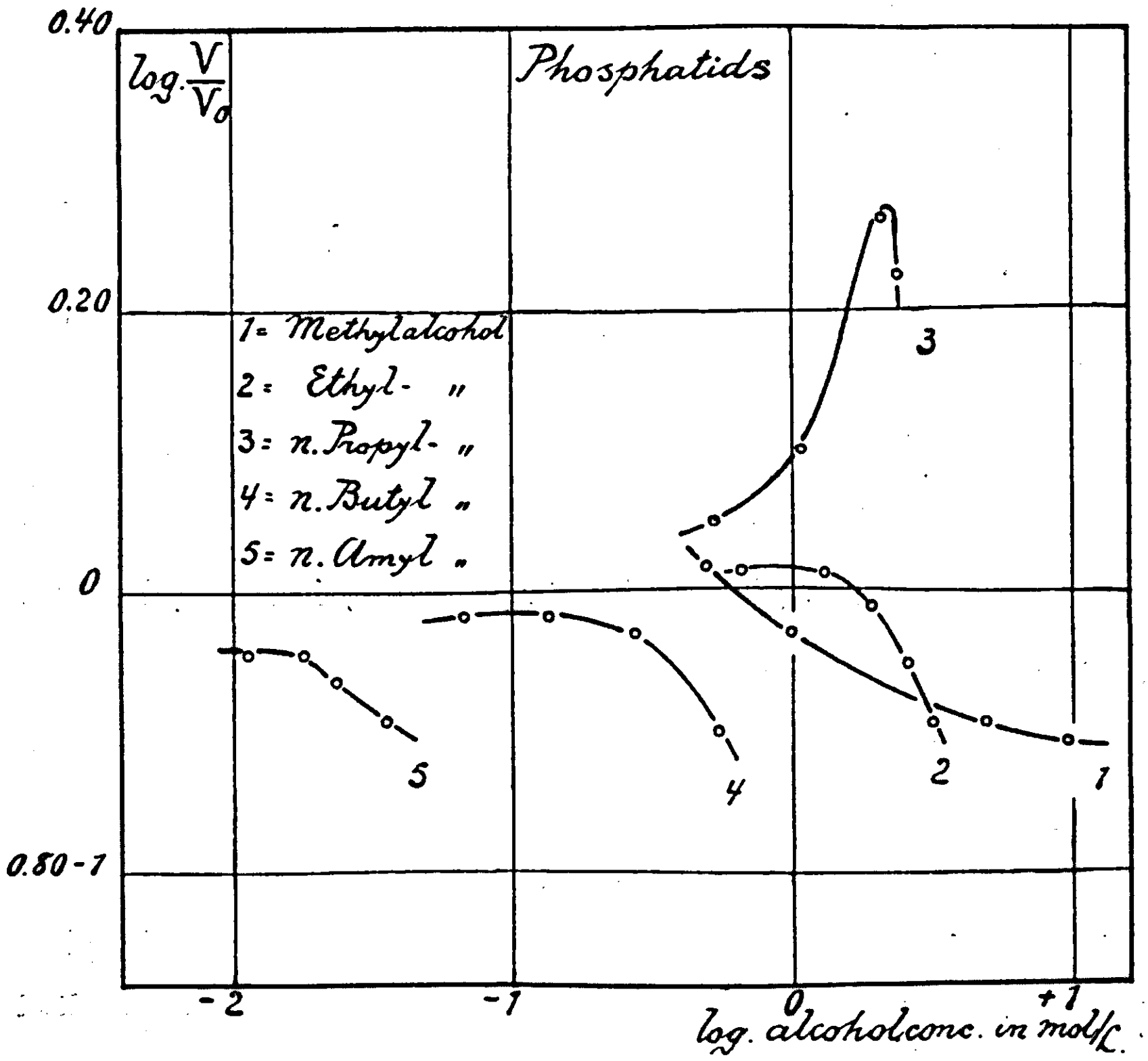
The purification-methods, already in use, are:

a. fractional precipitation with acetone from a solution in ether of the trade-product;

b. fractional separation, making use of the partition over two phases.

Instead of these methods we endeavoured to purify the material, by means of fractional precipitation of components from an alcoholic solution of the trade-product at lower temperature.

Graph IV.



This method was successful. The phosphatids obtained were for the greater part desensitized. Their sols, however, yielded coacervates with $CaCl_2$ at 40° C.

On heating the dialysed sol for ½ hour at 90° C, a sol is obtained which yields clear coacervates with $CaCl_2$ in suitable concentration at room temperature.

In order to be able to experiment at one temperature, it is necessary to obtain a sol that coacervates at room temperature.

Experiments in which the coacervate was condensed at 40° C within 10 minutes and was afterwards cooled down to room temperature, were unsuccesful, as the volumes appeared not to be reproduceable. During the cooling — no matter how quickly performed by means of waterbaths — the coacervate droplets change in volume on account of absorption of solvate. The volumina of coacervate appear to be very sensitive to temperature (Chapter III).

The objection still holds that we worked with undefined chemical compounds. It has to be considered, however, that we are not concerned with the study of the properties of a chemical compound here, but with the study of the way in which various compounds are mutually related. Moreover, not all the components of the protoplasmic membrane are known. It may be expected, however, that also decomposition-products of the phosphatids will act as sensitizers like, apparently, the decomposition-products in the heated sol do.

A first requirement is.... clear coacervates, the volumes of which are exactly measurable, which requirement is fulfilled by the heating treatment.

Our work was done with sols [29]) prepared in this way. The results [30]) are reproduced in graph IV, which is plotted on the same scale as graph III.

§ 3. *Comparison of phosphatid-coacervates prepared from emulsion and from sol.*

On comparing it appears that:

a. *Methyl- and ethyl alcohol* have a volume-reducing effect

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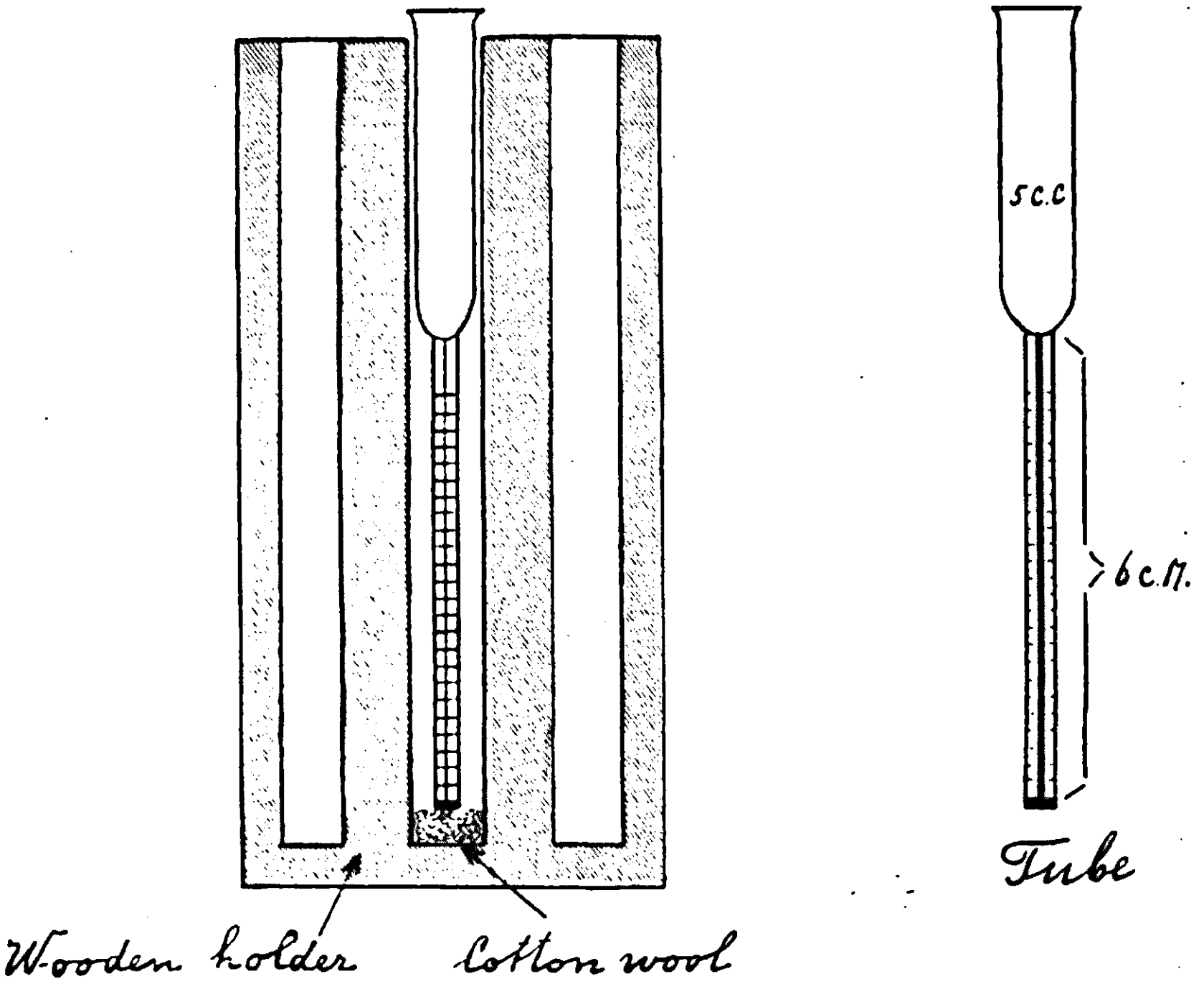
29) Recipe: 20 gr. "Planticin alkohollöslich 90—95 %" of RIEDEL—DE HAËN is dissolved under continuous shaking at room temperature in 200 c.c. 96% alcohol. About 85% dissolves at 20° C. This solution is poured into an Erlemeyer of 200 c.c. which is then placed in a thermos bottle containing water at 5° C. After about 6 hours the part of the trade-product that does not dissolve at 5° C, has settled.

The clear alcoholic solution is poured into a clean Erlemeyer. Now, the

Figure I.



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200 c.c. alcoholic solution is poured into 800 c.c. H_2O with a thin jet, while the water is stirred thoroughly. This may be done best by spinning the Erlemeyer with H_2O with the hand. The clear sol obtained in this way is dialyzed for 3 to 4 × 24 hours. The alcohol free sol is heated for ½ hour at 90° C, after which it will keep for weeks and weeks, preserved in the ice-chest. This sol is coacervated at 20 m.eq. $CaCl_2$/l final concentration at room temperature. The concentration of the sols is ± 1%. It is advisable to precipitate the alcoholic solution not only at 5° C, as the manufacturer always changes the composition of the trade-product. It may be that an other precipitation temperature yields better results for the product in question.

[30]) Method: In a flask of 50 c.c., containing x c.c. of a $CaCl_2$ stock solution of 500 m.eq. $CaCl_2$/l + y c.c. of an alcoholic stock solution + 20— (x + y) c.c. H_2O dist., is pipetted 5 c.c. of the stock sol prepared according to the recipe. After shaking, 5 c.c. of the mixture is pipetted out of each flask into a tube, held in readiness, in the shape and dimensions here reproduced. (this procedure in duplicate). The tubes are placed in a wooden holder, which just fits in the steel holders of a big "Ecco" centrifuge. Each holder may contain 6 tubes and the centrifuge may handle 4 holders at the same time. This means that 24 tubes may be treated identically — including

on the coacervate prepared from the sol (graph IV) in concentrations which act on the coacervate prepared from the emulsion (graph III) by an increase in volume.

b. *n. propyl alcohol* gives with the "sol-coacervate" in higher concentration an equally great relative volume increase as with the "emulsion-coacervate". The largest, relative volume of both coacervates is obtained at the same alcohol concentration, at 0,35 on the logarithmic scale. The coacervate, prepared from emulsion, however, reaches a larger relative volume than that prepared from the sol (log $\frac{V}{V_o}$ is greater for the "emulsion-" than for the "sol-coacervate").

c. *n. butyl alcohol,* on the contrary, gives with the "sol-coacervate" at lower concentration an equally great relative volume-decrease as with the "emulsion-coacervate". The curve for n. butyl alcohol of graph IV is situated to the left of that of graph III.

d. The *n. amyl alcohol* curve of graph IV is situated to the right of that of graph III.

Exept for n. amyl alcohol the curves of graph IV show more tendency to drop than those of graph III, in other words, the "sol-coacervate" has a stronger tendency to decrease its volume than the "emulsion-coacervate".

This cannot be explained by the fact that the "emulsion-

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duplicates, — i.e. 12 points of a series (see figure I).

After pipetting the mixtures into the tubes, we leave them for ½ hour at room temperature before centrifuging for 20 minutes at 2000 revolutions per minute.

After centrifuging the tubes are placed in racks at a temperature which is some degrees below room temperature (e.g. in a cellar) and the volumes are read after 12 hours. Before reading the tubes are turned again at 2000 revolutions per minute for 10 minutes. By doing so, clear coacervates are obtained without vacuoles (which immediately originate at a small rise in temperature — see Chapter IV). Moreover, the whole coacervate is coalesced to a homogeneous layer, the volume of which may be easily read.

The reading itself happens with the aid of a hand-lens, care being taken to keep the meniscus always at the same height.

After some practice the error needs not be greater than 2 × 0,1 of the distance between two division marks, i.e. 0,002 c.c.

In general, the duplicates agree very well.

The tubes are cleaned by means of a drawn glass tube, connected to a suction-pump, the coacervate being sucked out of the calibrated lower end of the tubes. After this the lower end of the tube is cleaned with dest. water by means of a siphon with thinly drawn capillary. After the tube is washed out well, a little acetone is poured into it, after which the tube is sucked dry be means of a thinly drawn capillary connected to a suction-pump.

TABLE I

Charge-reversal of phosphatid coacervates with $CaCl_2$

preparation of the sol		Concentration $CaCl_2$ at the charge reversal *)
1. not purified		91 m.eq. $CaCl_2$/l
2. purified by means of temperature-treatment	a. 5.5° C	65 m.eq. $CaCl_2$/l
	b. —3.3° C	60 m.eq. $CaCl_2$/l

*) Concentration $CaCl_2$ of the equilibrium liquid, by which the kataphoretic velocity of the coacervate is zero. The quantity of $CaCl_2$ adsorbed by the colloid may be neglected for $CaCl_2$ [not for all salts].

coacervate" is richer in sensitizers [31]), as with methyl and ethyl alcohol even a reversed action takes place.

It may be possible that the alcohols in higher concentration influence the reversal of charge of the coacervates. This zero charge is for the "sol-coacervate" considerably lower than for the "emulsion-coacervate", as may appear from table I. Moreover, the "emulsion-coacervate" was condensed with 12 m. eq. $CaCl_2$ from 10 c.c. 4% emulsion (total volume 25 c.c.), whereas the "sol-coacervate" with 20 m. eq. $CaCl_2$ from 5 c.c. 1% sol (total volume 25 c.c.). Therefore, the latter is much nearer its zero charge than the former. For the "sol-coacervate" an influence on the charge-reversal may be therefore expected at lower concentration of alcohols than for the "emulsion-coacervate" which has been confirmed by experiment.

D. *Influence of alcohols on the dielectric.*

§ 1. *Preparation of phosphatid coacervate, arabinate films and quartz suspension.*

In order to decide, if the change in the electric properties of the coacervate by the presence of alcohols may indeed account for the change in volume, the reversal of charge of these coacervates with $CaCl_2$ was measured without and with alcohols in different concentration.

If the alcohols change the electric character of a coacervate, one is liable to think of changes in the dielectric and therefore, in principle, other colloids and non-colloids should be influenced in the same way.

31) In the emulsion all sensitizers are still present, whereas the phosphatid was partly deprived of these sensitizers by purification during the preparation of its sol.

For that reason the reversal of the charge with $CaCl_2$ was measured under the influence of alcohols on:

1. Phosphatid coacervates [32]).
2. Arabinate films on quartz [33]).

For every measurement from this stock-solution 2 c.c. is pipetted into flasks of 50 c.c. containing 45— (x + y) c.c. H_2O dist. + x c.c. alcohol + y c.c. $CaCl_2$ solution + 3 c.c. quartz suspension. Total volume 50 c.c. The quartz suspension is prepared every morning by shaking 2 gr. of quartz with 100 c.c. H_2O dist. in a measuring glass and by leaving the suspension to settle for ½ hour, after which it is poured off into an Erlemeyer.

3. Quartz [34]).

Phosphatid, arabinate and quartz are taken because they are resp. a lipophilic colloïd, a strong hydrophilic colloïd and a non-colloïd.

§ 2. *Discussion of the graphs.*

In graph V the log. of the concentration $CaCl_2$ at the reversal of charge is plotted against the log. of the mol. percentage alcohol. The points of every curve are obtained by interpolation of a series of minus and plus points (direction and kataphoretic velocity plotted as a function of the $CaCl_2$ concentration at constant quantity of alcohol) in the way shown in graph VI. Thus always the $CaCl_2$ concentration at which reversel of charge takes place, is interpolated from at least three points. The velocity zero is not measurable and measurements of too small velocities are inexact. The error by interpolation need not be greater than ± 0.03 in the logarithm. For an exact

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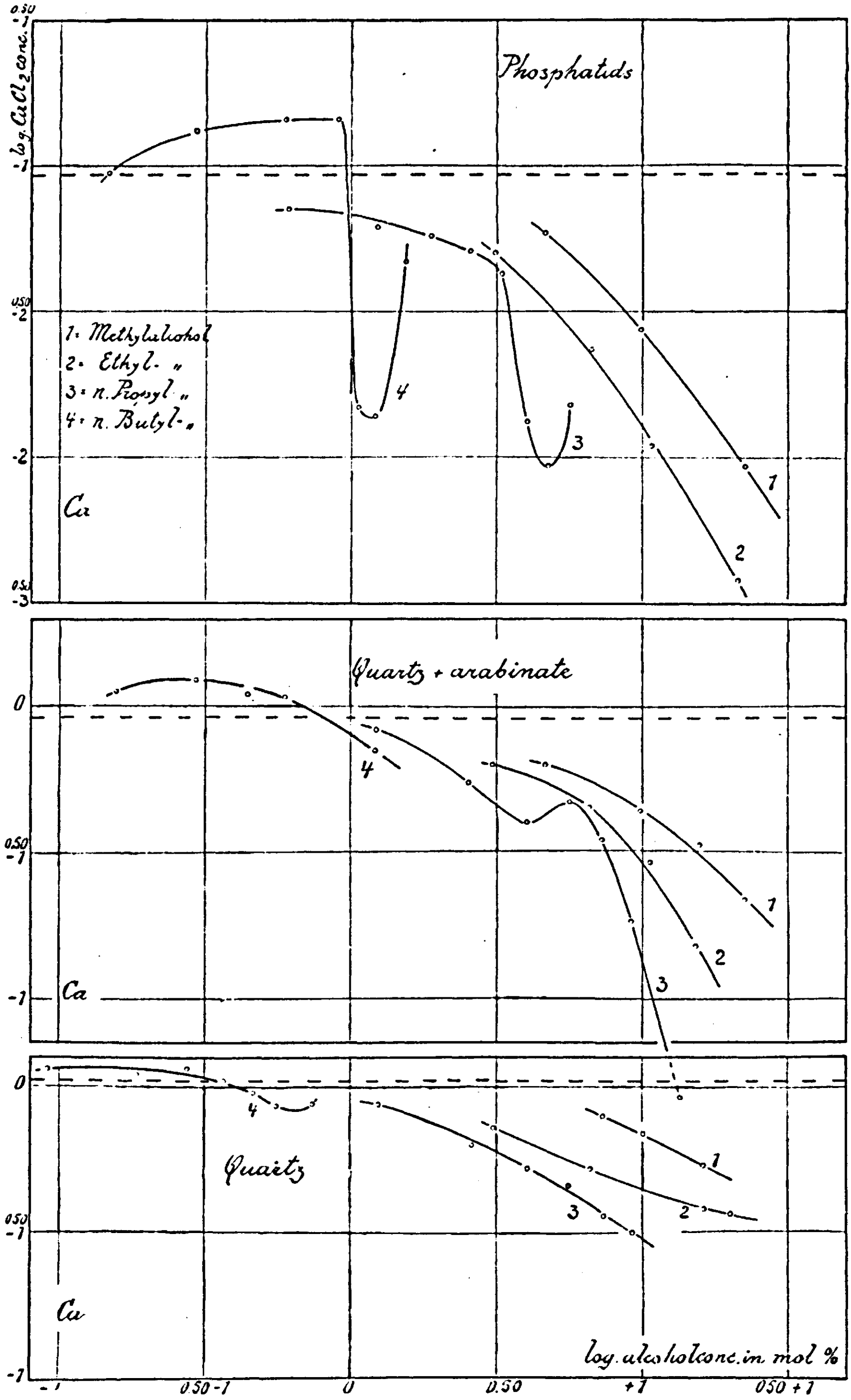
[32]) preparation: The phosphatid-coacervate was prepared as already described above, only with this difference that the sol was not heated. This was deemed unnecessary, as also in flocculations the reversal of charge may be measured very well. Moreover, here only the shape of the curves is concerned, which shape should be compared with the shape of the curves for other colloids and non-colloids. For every measurement 2,5 c.c. (5 c.c. of a dilution 1 : 1) of the stock-sol was pipetted into a mixture of 45— (x + y) c.c. H_2O dist. + x c.c. alcohol + y c.c. $CaCl_2$ solution. The stock-solution $CaCl_2$ contains 500 m.eq. $CaCl_2$/l.

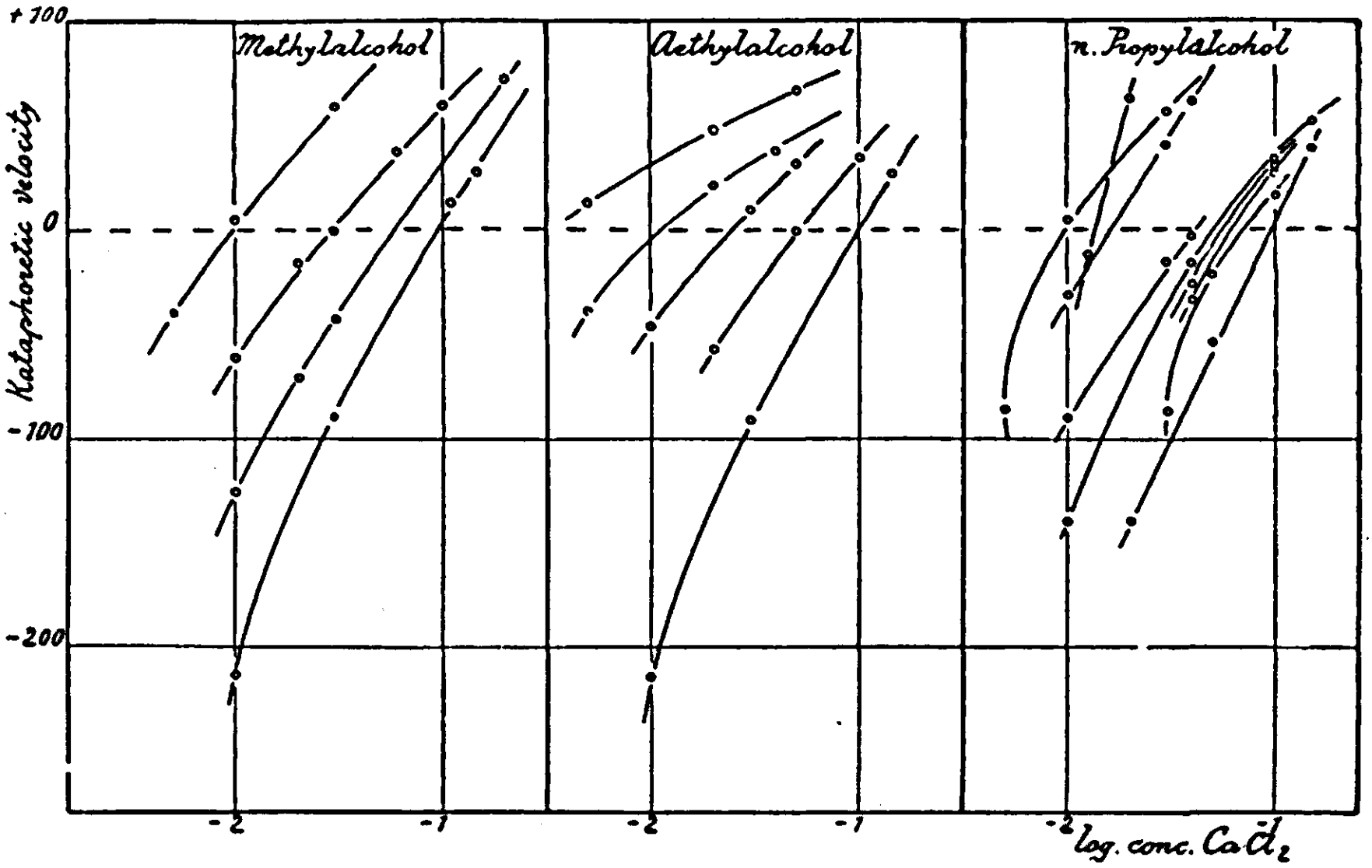
[33]) preparation: 10 gr. Na-arabinate are soaked about 2 hours in a volumetric flask of 200 c.c. in a small amount of dist. water at room temperature, where upon hot water is added, shaking the container. If the arabinate has been dissolved, after cooling, water is added up to the mark.

[34]) preparation: Preparation of stock-quartz suspension as indicated for measurements of arabinate films. From this stock-solution always 3 c.c. was pipetted into a mixture of 47— (x + y) c.c. H_2O dist. + x c.c. alcohol + y c.c. $CaCl_2$ (same solution as above).

Graph V.


Phosphatids
1. Methylalcohol
2. Ethyl- "
3. n. Propyl "
4. n. Butyl- "
Ca
Quartz + arabinate
Quartz
Ca
log. alcoholconc. in mol %
-1
0.50 -1
0
0.50
+1
0.50 +1

Graph VI.



description of the method we refer to the article in question by H. G. BUNGENBERG DE JONG and P. H. TEUNISSEN in Rec. des trav. chimiques des Pays Bas, t. 54, No. 5, 1935. The measurements always were performed at 1/5 cuvette-height.

Graph V shows that the curves for arabinate films and for quartz run more flatly than those for phosphatid-coacervates. The concentrations of $CaCl_2$ at the charge-reversal are, however, for arabinate and quartz about 10 times as high as for phosphatids. Therefore it may be possible that the high $CaCl_2$ concentration masks the similarity in shape of the curves for the two colloids and quartz. In order to investigate this point the charge-reversal for quartz was measured under influence of alcohols with Luteo (stock-solution of 11.145 gr./250 c.c. = 0,5 N in the refrigerator. This deluted 10 times before use, which dilution is used for the experiments). Luteo [$Co(NH_3)_6$ Cl_3] causes the charge-reversal of quartz in concentration of 5 1/4 m. eq.

The appearance of the shape of the phosphatid curves is seen clearly from graph VII.

Comparing the graphs V and VII we notice:

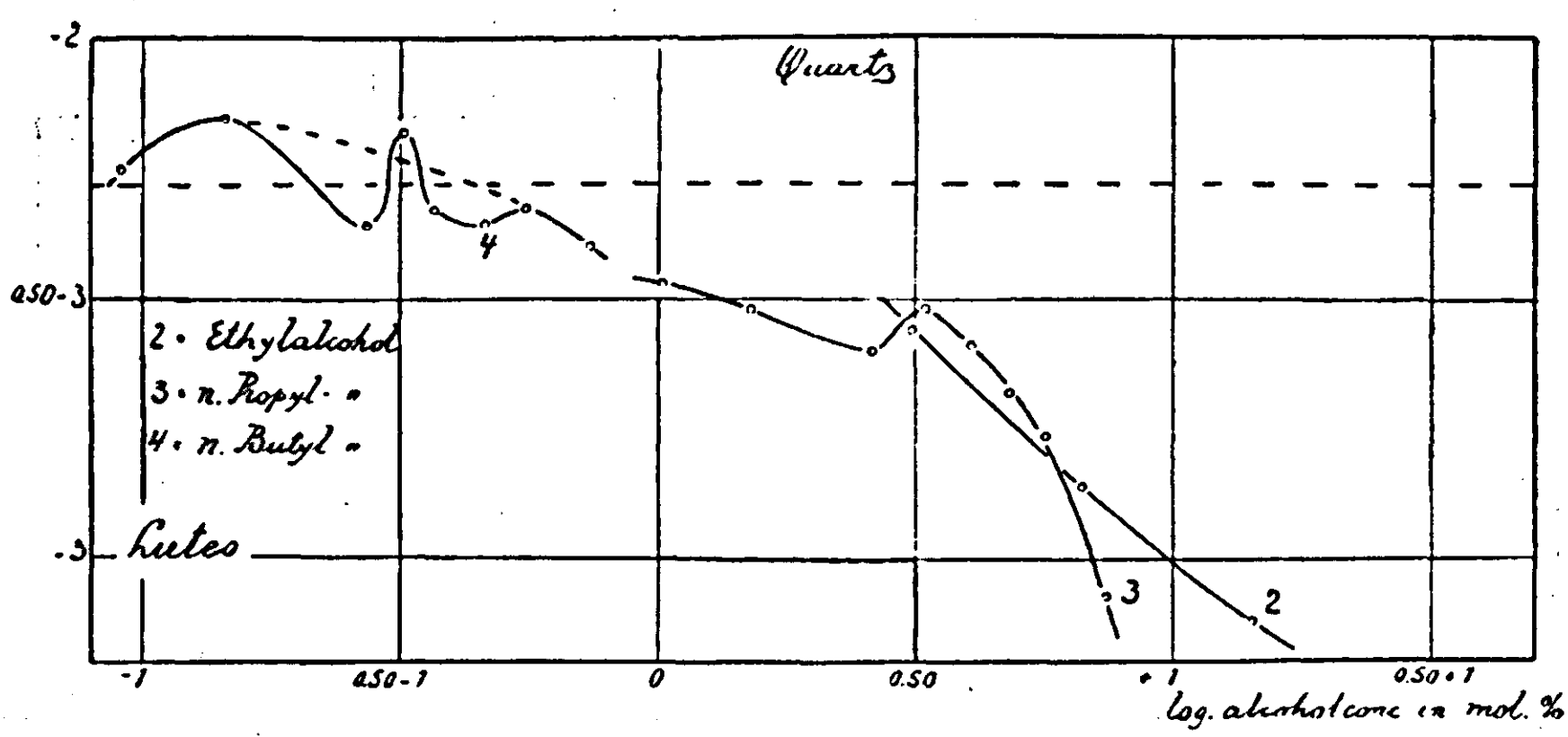
a. methyl- (in graph V) and ethyl alcohol cause, at increasing concentration, a regular decrease of the concentration of $CaCl_2$,

necessary for the reversal of charge of the colloids and of quartz.

b. n. propyl alcohol causes, for phosphatids at lower concentrations, a slight decrease, at higher concentrations a large decrease of the $CaCl_2$-concentration necessary for the reversal of charge of the coacervate. The sharp drop of the curve for phosphatid cannot be traced for arabinate and quartz.

At an alcohol concentration of 0.68 logarithmic mol. % for phosphatids and 0,70 for arabinate the curves rise again. This rise shows a strong tendency to disappear for higher salt-concentrations of the milieu (compare the curve of phosphatids → arabinate → quartz).

Graph VII.



The discontinuity in the alcohol/Luteo curve for quartz, may be only latent in the alcohol/$CaCl_2$ curve. Possibly the high salt-concentration masks this phenomenon. The influence of concentrations of n. propyl alcohol higher than 0,75 logarithmic mol % has not been checked with regard to phosphatids, as in that case the coacervates are counteracted. Measurements on phosphatid films on quartz were not carried out.

c. n. butyl alcohol causes, at low concentration, a rise of the $CaCl_2$ concentration, necessary for the reversal of charge of the colloids and quartz. The alcohol/luteo curve for quartz also shows the same course (see dotted line in graph VII). This first rise is followed by a drop. The n. propyl- and n. butyl alcohol curves for arabinate and quartz are still more level than those for the phosphatid-coacervate and do not show the apparent conformity as do the curves for phosphatid coacervates.

The alcohol/luteo curve for quartz shows secondary tops which cannot be interpreted. The points have been measured twice and appear real. The dotted line indicates how the normal course should be.

Where the curves of the colloids and the quartz agree fundamentally — secondary influences left out of consideration — one may conclude to an influence of the alcohols on the milieu. Apparently the colloid does not seem to count. A change in the properties of the milieu — independent of the colloid — in the sense of an easier or more difficult contact between oppositely charged ions present (resp. ion and ionogenous places), may only be attributed to the change of the dielectric constant of this milieu.

This idea is strengthened by the fact that the curve of the measured dielectric constant of water/alcohol mixtures as a function of alcohol-concentration agrees with the curves drawn by us. We have only data on mixtures of methyl- resp. ethyl alcohol/water mixtures.

It may be also observed from the graphs that the change in the dielectric is such, that it may be of conclusive importance for the structure of the condensed systems.

E. *Influence of alcohols on the volume of phosphatid-coacervates.*

§ 1. *The importance of the electric factor.*

A constant concentration of salt is always used, when measuring the volumes of coacervates as a function of the alcohol concentration, to which increasing quantities of an alcohol are added, while the total volume is kept constant. If by this addition of an alcohol the concentration of $CaCl_2$ necessary for the charge-reversal is decreased, the result will be a shrinking of the coacervate.

Near the zero charge the effective attraction of the particles in the coacervate increases and the coacervate gets poorer in solvate and occupies a smaller volume. As soon as the zero point of charge has been passed, the effective attraction decreases and the coacervate increases in volume [35]).

The more sensitized the coacervate from which we started,

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[35]) The point of view developed here applies only to systems close to their zero point of charge. Oleate-coacervates, which only at very high concentration of an alkali-salt attain their zero charge, cannot show this electrical influence, as alcohols shift the system comparatively slightly in the direction of the zero charge. In the graphs I and II this influence on the dielectric therefore is not expressed.

(and, therefore, the denser) the less obvious the relative decrease of volume and the more obvious the relative increase of volume will be. The same applies to the structure of systems as a consequence of their electrical condition.

The denser a system is, as a consequence of the approach to the zero charge, the more insensitive to a relative shrinking and the more sensitive to a relative expansion it will be under the influence of substances.

The opposite is true for the "other side" of the zero charge, or rather, at the other side of the maximum-density.

Moreover, a high concentration of an alcohol causes the system to disappear. If we want to decide, on the basis of changes in volume of the coacervate, to a change of the solvatation, the changes in volume are to be compared per weight-unit of colloid in the coacervate.

If a substance exerts a dissolving action on the coacervate, the total weight of colloid decreases and, in consequence, the volume of coacervate decreases also. This change of the volume has not to do with the solvatation of the system. In considering the curves this has to be taken into account.

§ 2. *Discussion of the influence of alcohols on phosphatid-coacervate-volumes.*

The curves of graphs X and XI represent relative volumes of coacervates as function of the alcohol concentration. Plotted are Log. $\frac{V}{V_o}$ against Log. concentration alcohol in mol./L.

The curves 1, 2, 3, 4, and 5 of the graphs X and XI show the course of relative volume-changes under influence of resp. methyl-, ethyl-, n. propyl-, n. butyl- and n. amyl alcohol. The curves 2, 2' and 2" of graph VIII show the course of relative changes in volume of coacervates condensed with 20, 60 and 200 m. eq. $CaCl_2$ under influence of ethyl alcohol. The sol was prepared according to the given recipe and the method was already described above.

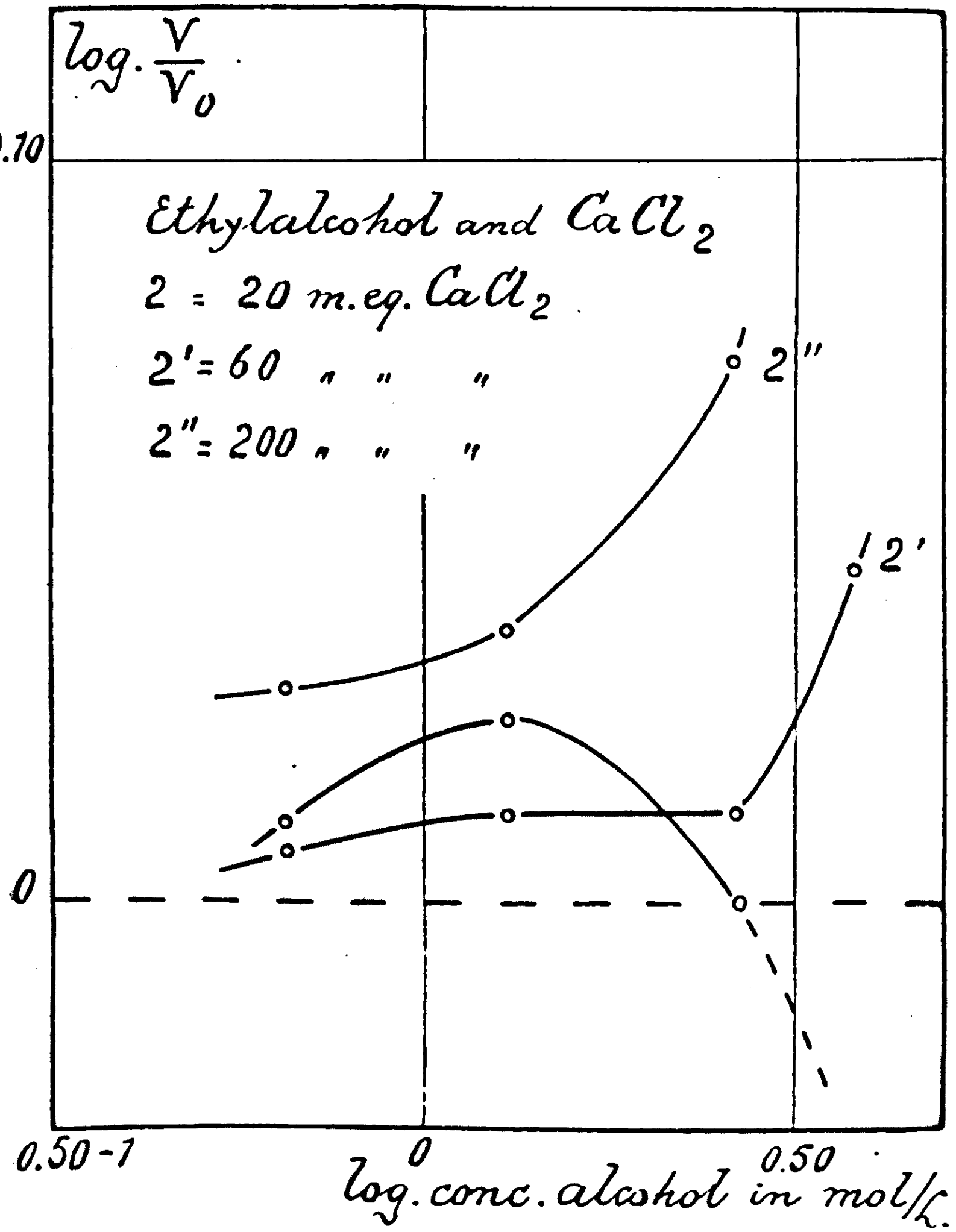
All measurements were made with the same sol that practically did not change during the time of the experiment.

Each point of a curve has been determined in duplicate and for each alcohol the whole series was determined on one day. In the graphs VIII—XI the zero level (coacervate-volume without alcohol) is indicated by a dotted line. In order to be able to compare the action of various alcohols, a series of 5 different alcohols, at one definite $CaCl_2$-concentration, was started simultaneously on one day. In this way we might compare directly:

a. the influence of an alcohol at different concentration $CaCl_2$ (= different electric condition of the coacervate).
b. the influence of the 5 n. alcohols at one concentration $CaCl_2$ (= one electric condition of the coacervate).

The influence of an alcohol on the volume of coacervates was

Graph VIII.



always examined at 20, 60 and 200 m. eq. $CaCl_2$ in the equilibrium-liquid. As the electric condition of the system is a function of this concentration, the action of the alcohols is, in this way, examined at 3 conditions of charge. The concentrations are chosen in such a way that in aqueous milieu they are logarithmically at an equal distance on both sides of the concentration of zero charge and one concentration is a little smaller than the concentration of the charge-reversal. In such a way a negative system (20 m. eq. $CaCl_2$), a positive system (200 m. eq. $CaCl_2$) and a feebly negative system (60 m. eq. $CaCl_2$) are obtained. The concentration of the charge-reversal itself is 70 m. eq. $CaCl_2$ for this phosphatid system.

a. From graph VIII which shows the first part of the three curves for ethyl alcohol may be observed the opening up action on the positive system (scale abscissa: ordinate 1 : 10). On the negative coacervate ethyl alcohol has, in lower concentration, an opening up and later a condensing action, while the feebly-negative coacervate occupies an intermediate position. From this the importance of the electric condition of the system may be clearly seen.

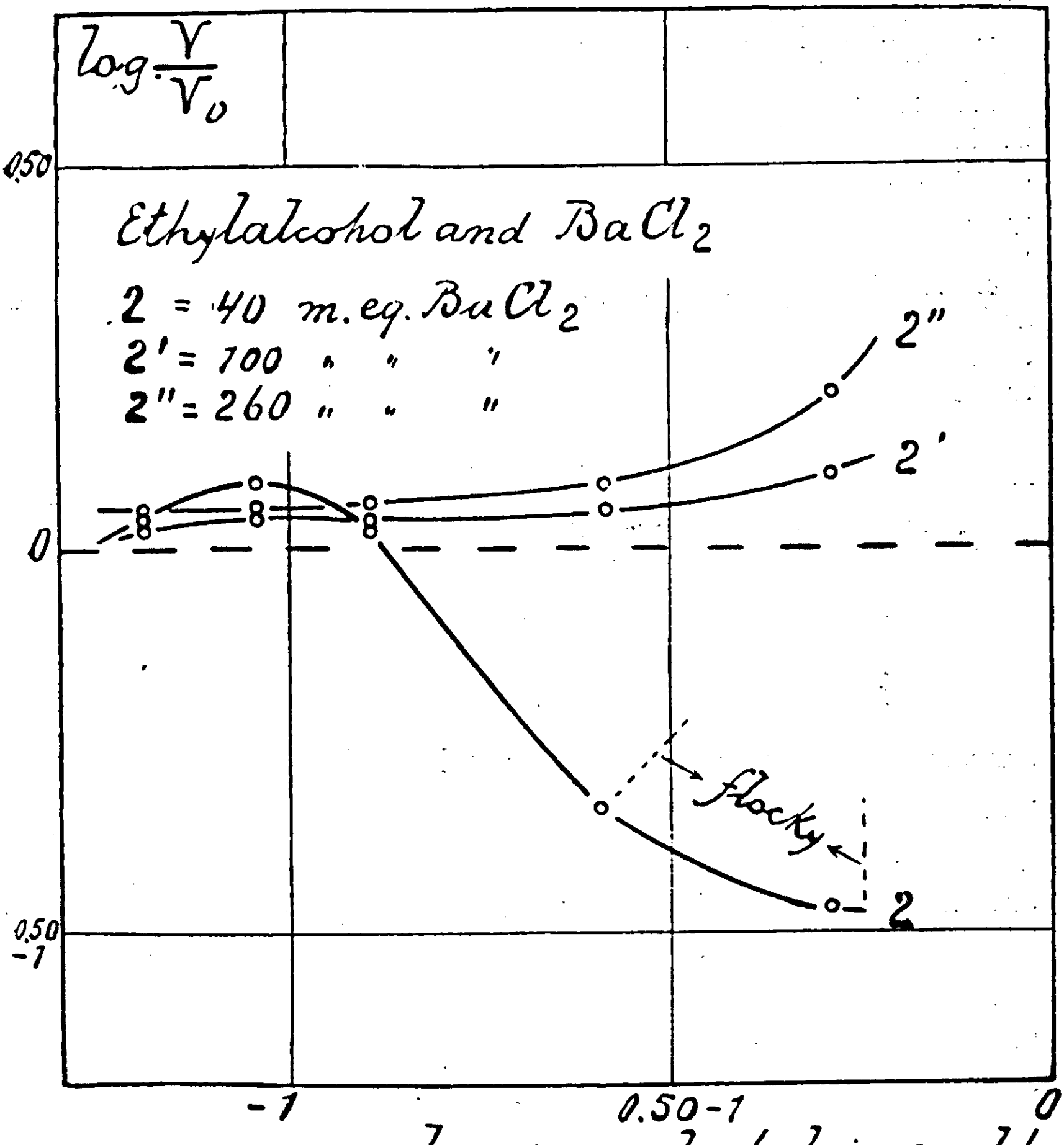
The positive system only may react on the ethyl alcohol by increase of volume on account both of the opening up action of the alcohol and the system becoming more positive (change of the dielectric). The negative system only may increase in volume at low alcohol concentration through the opening up action of the alcohol.

However, at the same time this alcohol brings the system to its reversal of charge, by which the effective attraction of the particles increases and the system decreases in volume.

In concentrations higher than 6 mol. per litre the alcohol dissolves the system which has to be taken into account, especially for the positive system which by its charge facilitates the dissolution. At these alcohol concentrations the coacervate is seen to form 2 to 3 layers, gradually merging into each other, the total volume of which layers is not measurable. More illustrative is graph IX, where the coacervate was condensed with resp. 40, 100 and 260 m. eq. $BaCl_2$. For, with Ba coacervates are obtained richer in solvate, that is to say, a larger volume of coacervate is obtained from an equal amount of phosphatid-sol.

b. For the interpretation of the methyl alcohol curves (graphs X and XI) the same idea may be maintained. Concentrations higher than 8 mol./l effect a dissolution of the coacervate —

Graph IX.



especially of the positive coacervate — as may appear from the following determinations of the "equilibrium liquid" at different alcohol concentration and 200 m. eq. $CaCl_2$. (11.1 m. gr. is deduced from the dryweights per gr. equilibrium liquid).

3.92 mol./l methyl alcohol — 8.38 m. gr. phosphatids/gram "equilibrium liquid"

11.76 mol./l methyl alcohol — 9.50 m. gr. phosphatids/gram "equilibrium liquid"

16.66 mol./l methyl alcohol — 11.00 m. gr. phosphatids/gram "equilibrium liquid"

Also the negative system is subject to dissolution after passing the point of zero charge (5 mol./l).

The negative system does not show any increase of volume in the lower alcohol concentration, as is the case with ethyl alcohol. The action of the methyl alcohol on the electric part of the phosphatids is in this system stronger than the action on the carbon chains.

c. All the curves for n. propyl alcohol ascend. The opening up action of this alcohol is so strong that the approach of the zero charge [the reversal of charge [36]) lies on the rising branch of the curve] does not result in a drop of the line [37]). Only in

TABLE II

conc. n. propyl alcohol in mol./l	m.eq. $CaCl_2$/l in the "equilibrium liquid"	dry weight in m.gr. phosphatids/gram "equilibrium liquid"
2.39	20	0.54
2.66	20	2.13
2.39	200	7.7
2.66	200	9.1

concentrations where dissolution takes place (see table), the curves drop (graphs X and XI).

From the figures of the analyses in the table appears that the positive system is counteracted more rapidly than the negative one. The strong opening up action of n. propyl-alcohol is connected with its relatively favourable distribution over both phases.

In discussing the action of alcohols on a system, the action per molecule has to be distinguished from the total action, being the effect which is graphically represented. It could be concluded for example from the position of the curves of graph I that n. propyl-alcohol has a stronger opening up action than methyl-alcohol. This, however, is not true. Methyl alcohol has,

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[36]) The charge reversal was not determined on the coacervate, which served for the determination of volume. A coacervate, freshly prepared from the stock sol was used to determine this charge-reversal. The difference between the two coacervates cannot have been great.

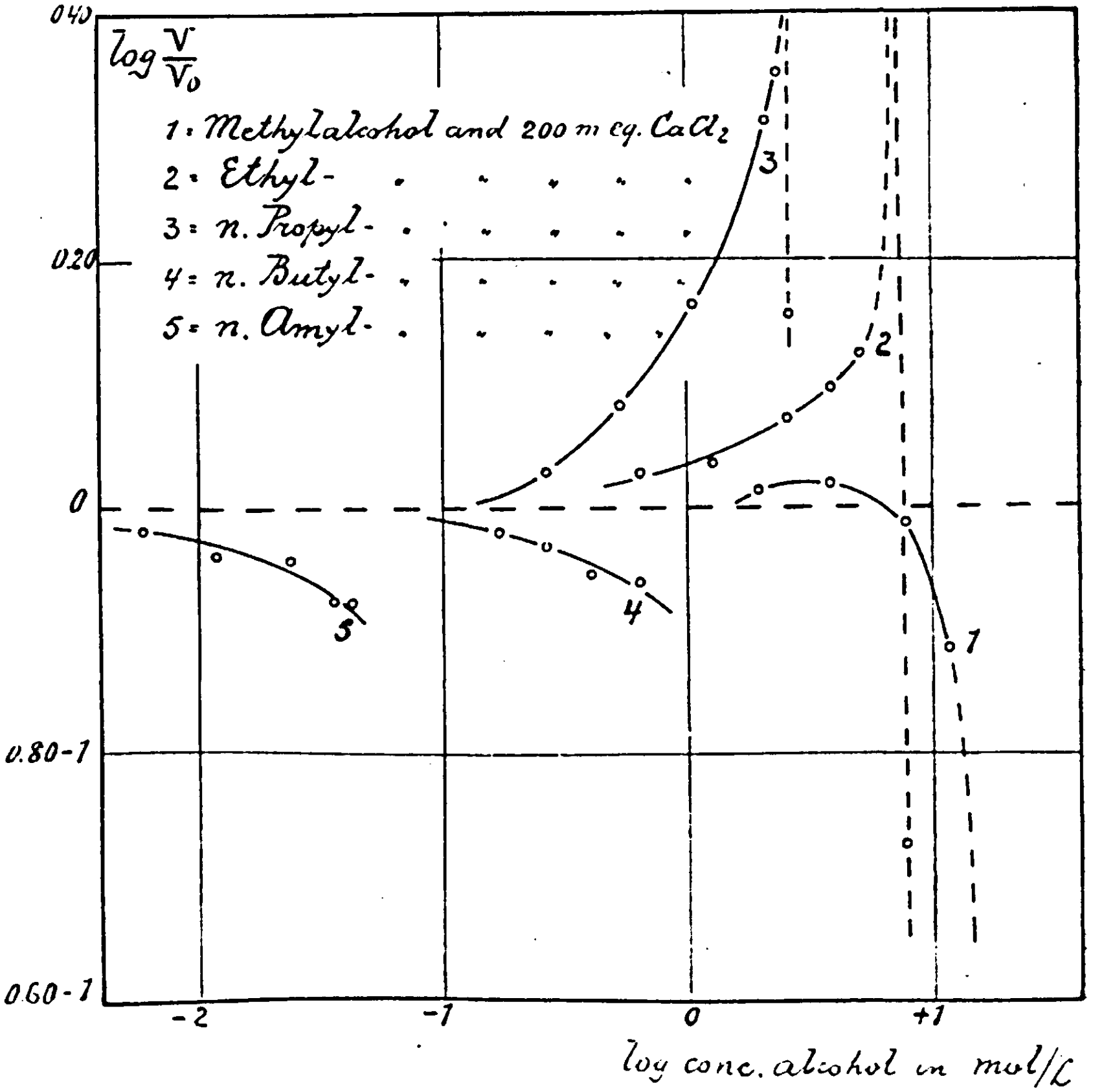
[37]) This fact needs not be in contradiction with the curve shown in Graph III and the discussion given at the place on the relation between charge-reversal of the system and form of this curve. The phosphatid coacervate discussed at that place has a higher density of charge (e.i. the number of charge-equivalents per gram of substance) and the electric factor may exert, therefore, a more prominent influence.

per molecule a stronger opening up action, but there will be found, starting from the same concentration of methyl- and n. propyl alcohol, per weight-unit of coacervate less methyl- than n. propyl alcohol. In consequence, the total effect of the n. propyl-alcohol is stronger than that of the methyl-alcohol.

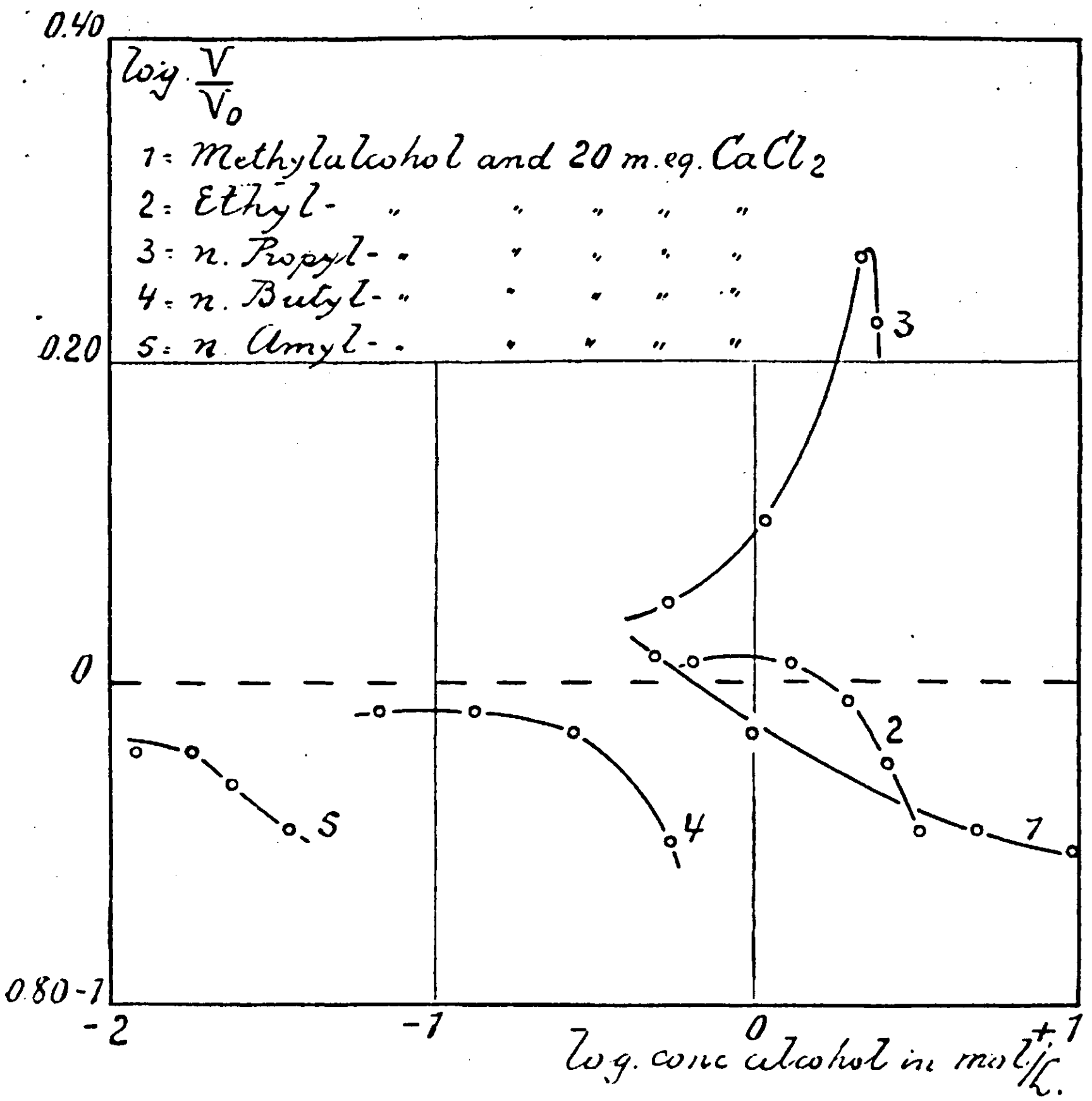
d. The curves for n. butyl and n. amyl alcohol do not suggest much discussion. In all systems they cause, as expected, a shrinkage. The shrinkage of the negative system is stronger than that of the positive system (graph X and XI), favoured as it is by the approach of the reversal of charge at increasing alcohol concentration.

On comparing the action of the alcohols, also in this system

Graph X.



Graph XI.



methyl alcohol is observed to have a less strong opening up action than ethyl alcohol, which in its turn opens less strongly than n. propyl-alcohol. Methyl-alcohol has a less pronounced opening up action for the negative system. This opening up happens only in the positive system, where the electric state does not oppose an expansion at increasing alcohol concentration.

Ethyl-alcohol already shows this opening up action in the negative system, but is even more pronounced in the positive system, because, in the latter case, the action is not counteracted by the electric state of the system.

N. propyl-alcohol exerts an opening up action at all the electric conditions of the system tested, while n. butyl- and n. amyl-

alcohol exert a condensing action.

Summarizing, we may say that the action of an alcohol on a condensed system depends on its influence upon the interaction of the carbon chains of the components of the system and on its influence upon the electric condition of the system.

The behaviour of the interacting C-chains of the system components is also influenced by the presence of sensitizers.

The stronger the system is sensitized, the stronger the opening up action, exerted by an alcohol (which action increases the distance between the components) may be.

The influence on the electric state is the more pronounced the closer the system is to its zero point of charge and the higher the electrolyte-concentration at which this zero point is situated, which means that this influence increases with the density of charge of the system.

CHAPTER III

The influence of alcohols on amorphous colloid-systems.

A. *Influence of temperature on amorphous and oriented systems.*

§ 1. *Description of the experiments.*

Chapter II dealt with the influence of sensitizers and charge on condensed systems. The significance of the condition of the system whether amorphous or directed will be discussed now, for it is conceivable that an oriented system shows an other behaviour than an amorphous condensed system. In Chapter II was observed that the more compact the system is, the greater the resistance is against proceeding condensation and the easier it will be opened. An oriented system is more compact than the amorphous coacervates and what has a condensing action on amorphous systems, may have an opening up action on oriented systems.

The influence of the temperature on both forms gave the first clue. When a sol is condensed to a coacervate at different temperatures [38]) in a special type of calibrated tube (see illustration),

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[38]) *Method:* x c.c. $CaCl_2$ of 500 m.eq./l + (5—x) c.c. distilled water is brought in the tubes which tubes are placed in the thermostat. To each tube is added 20 c.c. of phosphatid sol prepared as indicated in Chapter II (but not heated), which is brought previously to the same temperature as that of the liquid in the tubes. The mixture is shaken thoroughly and left for ½ hour after which each tube is shaken again.

we see that at temperatures below 35° C we find in the coacervate particularly in the neighbourhood of the point of zero charge a marked flocculation. At 35° C this flocculation disappears entirely. The temperature at which flocculation disappears depends upon the nature of the components of the system and of the concentration of $CaCl_2$. It is known from the work on soap coacervates that the flocculation which originates in the coacervate (for instance, by addition of an active sensitizer), often shows birefrigence which shows a transition from the amorphous to the oriented state. Even, when birefringence is not observed, we may assume an oriented system, for the intensity of birefringence depends upon the thickness of the oriented layer traversed by the polarized light. The flocculations which occur when a coacervate is slowly cooled, are indeed feebly birefringent, more or less oriented systems. We find, under certain con-

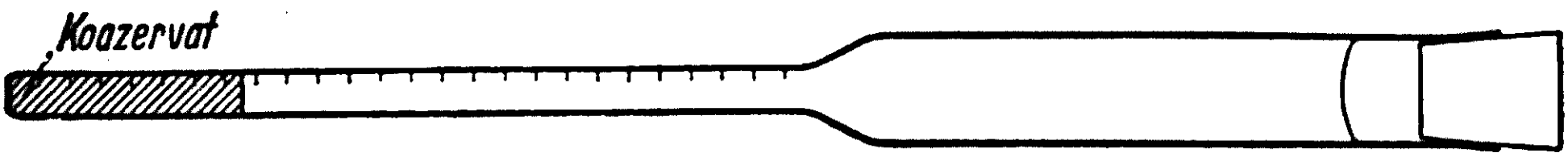

Figure II.

ditions, two co-existing phases of condensed systems.

The experiments were done as follows:

a. Series with increasing $CaCl_2$ concentrations at 25° C. 10, 15, 20, 25, 30, 35, 40, 50, 60, 80 m.eq. $CaCl_2$/l were used. The coacervate volumes were determined after 20 hours. After the treatment given under series c. readings were made every 3 hours.

b. The same series was kept at 40° C and the resp. volumes compared with those obtained in series a.

c. After reading the coacervate volumes at 25° C, the temperature was changed to 40° C and vice versa. The change of volume as a function of time was observed.

The results of these measurements are given in the accompanying graphs (p. 751 and 753) [39]).

§ 2. *Discussion of the graphs.*

From these graphs may be seen that:

a. Increase of temperature causes a decrease of coacervate volume and the disappearance of flocculation.

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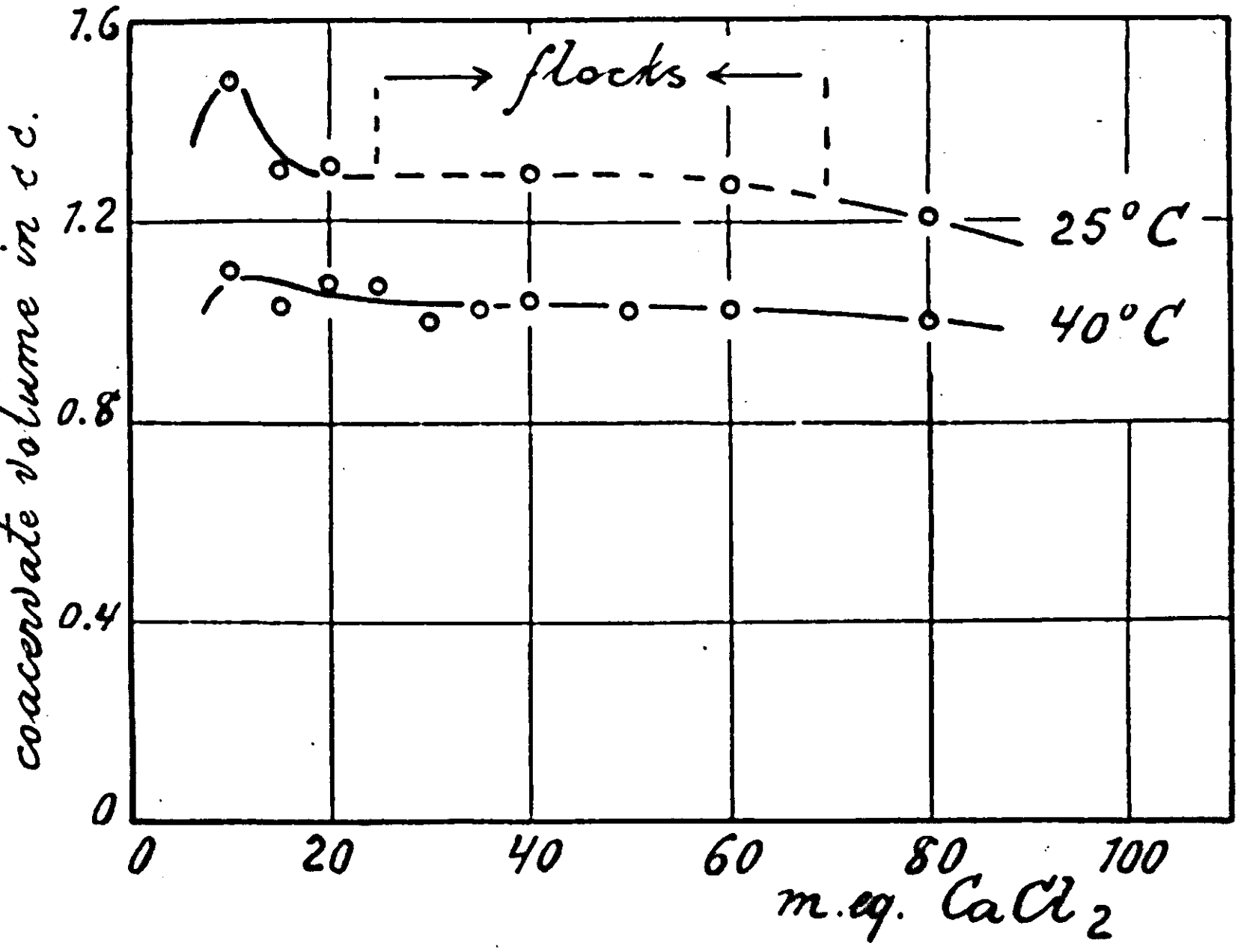
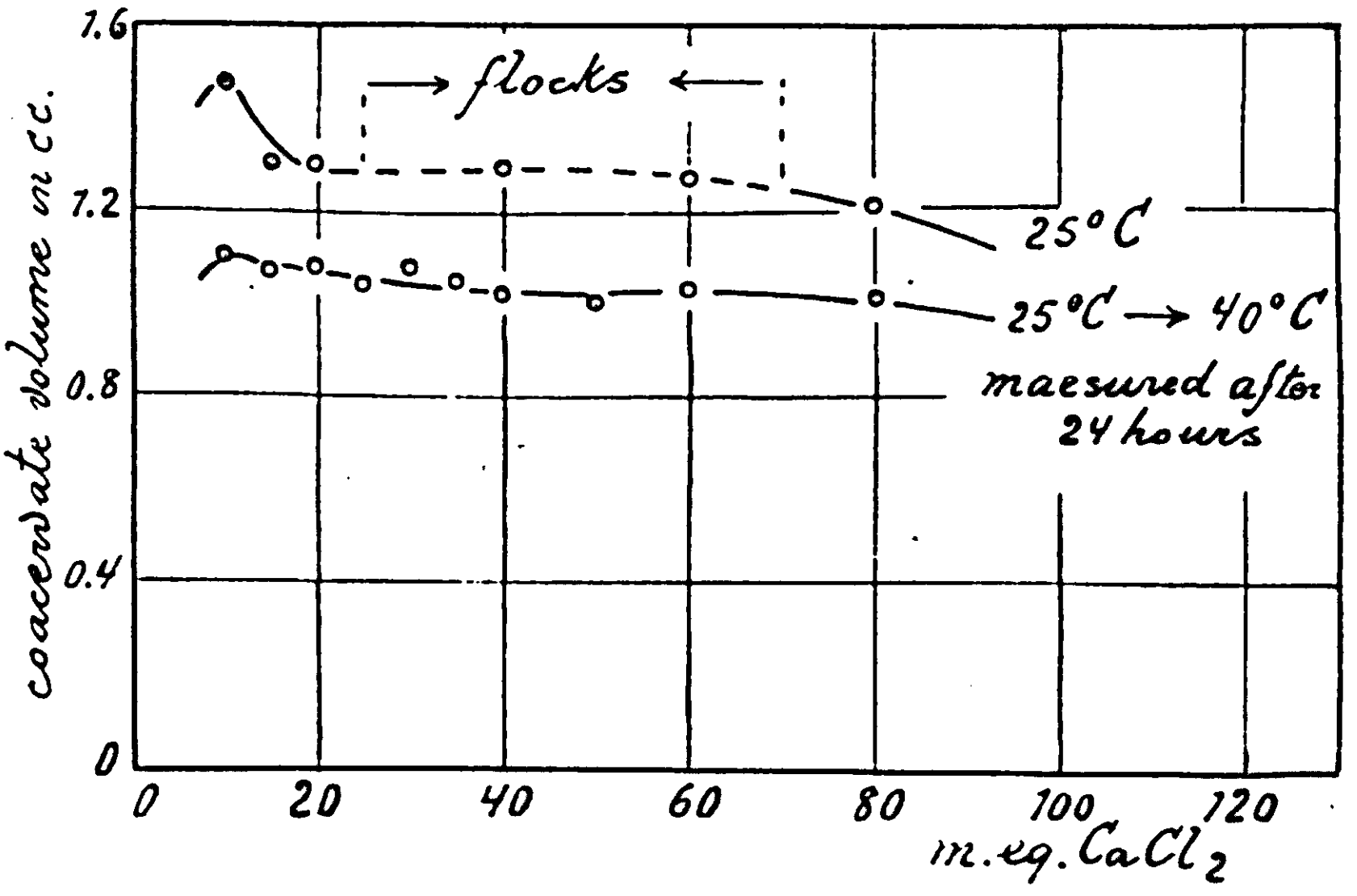
[39]) The deviations are caused by the adherence of the coacervate droplets to the wall of the vessel, preventing the droplets to coalesce to a layer. As these deviations prevent us to obtain a clear picture of the process, the method was abandonned.

Graph XII.



Graph XIII.



b. The temperature influence is fully reversible, if the coacervate is thoroughly shaken on transitions from one temperature to the other. If not shaken, the change in volume and the eventual change in state (amorphous to oriented), is much retarded. Especially the increasing volume on cooling is slowed down, while the solvate has to diffuse through a saturated top-layer. The decrease in volume on heating takes much less time, because vacuoles occur throughout the coacervate which are filled with the so-called equilibrium liquid (see Chapter II). These vacuoles coalesce forming large ones which ascend to the boundary coacervate/equilibrium liquid, having a low specific gravity and are, in this way, incorporated into the latter. The coac.-volume at 40° C averages 1,05 c.c. and at 25° C 1,30 c.c. (graph XII). After transfer and shaking the volumes are 1,05 c.c. (for 25° C → 40° C) resp. 1,20 c.c. (for 40° C → 25° C). Readings were made 24 hours after transfer and shaking (see graphs XIII and XIV).

c. At temperatures above 40° C the coacervate volume still decreases with temperature. At temperatures below 25° C the coacervate volumes still increase with temperature. The region in which flocculation occurs (conc. of $CaCl_2$ in which flocculation occurs), also increases with decrease in temperature. Graph XV gives a schematical representation of:

a. The volume changes of the coacervate as a function of the temperature.

b. The boundary of the region in which both forms of condensed systems are co-existent.

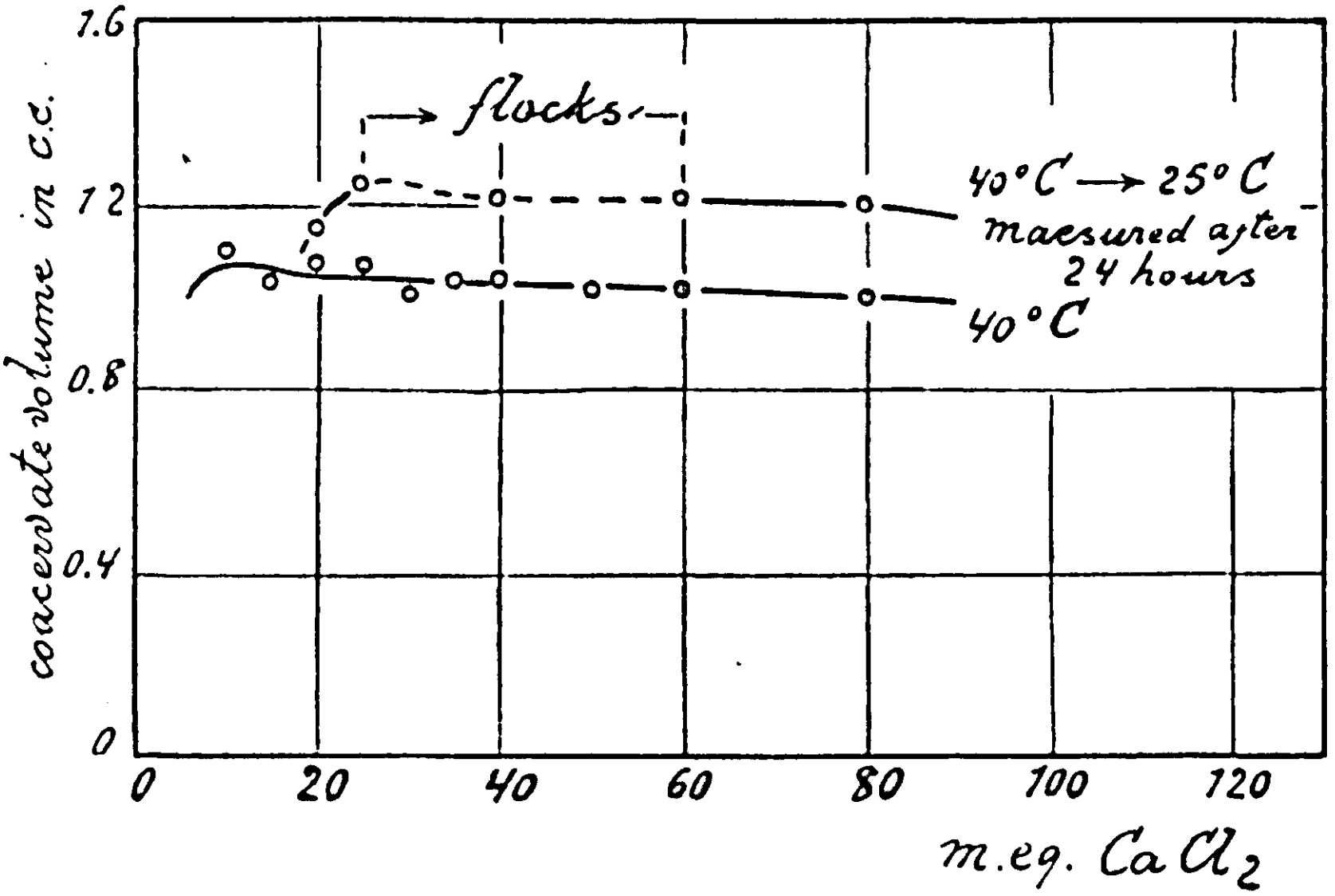
c. Decrease of the number of flocks with increasing temperature.

Decrease in volume of the coacervate means desolvatation, for increase in temperature causes a decrease in hydratation of the essential components. From volume changes of the flocky mass nothing can be concluded concerning its state of solvatation, for the volume of such a flocky mass is dependent on:

a. the way in which the flocks are accumulated;
b. the solvatation of the flocks;
c. the quantity of phosphatid that occurs as flock.

The last point still requires some comment. In Chapter II we saw, that our considerations concerning soap- and phosphatid coacervates started from the fact that the total quantity of colloid in the coacervate does not change markedly. Where this happens indeed, no conclusion can be drawn about solvatation of the

Graph XIV.



coacervate from volume-measurements, which holds good as well for the flocky mass in our coacervate. A volume-decrease at rising temperature does not mean a desolvatation of the flocks. They entirely disappear at rising temperature and pass on into coacervate. The quantity of phosphatid, present as a flock, markedly decreases at rising temperature. The improvised line for the volumes of the flocks has been indicated by a dotted line.

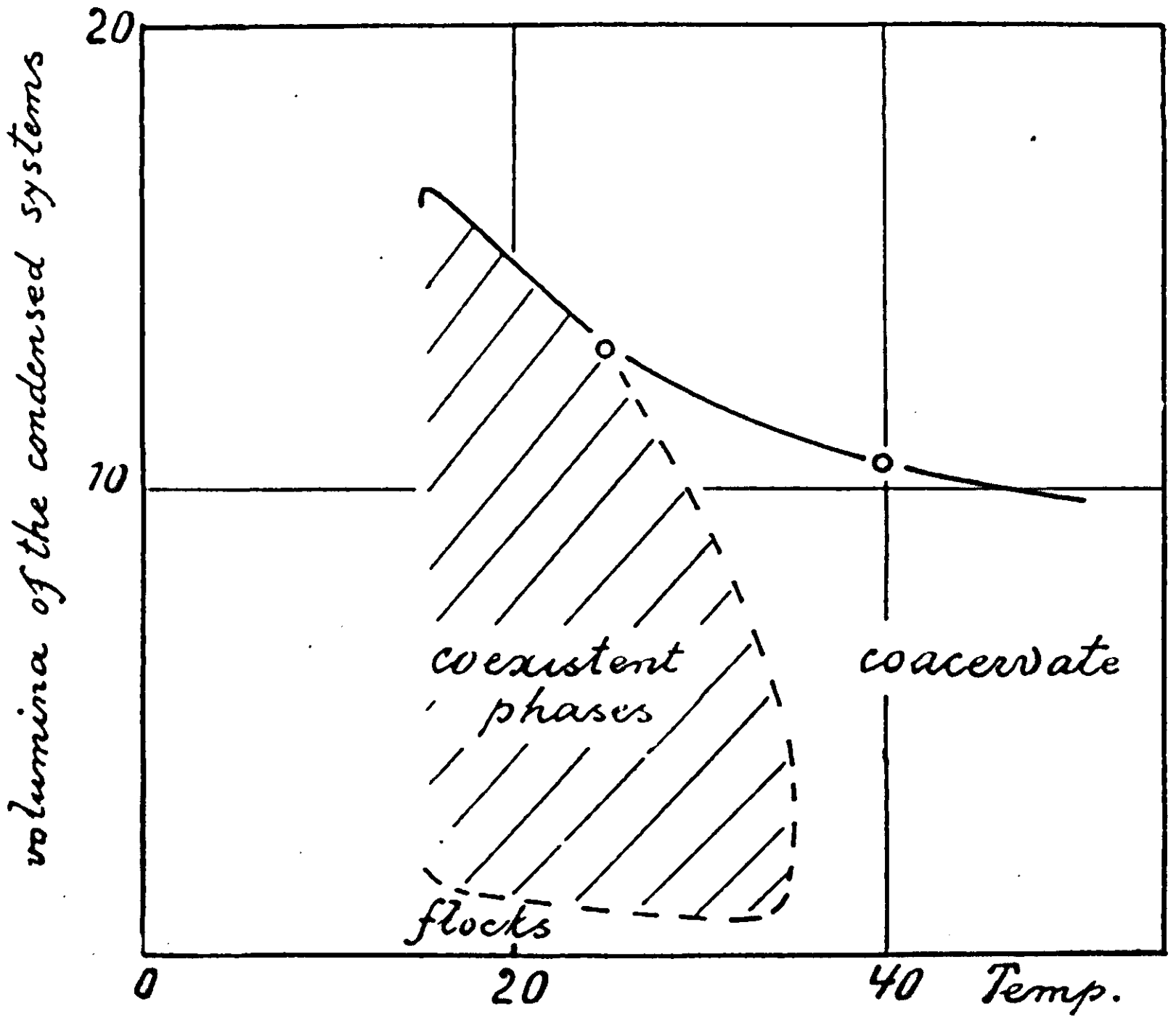
The kinetic energy of the constituents increases with the temperature and their orientation decreases. The intermolecular distance is enlarged and liquid may be incorporated. The system has become amorphous and changed into a coacervate.

The boundary region of both forms of condensed systems may be therefore important biologically, because of its many possibilities.

§ 3. *Description of oriented phosphatid systems.*

In phosphatid coacervates often originate highly birefringent spherules, if such a coacervate originates at a $CaCl_2$ concentration in the neighbourhood of its point of zero charge and is cooled from its temperature of formation (40° C) down to room

Graph XV.



temperature. After a few hours these birefringent spherules are then formed which may be observed with a hand-lens. They may be separated from the coacervate by centrifuging and washing with a solution of $CaCl_2$ and keep for weeks in the ice-chest.

Between crossed nicols in orthoscopic view the spherules proved to be sphaero-crystals.

The radial needles show straight extinction which is positive as shown by a gyps plate. The refractive index is larger than that of the coacervate. This index was determined using:

a. the method BRUN-KLEY.

Using parallel light (central cone) and the medium objective, a particle is focused and the tube is raised. If the luminous contour (BECKE's line) of the particle moves centripetally, the particle shows a higher refractive index than the medium. If, on raising the tube, the luminous contour moves centrifugally,

the medium shows a higher refractive index than that of the particle.

b. Method SCHRÖDER VAN DE KOLK.

Convergent light (large condensor cone) and a low objective is used. After focusing a finger of the left hand is moved underneath the condensor from left tot right, a shadow may be observed either at the front or at the back of the particle. A shadow at the front (space between particle and finger) means that the refractive index of the particle is higher than that of the medium. This method is not as reliable as the first one. The position of the condensor is of great importance. It is well to test the method with crystals and a medium with known refractive indices.

If two spherules make contact, even if their partitions disappear, both spherules retain their individual structure for, under crossed nicols, the two crosses remain. The spherules are often joined by birefringent fibres and at the margins myeline-tubes occur. The description of the structures will be given in an other article.

As might have been expected, the spherolytes loose their birefringence on heating. The temperature in which this phenomenon occurs within a given time, varies with the $CaCl_2$ concentration of the system and the presence of organic compounds. The possibility is given here to investigate the influence of chemical compounds upon oriented systems.

B. *Influence of alcohols on oriented systems.*

§ 1. *Description of the liquid crystalline oleate-system.*

The influence of various alcohols on directed systems was investigated with liquid crystalline systems [40]) of "Na-oleate: oleic acid".

As mentioned before, the so-called acid soaps — e.g. K-oleate at pH 7.8 — form birefringent systems. An attempt was made to experiment with this flocculent anisotropic mass. Acidification of the oleate was carried out subsequently with boric acid, biborate, $KHCO_3$ and in a more systematic way, with $NHCO_3$ — Na_2CO_3 buffers.

The latter method was sucessfull. In a pH region 9,6—10,0

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[40]) As to opinions upon nature and definition of liquid crystals, we refer to Zeitschrift für Kristallographie, Bd. 79, 1931, and to Handwörterbuch der Naturwissenschaften, Bd. 5, p. 1161. The occurrence of oriented systems in living organisms is treated in Protoplasma-monographs No. 11, 1937. Compare also H. FREUNDLICH — Kapillarchemie Bd. II, p. 690, 1932.

with NaCl striking anisotropic needles of liquid nature [41]) appeared.

The buffers serve to obtain a suitable proportion oleate : oleic-acid. The oleic-acid serves as sensitizer. The needle shape of the crystals means that the molecular directive forces exceeds the interfacial tension. Contact of two needles causes their parallel direction and their sudden co-alescence (like liquid drops) without changing their crystalline character. Accretion occurs parallel to the longitudinal axis of the crystal which may be related to the structure of the liquid crystal. If two needles are perpendicularly in contact no co-alescence occurs. The crystals are positive, uni-axial and show a refractive index higher than that of the medium. The liquid crystalline needles disappear on heating and in excess NaCl. Cooling, or lowering of the NaCl concentration causes them to reappear.

§ 2. *Description of cloudiness measurements.*

The disappearance of the needles, so called extinction of the system, may be measured with the extinctometer of MOLL which gives the percentual light absorbtion of a suspension. These values we shall call cloudiness. This cloudiness depends upon both size and number of the needles. Both magnitudes, varying continually, all samples have to be treated in exactly the same way in order to obtain reproducible results. The series has to be started simultaneously, every flask has to be shaken in the same way and has to be measured after the same settling time. In our series measurements were made a half hour after the introduction of the Na-oleate solution. The measurement takes 90 seconds, washing of the cuvets included. The introduction of soap solution

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41) recipe: The needles are obtained as follows: a 0.05 mol. solution Na-oleate (Na-oleinicum medicinale pur. pulv. — Merck) serves as stock-solution. 4 c.c. portions of this solution are pipetted into steamed flasks of 50 c.c. containing 10 c.c. buffer + x c.c. NaCl of 4 N. + (36—x) c.c. H_2O dist. After addition of the oleate the total volume will be 50 c.c. In one series the pH is constant within ± 0.3 unit. The mixture is shaken immediately after addition of the soap solution. At pH = 9,73 and 4 c.c. NaCl 4 N after one minute fine needles may be observed under the microscope, too small to show anisotropy. Fast growth of the crystals enables us to observe anisotropy after 5 minutes. After 10 minutes the needles are coalesced in patterns.

Data of KOLTHOFF were used to prepare a graph in which the abscissa represents the number of c.c. Na_2CO_3, 0.2 mol. present in a buffer mixture of 10 c.c. (x c.c. Na_2CO_3 0.2 mol. + (10—x) c.c. $NaHCO_2$ 0.2 mol.). pH is given on the ordinate. Measurements of the pH with the glass electrode confirmed the data obtained graphically.

has to take place in intervals of 90 seconds in order to be able to measure every sample after half an hour.

The table gives relation of the cloudiness at the given pH as a function of NaCl concentration. In order to investigate the influence of the alcohol on the disappearance of the liquid crystals, a fixed pH of 9,73 was chosen. The system is liquid and reproducible. The disappearance of the liquid crystals by an excess of NaCl, may be accounted for by the protective action of the anions of the electrolytes added. Alcohols in increasing

TABLE III

Series with $NaHCO_3$—Na_2CO_3 buffer

pH 9.47 **Temp. 17.5° C.**

No.	Na in m.eq./l	Cloudiness measurement after ½ hour
1	320 + 103 = 423	83.00
2	640 + 103 = 743	87.10
3	960 + 103 = 1063	83.00
4	1280 + 103 = 1383	77.00
5	1600 + 103 = 1703	66.00
6	2080 + 103 = 2183	58.00

pH 9.62 **Temp. 17.5° C.**

No.	Na in m.eq./l	Cloudiness measurement after ½ hour
1	320 + 107 = 427	73.00
2	640 + 107 = 747	87.00
3	960 + 107 = 1067	84.00
4	1280 + 107 = 1387	80.00
5	1600 + 107 = 1707	73.00
6	2080 + 107 = 2187	62.00

pH 9.73 **Temp. 17.0° C.**

No.	Na in m.eq./l	Cloudiness measurement after ½ hour
1	320 + 112 = 432	70.50
2	640 + 112 = 752	68.00
3	960 + 112 = 1072	71.50
4	1280 + 112 = 1392	75.00
5	1600 + 112 = 1712	75.25
6	2080 + 112 = 2192	77.50

	pH 9.80	Temp. 17.0° C.
No.	Na in m.eq./l	Cloudiness measurement after ½ hour
1	320 + 116 = 436	66.25
2	640 + 116 = 756	79.00
3	960 + 116 = 1076	74.00
4	1280 + 116 = 1396	74.00
5	1600 + 116 = 1716	78.00
6	2080 + 116 = 2196	68.00

	pH 9.90	Temp. 17.0° C.
No.	Na in m.eq./l	Cloudiness measurement after ½ hour
1	320 + 120 = 440	32.00
2	640 + 120 = 760	61.00
3	960 + 120 = 1080	52.25
4	1280 + 120 = 1400	61.00
5	1600 + 120 = 1720	63.00
6	2080 + 120 = 2200	73.00

concentration cause the disappearance of the system [42]).

The cloudiness decreases and reaches zero value with increasing concentration of alcohol. A solvatation and final desintegration similar to that treated in Chapter II for the influence of alcohols on coacervates may account for this phenomenon (the small distance between the components in the directed system may exert its influence as we shall see later on). As chemical analyses is too coarse and x ray analyses only yielded the water diagram, no direct method could be used to determine the degree of solvatation as a function of the alcohol concentration.

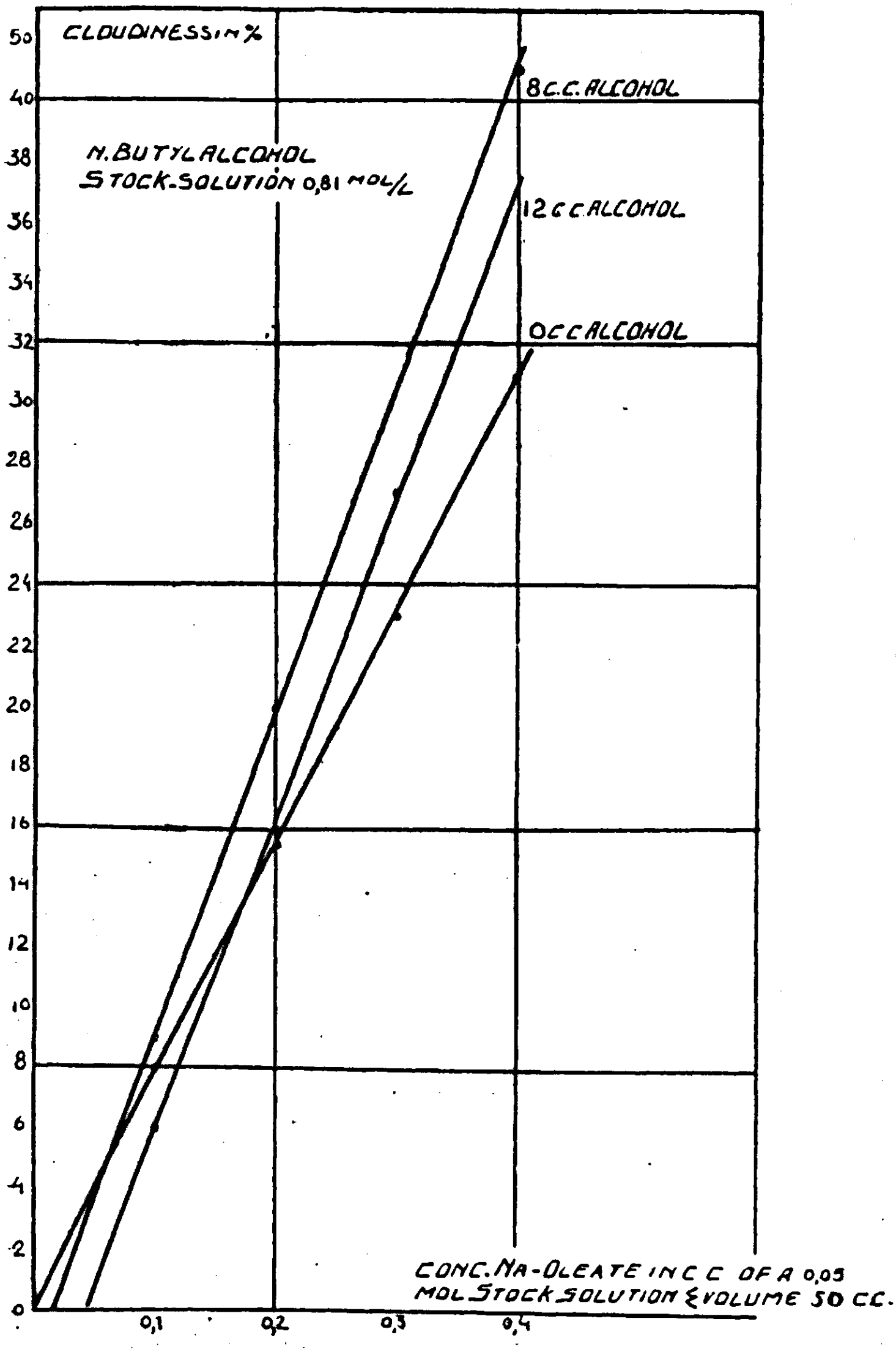
§ 3. *Indirect measurement of the solvatation of oriented oleate-system.*

Finally, the following method was used to test the above account of the phenomenon. Soap coacervates almost identical in composition with that of the liquid needles (different only by lower oleicacid-content and the presence of K) show symbate relation of the solvate in the soap phase and the soap-content in the "equilibrium liquid". The validity of this relation may therefore

[42]) Recipe: 4 c.c. soap solution + 10 c.c. buffer + x c.c. NaCl + y c.c. alcohol + 36— (x + y) c.c. dist. water. Total volume is 50 c.c.

also be expected for liquid crystalline systems. The analyses of the soap-content in the "equilibrium liquid" may be a measure of the solvatation of the needles. The soap concentration, being

Graph XVI.



too small for chemical analyses (4 m. mol.), an indirect measure has to be used. The cloudiness caused by increasing soap concentration under similar conditions [43]) is measured. The relation is given in the accompanying graph XVI. The points are situated on a straight line through the origin. This means that the lowest soap concentration causes cloudiness which means that liquid crystals are formed. Similar lines may be constructed after the addition of a constant amount of alcohol. The straight line intersect the abscissa at a distance x of the origin. This means that only soap concentrations larger than x lead to the formation of liquid crystals. Before this crystalline system is formed, the equilibrium liquid has to contain a certain soap concentration. This concentration increases with the increasing alcohol concentration, as is shown in the graph for n. butyl alcohol. Inversily, the conclusion is warranted that the solvatation of the crystalline phase increases with the concentration of the n. butyl alcohol, until not only the orientation of the soap molecules is lost, but the whole system disintegrated. It appears from the above that normal butyl alcohol exerts an opening up action upon liquid soap crystals, while it has the opposite action on soap coacervates. Inasmuch as n. butyl-, methyl-, ethyl- and n. propyl-alcohol show a decreasing cloudiness of the system in increasing concentration, we might conclude that these substances exert an opening up action.

§ 4. *Discussion of the influence of alcohols upon the oriented oleate system.*

From the graphs we see that:

a. Methyl-, ethyl-, n. propyl- and n. butylalcohol destroy the liquid crystalline systems. They, therefore, exert an opening up action on oriented systems. On amorphous systems n. butanol exerts an opposite action (Chapter II) like n. pentanol. The latter substance most probably also shows an opening up action on oriented systems, which could not be traced far enough because of its low solubility (graph XVII).

b. Higher NaCl concentration requires a higher alcohol concentration to destroy the crystalline system (graph XVIII).

c. The same pertains to increasing soap concentrations (graph XIX).

Summarizing, we may formulate the results as follows:

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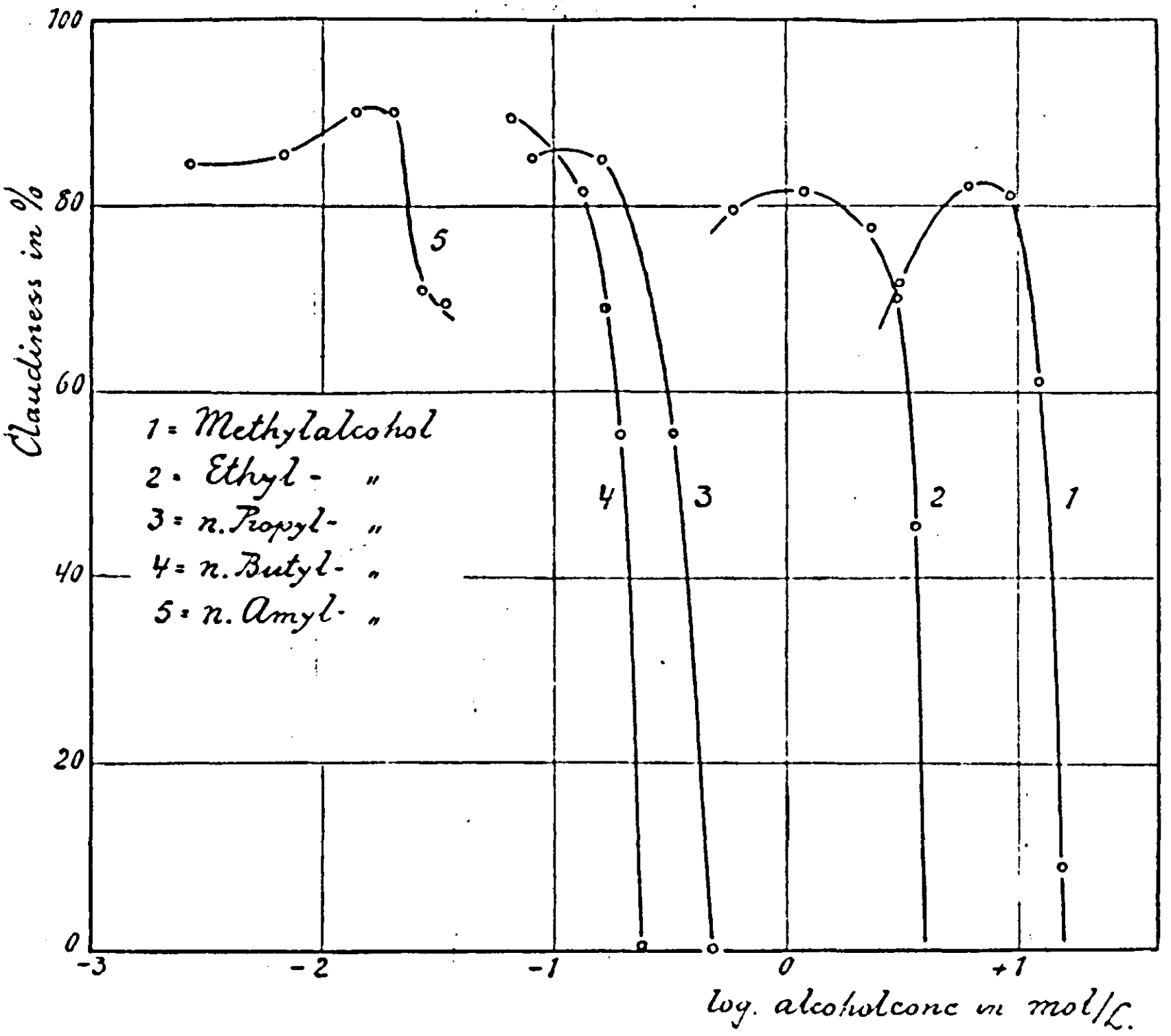
43) Constant concentration NaCl and constant pH = 9,73.

Graph XVII.



a. amorphous and oriented systems show a different reaction in relation to chemical compounds.

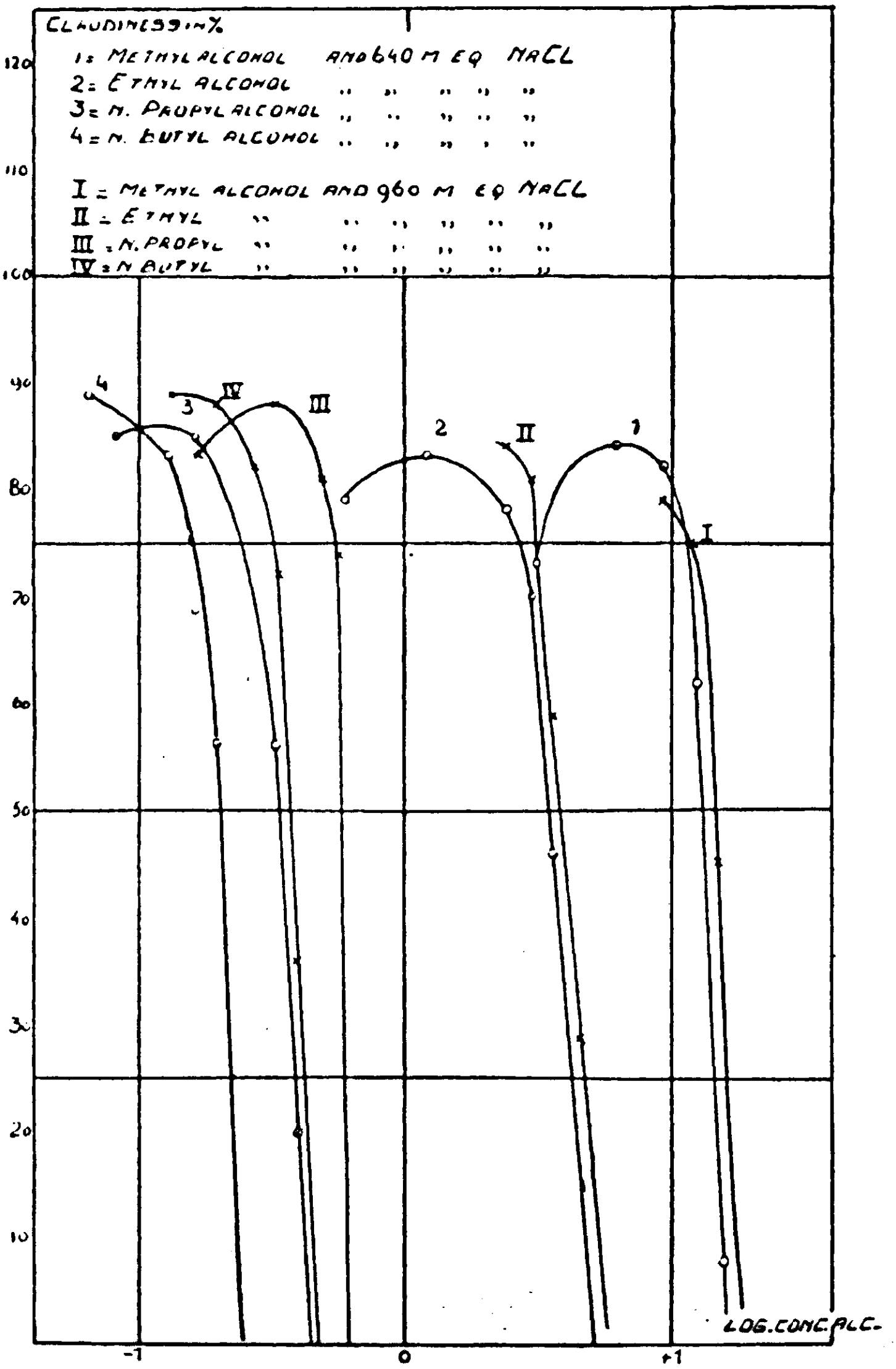
b. the distractive action of alcohols shows qualitatively an analogon with TRAUBE'S rule.

c. the resistance of the system against alcohols and temperature increases with the NaCl concentration within the region of the system.

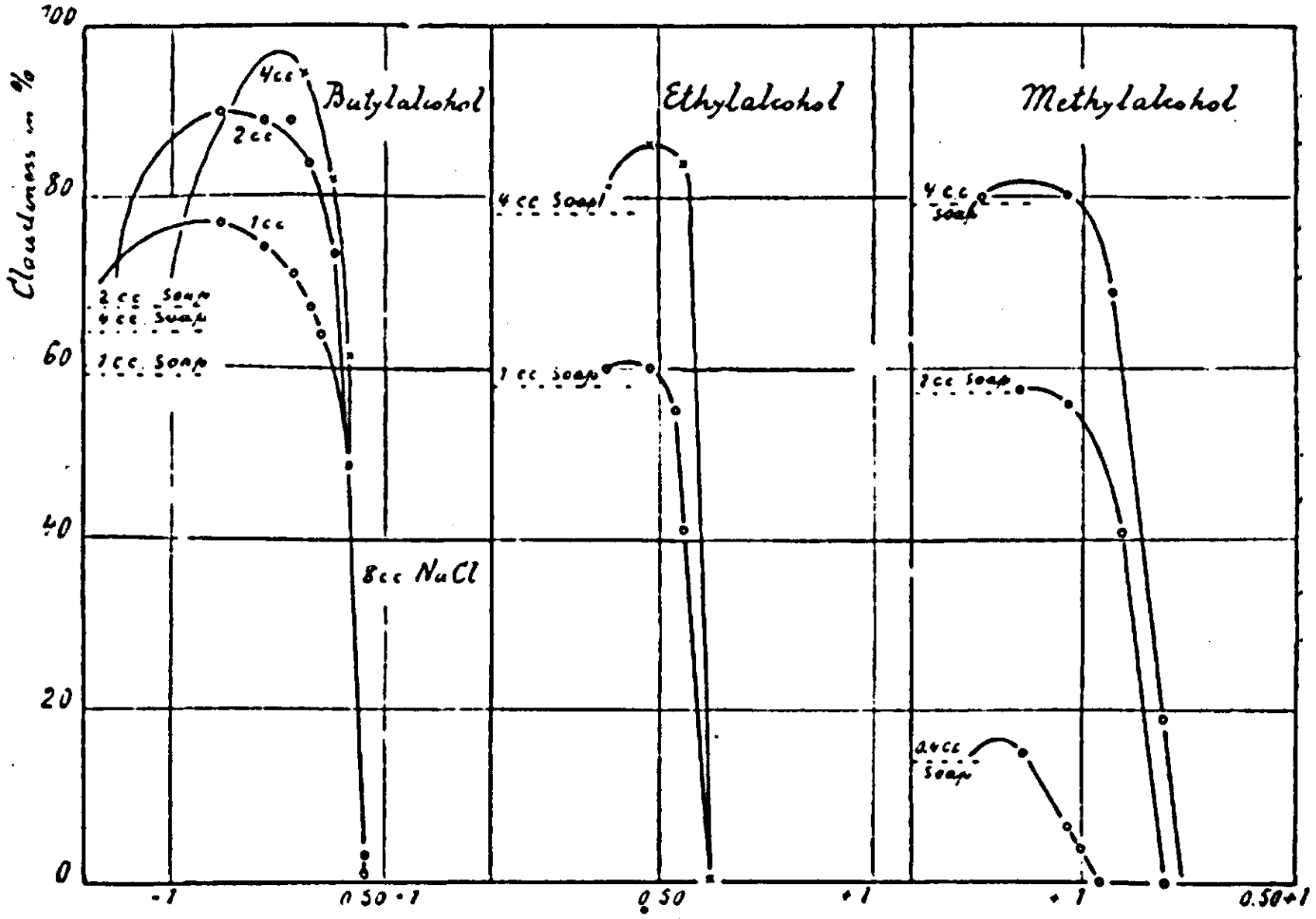
The validity of this rule appears from the fact that addition of NaCl to a system which had been previously destroyed by an alcohol, will cause its reappearance. A system originated in a final concentration of 1,28 N NaCl still persists at 80° C, while a similar system at a final concentration of 0,64 N NaCl is destroyed at this temperature.

d. a higher soap concentration requires more alcohol for

Graph XVIII.


CLAUDINESS in %
1 = METHYL ALCOHOL AND 640 M EQ NACL
2 = ETHYL ALCOHOL " " " " "
3 = N. PROPYL ALCOHOL " " " " "
4 = N. BUTYL ALCOHOL " " " " "
I = METHYL ALCOHOL AND 960 M EQ NACL
II = ETHYL " " " " " "
III = N. PROPYL " " " " " "
IV = N BUTYL " " " " " "
120
110
100
90
80
70
60
50
40
30
20
10
4
3
IV
III
2
II
1
I
-1
0
+1
LOG. CONC. MOL.-

Graph XIX.



equivalent action (equal cloudiness means equal degree of desintegration). In the higher homologues this effect is less pronounced.

Point a is of importance for me and can be interpreted as follows:

C. *Discussion.*

On comparing the results of the research of the influence of alcohols upon oriented oleate systems with those upon oleate coacervates, the reversal in behaviour of n. butylalcohol and n. amylalcohol is striking. In Chapter II the influence upon the action of alcohols was considered by addition of a sensitizer. The denser an oleate system is, the easier the opening up action of an alcohol is (the relative volumes $\frac{V}{V_o}$ increase more quickly as a function of the alcohol concentration) and the more difficult the condensing action ($\frac{V}{V_o}$ decreases more slowly with the alcohol concentration). As an oriented system is more compact than coacervates, the reverse action of n. butylalcohol and n. amylalcohol is conceivable on the basis of the above-mentioned prin-

ciple. The action of alcohols upon a system does not only depend of its constitution, but also of the nature and state of the system. For n. butylalcohol, on account of what is known about soap coacervates (thus about amorphous systems), a shrinking action should be expected. In these amorphous systems the particles move in respect of each other more freely than in oriented systems. In amorphous systems n. butylalcohol may act as a cohesion intermediary, as it restricts the movement of the components of the system. In a directed system the molecules are by nature less mobile and n. butylalcohol can only increase the mobility. In, for instance, the liquid crystalline system of "oleate : oleic-acid", carbon chains of 18 C atoms are in direct, non-intermediate, interaction. If n. butylalcohol molecules interfere in these carbon chains, the direct interaction is broken and takes place via the C chains of the butylalcohol, that is to say via chains of 4 C atoms, which are in respect of one another relatively free. Therefore the orientation of the system can only decrease.

A compound as n. butylalcohol which may bring the components closer together in an amorphous system, may, show just the opposite action in an oriented system.

D. *Liquid crystals as a model for the protoplasmic membrane.*

The above account for the liquid crystalline needles is of importance for physiology. The following conclusions are drawn by CHAMBERS [44]) from coalescence [45]) experiments with unfertilized Arbacia eggs and with oil droplets: "the fact that there are so many degrees of apparent fluidity in cell surface as shown by our penetrating oil drop data, suggests the improbablity of a crystalline cell surface. It is more probable that the cell surface has the characteristics of an amorphous solid potentially pos-

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44) CHAMBERS and KOPAC — Journ. of Cellular and Comp. physiology 9, 331, 1937.

45) This author implies with coalescence the penetration of oil droplets into eggs under certain conditions. The egg behaves in this process as a liquid sphere. Contact of an oil droplet and an egg represents a system of two liquid spheres. The occurrence and the mode of coalescence depends upon the interfacial tension of the interface egg/medium and oil/medium which we shall indicate by T 2/3 and T 1/3 resp. The principle of minimal free energy is realized in the case of coalescence. If T 1/3 $<$ T 1/2 $<$ T 2/3, the oil droplet incorporates the egg, provided that the resp. volumes show a suitable proportion. If, however, T 2/3 $<$ T 1/2 $<$ T 1/3, the egg absorbs the oil droplet. If the egg behaves like a solid body, no coalescence takes place, but the so-called "capping" occurs. The oil droplet is flattened against the egg and decreases in this way the total free energy. This phenomenon was observed in Amoeba dubia and in eggs of Asterias.

sessing many degrees of state".

Summarizing, the results of his experiments CHAMBERS states that wherever coalescence occurs "the physical state of the cell surface is essentially that of a liquid".

Where the so-called "capping" occurs "the cell surface is solid".

HARVEY [46]) concludes from centrifuge experiments with Arbacia eggs, showing clean fragments by centrifugal force, that the protoplasmic membrane shows a liquid character.

KOPAC [47]) protected oil droplets by means of a plastic membrane and could not observe coalescence of these droplets. Coalescence of two spherical bodies seems, therefore, to imply the assumption of an amorphous liquid surface film.

This conclusion seems to be unwarranted, when we take into account the behaviour of liquid crystals in thin liquid condition. Liquid crystals of Na-oleate coalesce easily and — like Arbacia eggs and oil droplets — suddenly on contact. If the crystal has increased in size by coalescence, the state passes from liquid into plastic. This transition may also be caused by other factors. The liquid crystals may be compared, in this respect, with amorphous solid substances, which on heating changes from highly viscous to thin liquid. This change in viscosity requires a certain amount of heat. A liquid Na-oleate crystal, however, may be changed from the liquid into the plastic stage or vice versa, without measurable heat exchange. The phosphatids may be also obtained in a birefringent state in which the molecules are, therefore, directed, while the substance still shows a liquid character (myelins).

CHAPTER IV

Phosphatids as membrane components.

A. *Arguments for the assumption of phosphatids as protoplasm-membrane-components.*

Chapter II and III dealt with the influence of alcohols on systems of oleate and phosphatids + sensitizers. In Chapter II it was said that the research should take place on systems of those colloids which may be assumed as membrane components.

Certain data seem to point to the possibility of phosphatids being component-parts of the membrane, which only means

46) HARVEY — Journ. of Cellular and Comp. physiology 8, 251, 1936.
47) KOPAC — Biol. Bulletin 71, 398, 1936.

that phosphatids constitute an integral part of this membrane.

a. The first indication as to the possible role of phosphatids may be deduced from BUNGENBERG DE JONG's experiences with coacervates of bio-colloids. This author pointed out the possible change of water-permeability by the addition of ions as a function of the concentration of these ions. If, for instance, a sol is condensed into a coacervate by the addition of a certain quantity of a suitable salt, in general the addition of more salt may cause the disappearance of the coacervate [48]). As pointed out in Chapter II the colloidal particles [49]), which in a sol repel each other mutually as if in the gaseous state, condense by the addition of a suitable salt in low concentration. If a coacervate originates, its condensation may proceed by the addition of a little more salt; "equilibrium liquid" is excreted which may be seen in the vacuoles originating in the coacervate [50]). The coacervate condenses and contains less solvent. By the addition of still more salt, the coacervate starts taking in so much solvent that the colloidal particles are dispersed and again reach their gaseous state. The coacervate has disappeared.

For physiology this point of view is of importance. A continuous desolvatation means that the coacervate gets a lower percentage of water. If water has to diffuse through such a system, the resistance to be overcome is greater according to the decrease of the percentage of solvate of the system. At the disintegration of the coacervate the reverse takes place, as the percentage of solvate increases. These ideas lead to a curve with a minimum, if in experiments on organisms the permeability to water is plotted against the ionic concentration [51]). At first, at an increasing ionic concentration the permeability for water decreases, and again increases if the increase of the ionic concentration continues. In fact, DE HAAN [52]), using cells of Allium Cepa, obtained such curves, the minimum of which are in the

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[48]) The disappearance of the coacervate already formed should be distinguished from the sol treated with an excess of salt and which thereby is prevented from forming a coacervate.

[49]) Whether these particles are thought of as micellae or as molecules, seems irrelevant. In general, it may be advisable to take molecules here as kinetic units.

[50]) H. G. BUNGENBERG DE JONG — La coacervation, les coacervats et leur importance en biologie — Tome I et II — Exposés de Biologie VI et VII 1936. Hermann et Cie, Editeurs, Paris.

[51]) As most of the membranes are negatively charged, a curve as described above may be expected where the cationic action prevails over the anionic action.

[52]) I. DE HAAN — Protoplasma 24, 186, 1935.

same region of salt concentration as the zero charge of the phosphatid coacervates. For phosphatid coacervates reach their zero charge, dependent upon the mode of preparation, between 10—100 m. aeq. $CaCl_2$. The zero charge nearly coincides with the minimum hydratation.

In this respect, the membrane acts as a coacervate, or more accurately, as a coacervate that reacts upon *small* differences in ionic concentration, as physiologically only a relatively small concentration range may be tested. Coacervates, consisting of non-amphoteric lipoids, cannot react structurally upon these changes in concentration — because, under usual physiological conditions, they are far removed from their zero charge and their charge increases only slightly with the salt ($CaCl_2$) concentration, — like the biological object does.

If, on the contrary, a coacervate is taken, chiefly consisting of amphoteric lipoids (or more specially of phosphatids, as the zero charge of proteins is too high), it reacts in the same way as the biological object. However, a system consisting of pure phosphatids, even with many Cephalins, would react within too narrow limits of ionic concentration, for purified phosphatids reach their zero charge at too low cationic concentration. As is known from colloid-chemistry, a phosphatid coacervate cannot originate from a sol by the addition of a salt without adding "sensitizers".

Also coacervates of proteins, nucleinates and arabinates may be sensitized by acetone and ethylalcohol, but phosphatid coacervates are, moreover, strongly sensitized by relatively water-insoluble substances, accompanying the phosphatids in nature. As such may be mentioned cholesterol, trioleïn, fatty acids, as far as they are not dissociated. A coacervate, consisting of phosphatids, sensitizers, the phosphatidic and fatty acids mentioned above and ions, reacts on changes in cationic concentration like the biological object tested by DE HAAN, and may have the charge of the protoplasmic boundaries. This does not mean that the protoplasmic membrane is a phosphatid coacervate, but only that amphoteric lipoids must be integral components of the membrane, if it is to act according to DE HAAN's observations [53]).

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[53]) The theory developed above for condensation into a coacervate and annullation of the same, was only tested later in regard to the water percentage to be expected for phosphatid coacervates. Now we have succeeded in measuring this water-percentage on the volumes of phosphatid coacervates. Plotting volumes against cationic concentration, minimum curves are obtained.

It is difficult to assume that the protoplasmic membrane should be a coacervate, as the latter, on account of its too-high percentage of solvent, should not offer sufficient resistance to the water-permeation. The permeability to water is known to be so small that a very hydrophobic system has to be assumed as a regulator of the water-permeation. It is known that a phosphatid coacervate, when becoming poorer in solvent, may change into a birefringent oriented system. Such an oriented system is hydrophobic enough to be able to function as a membrane.

b. If the protoplasmic membrane is such a system, the charge on the interface is of great importance for its structure. The smaller the non-compensated surplus of the charge, the more compact the molecular packing and consequently the less permeable for lipophobic substances the membrane is. If the non-compensated surplus of the charge increases in the positive or the negative direction, the molecular packing will become looser, as the components are going to repel each other as a result of a greater equally directed charge [54]). This means in our theory that the system becomes richer in solvent.

The more compact the structure of such a system, the more sensitive it is to relative changes in the mutual distance between the components. The phosphatidic systems have a small density of charge [55]), as the pure phosphatids are electrically almost neutral. (The charge of the system is mainly to be ascribed to fatty- and phosphatidic acids present). Moreover, on account of specific properties of the phosphate group they reach their zero charges more easily than components with Carboxyl groups [56]). Therefore, the phosphatid systems in question easily change their electrical condition and, consequently, their structure under the influence of cations.

The charge of the membrane, therefore, is not only of importance — by its sign and its amount — for the ionic permeation, but also — by its influence on the molecular packing of the membrane — for the passage of non-electrolytes. Assuming that a non-amphoteric lipoid, e.g. an oleate, forms the membrane,

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54) As appeared from Chapter II, other factors may prevail through which, however, decrease of the charge-surplus does not coincide with increase of the density.

55) With density of charge we mean the number of charge-equivalents per surface-unit of the particle, or more accurately, per unit of weight. If all particles have the same size and composition, it is also a measure for the number of equivalents per surface-unit of the particles.

56) P. H. Teunissen — Lyophiele niet amphotere bio-colloïden beschouwd als electrolyten — Thesis, Leiden 1936.

thus assuming an oriented system of oleates this system cannot, therefore, react in regard to the water permeation, like the protoplasmic membrane, on such small changes of the cationic concentration of the milieu. Such an oleate system reacts slowly on changes of the cationic concentration of the milieu, both because of its density of charge and because of the nature of its ionogenic parts (Carboxyl). The same rules hold for a system consisting of oleate and oleic-acid, the so-called acetous soaps.

c. Based on the supposition that H^+ reacts relatively more strongly on ampholytes than other ions, a great influence of pH may be expected on phenomena of permeability. Experimental data, however, are not at my disposal.

To my knowledge only the influence of pH has been measured on erythrocytes, i.e. for positive membranes.

d. The sensitiveness of phosphatid coacervates to all sorts of influences make these phosphatids pre-eminently suitable as a membrane substance.

e. The molecular structure of the phosphatids lends itself well for the structure of a stable membrane.

DANIELLI [57]) has tried to estimate the stability of various thin membranes — so-called films between two aqueous phases — composed of lipoids to which proteins may be adsorbed. If, on the basis of capacity-measurements, the maximal thickness of the membrane is calculated to be 60 tot 80 Å and the minimum 30 Å, this membrane must be 1 tot 5 molecules thick, taking into consideration that the molecules of the membrane, if they are to maintain themselves as a membrane in an aqueous milieu, have to be very water-insoluble and consequently, rich in C and therefore large. As the cell, without changing its permeability, may enlarge the surface at osmotic expansion, the protoplasmic membrane must be more than 1 molecule thick. Under natural circumstances it cannot have its minimum thickness, as enlargment of the surface cannot be thought of without laesion, because it has hardly any elasticity [58]).

Discussing the membrane-stability, DANIELLI suggests as building blocs molecules with 1 or 2 polar groups (hydratation centra), symbolically represented resp. as —o and o—o. The 6 possible combinations are symbolically represented below.

Cases b, c, d and e may contain less oriented layers and may therefore be thicker than 2 molecules. Moreover, DANIELLI assumes that the film always adsorbs proteins as covering layers.

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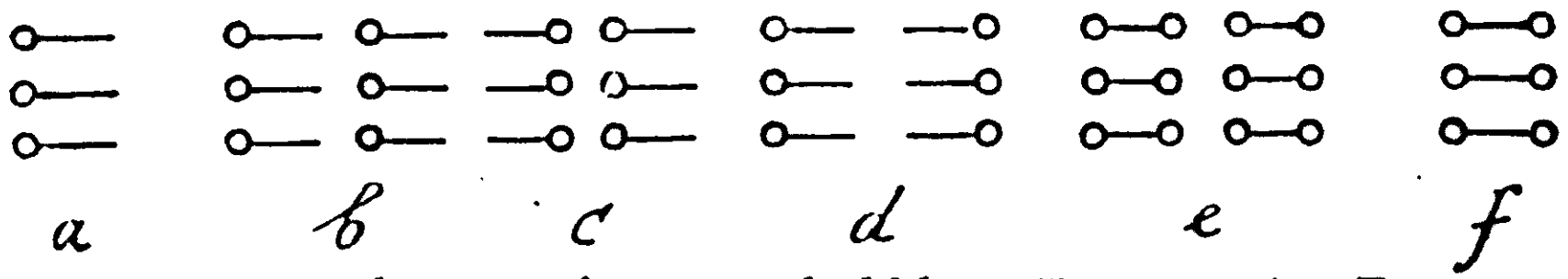
57) J. DANIELLI — Journ. of Cell. and Comp. Physiol. 7, 393. 1935/36.
58) E. N. HARVEY — Journ. of Cell. and Comp. Physiol. 4, 35, 1933.

Figure III



Case c is known for soap-bubbles (LAWRENCE). Between two aqueous phases, however, this structure is unknown.

Cases a and f are known at the interface water/air, in which case especially a is stable. Thought between two aqueous phases all these film structures are *meta*stable and easily change into drops with less free energy. The stability, however, may be estimated. For the stability of a film is of importance: 1. the orientation by means of polar groups and 2. the mutual attractive forces of the C-chains. The orientation of the polar groups can only be effected, if they come into contact with water or other polar groups. Then the attraction between similar polar groups always is smaller than between water and the polar part of the molecule [59]). Owing to this those films, the polar molecular part of which is in water, are most stable. Therefore, between two aqueous phases the cases d, e and f are the most probable, also on account of measurements on naked protoplasts, measuring the sum of surface-tension and elasticity.

For HARVEY and COLE found as maximal value for the sum of surface-tension and -elasticity of unfertilised Arbacia eggs 0.25 dyne/cm [60]). From this DANIELLI [61]) concludes that the interface must have adsorbed proteins and that the cases a, b and c — all containing at least one "hydrocarbon-protein-water" layer at high tension — are improbable.

DANIELLI has realized the possibility d, but in the form of a much thicker membrane. GORTER too accepts this structure for the cellular membrane of the erythrocytes, but without adsorbed proteins. (See p. 397—400 of DANIELLI's first mentioned publication).

B. *Models of the protoplasm membrane.*

Now DANIELLI has considered molecules of a certain structure, viz. molecules with one polar group at one or both sides of the

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59) The fact that such polar substances dissolve in water, points in that direction.

60) 0.5 dyne/cm for the sum of the tensions at both interfaces of the membrane.

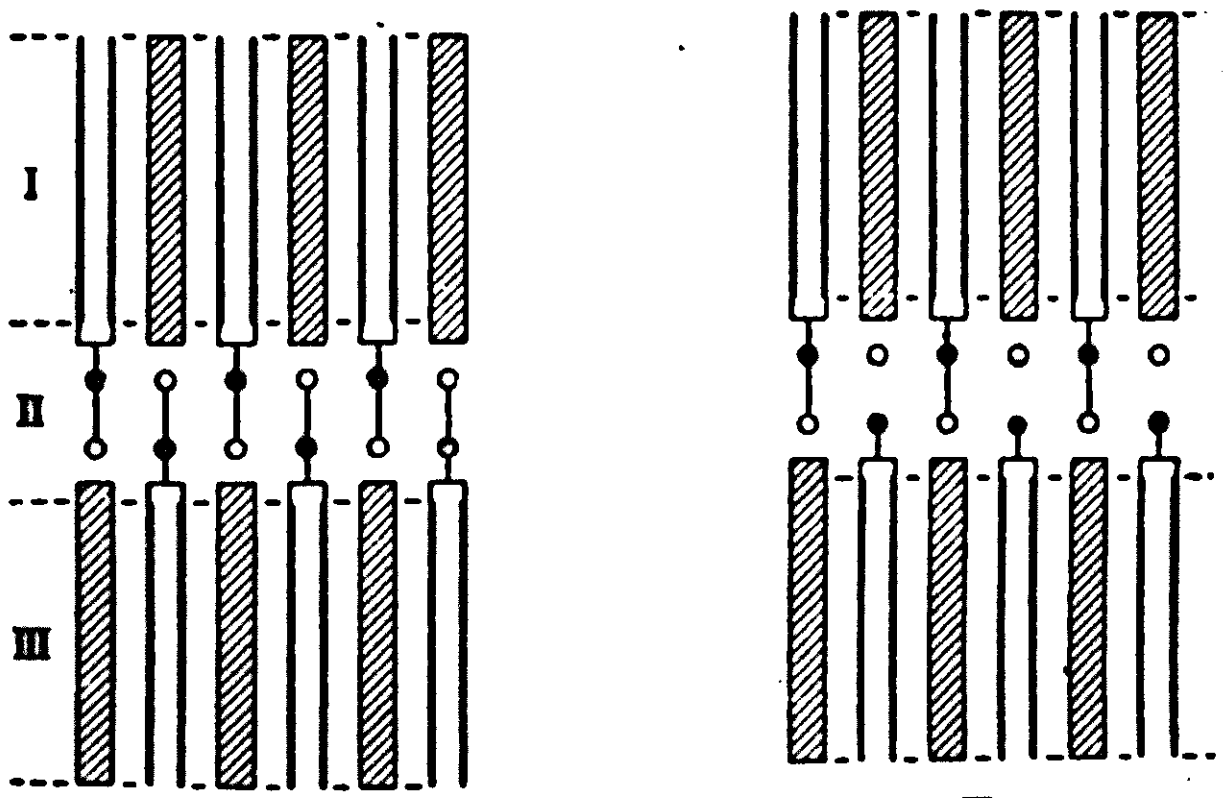
61) J. DANIELLI and E. N. HARVEY — Journal of cellular and comparative Physiology 5, 483, 1934/1935.

C-chain, and judging the stability of possibly formed membranes, he has only taken into account the directive force of 1. polar groups and 2. C-chains. However, if we take phosphatids, a third directive force will be added which in magnitude by far surpasses both the first-mentioned forces, to wit the electrical attraction in consequence of the amphoteric character of the phosphatids.

If a "Zwitterion" is represented symbolically by 

the mutual attraction of the — and + groups is introduced as stability-factor in the formation of the membrane. The study of the behaviour of phosphatid coacervate drops lead BUNGENBERG DE JONG to construct "possible structures of the protoplasmic membrane with phosphatids as their essential components". The first model [62]), reproduced below (A), represents the phos-

Figure IV.



A B

phatid "Zwitterions", arranged in such a way that the positive and negative group of each "Zwitterion" respectively lies between the negative and positive groups of neighbouring "Zwitterions". The two-dimensional model has to be imagined three-dimensional. The so-called sensitizers are situated between the hydrocarbon-chains.

An objection to this model is that the contractive force of ions of e.g. alkaline earths at low concentration — physiologically appearing from the decrease of the permeability to water — is not

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[62]) H. G. BUNGENBERG DE JONG and J. BONNER — Protoplasma 24, No. 2, 1935.

conceivable without further consideration. The electrical compensation of the negative zwitterionic groups which does not completely take place by the positive groups, could be supposed to take place better by addition of bivalent cations in the form of neutral salts, e.g. $CaCl_2$. Then Ca should screen off the negative charge of the "Zwitterions" better than Cl does the positive charge [63]). In BUNGENBERG DE JONG's opinion this assumption is improbable on account of the small mutual distance between the membrane-components. As the negative charge of the membrane of most protoplasts may only for the smaller part account for the occurrence of cephalins, BUNGENBERG DE JONG already suggested the possibility of fatty acids, but especially of phosphatidic acids acting as membrane components. However, these components could not be taken up in the pattern without interrupting the continuity. Only if coacervates of the type "Zwitterion-colloid anion-crystalloid cation" were realized [64]), we might consider the second possibility of the model reproduced above (B) [65]).

This model fulfills the requirement of sensitivety to cations. As appeared from the research on phosphatid coacervates, Ca has a stronger condensing action than e.g. Na and K. Compared with systems containing Na or K, Ca in the system causes condensation, on account of which the system becomes poorer in water, and consequently decreases its permeability to water. The antagonistic action of alkaline earths- and alkali-ions on the permeability to water is, like the ionic interchange, obvious in this model, which acts as a permutoid system. Two of the three membrane-components are mutually bound by great cohesive forces (VAN DER WAALS forces between C-chains mutually, both of the phosphatid Zwitterions and of sensitizers). The third, however, the crystalloid cation, is not bound by such forces and consequently will be interchangeable with cations from the milieu. Now there is place for the regulating cations, as they are taken into the pattern as membrane-components.

For the origination of these double-films phosphatidic acid is not necessary, but only a colloid anion. Therefore, in principle, the occurrence of proteid anions as membrane components is possible according to this model. As these double-films probably

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63) Here the membrane must be pictured spatially in order to represent the protective screening-off action of Ca^{++} and Cl^{-} resp.

64) H. G. BUNGENBERG DE JONG, G. G. P. SAUBERT — Biochemische Zeitsch. 228, 1 and 13, 1936.

65) H. G. BUNGENBERG DE JONG, G. G. P. SAUBERT — Protoplasma 28, Heft 3, 1937.

have a high interfacial-tension, which is very low for naked protoplasts, probably a layer of adsorbed proteins will have to be assumed. There is no objection against this assumption, as it is known from measurements of kataphoretic velocities that phosphatid coacervates are slightly covered by Na- or NH_4 arabinate and probably also by proteins [66]).

Such a phosphatid coacervate covered by arabinate, taken as an unit, electrically acts like an arabinate coacervate, while the phosphatid coacervate maintains under such a cover its own electrical behaviour [67]). This is of great importance for physiology, as from this it may be conceived, how a naked protoplast may act in regard to its permeability to water, as if the membrane were a phosphatid coacervate, while the kataphoretic behaviour of the protoplast — i.e. the electrical behaviour of the protoplasmic boundary — is different. This phenomenon may be pictured, assuming that the membrane, regulating permeability, is covered by a film of non-lipoids. This cover needs not alter the properties of the membrane underneath it. It may be observed that DANIELLI's patterns, from the electrical point of view, are very unfavourable, as increase of the charge to + or — direction should result in disintegration of his models. In respect to their electrical behaviour BUNGENBERG DE JONG's patterns are more stable.

The word "model" is not used here in the sense of an imitation of a live reality. Science is concerned with the *relation* of different series of observed phenomena. We are dealing with the study of so-called "complex-relations", the trend of which relations gives rise primarily to a mental image, keeping in mind however, that every mental picture is "thought" and as such exists as subject of thought. As, therefore, imagining is also subjective, we are responsible for its nature. In order to recall all that is worth keeping of a certain happening, we schematize the image formed by thought into an illustration, which is therefore an image of the second instance. In this sense the pictures given above represent "models" of the protoplasmic membrane: a graphical shorthand, not unlike Bohr's atom-models. The method is a reminder of those elements of our thought-product that could not be schematized into an illustration because they might become "petrified". The illustration, the "image of the second

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66) Some proteinoid substances have a great surface activity for the interface lipoid/water.

67) J. G. WAKKIE — Onderzoekingen aan phosphatid systemen naar aanleiding van eenige biologische problemen — Thesis, Leiden 1936.

instance" is never able to represent that of which thought is capable — to wit its dynamical aspect.

The designed picture therefore always must remain imperfect and be continuously subject to change for the course of our thinking, which precisely manifests itself as this permanent change.

By this permanent sequence of images, by this discontinuous proces, the continuous demonstrates itself as the unchangeable way in which the changeable changes.

In this sense the statical nature of the "model" is not only admissible, but even necessary in order to witness its dynamical aspect; the proces being recognized as such by a sequence of states. In science the quintessence is; to recognize order (i.e. the unchangeable) in the chaos of changing images.

Thus arises the second sense, in which the word "model" is used. For when we carry out measurements on colloids (e.g. of the alcoholic influence upon the zero charge with $CaCl_2$) and we call this object of research a colloid "model", this model is not an illustration. We only try to find from the observed changes under the influence of factors the order which dominates the colloid-chemical process, the so-called complex-relations. As in physiology the study of complex-relations is also considered, but — because of the abundance of simultaneous factors — more complicated than those concerned in the "model", the meaning of the so-called model research is obvious. The complexity of processes which the living object shows are related to more simple complexes, in order to reveal stabilities which will have to be estimated also in biology, if only in hidden and unrecognizable form.

4. Assuming the protoplasmic membrane as an oriented system of phosphatids, the influence of elementary ions in low concentration upon the permeability to water is conceivable as an influence upon the electrical conditions and consequently on the compactness of the system. The influence of the ionic concentration-range upon the permeability to water, to be expected on the basis of this assumption has been confirmed experimentally by DE HAAN and others.

The influence of composed ions and non-electrolyts is more difficult to interpret. From the colloid-chemical part of this article appeared that organic non-electrolyts cause an action on the C-chains and by change of the diëlectric on the electric condition of the membrane components. Their action is binomial and may be in two directions such that both directions influence

the membrane structure to similar or opposite effect. In the latter case the membrane structure may remain unchanged and consequently the permeability to water constant. For the determination of the permeation velocity of substances according to the plasmometrical method this is of great importance.

For if at the permeation of a substance the membrane changes structurally [68]) and therefore influences the permeability to water, the plasmometrical method cannot be used in order to compare the permeation velocity of substances. As appears from the fact that the relative permeation velocity of substances, determined according to the "direct" method [69]), agrees with that determined according to the plasmometrical one, the membrane does practically not alter for the substances tested.

In order to examine, if the protoplasmic membrane reacts at ascending concentrations of permeating substances, the permeability to water under influence of alcohols was checked (Chapter V).

CHAPTER V

The permeability of Chara ceratophylla to alcohol/water mixtures.

A. *Introduction.*

The permeability to water has not been investigated with the same zeal as that for other chemical compounds, although the use of the plasmometrical method should inevitably have led up to it.

STILES in his book on Permeability (1924) discussed in the chapter concerning the permeability to water chiefly his own work, just because so little had been done on this subject. In 1930 HÖFLER and HUBER published a treatise on the permeability to water and since then more attention has been paid to this detail [70]).

The methods followed for this research are:

a) the plasmometrical method (DE HAAN, HÖFLER and HUBER)
b) the weighing of tissue parts (STILES, BAPTISTE)
c) measurement of volume-changes of cells (Mc. CUTCHEON and LUCKÉ; LEVITT, SEARTH and GIBBS; STUWART and JACOBS; TOKO RO FUKUDA).

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68) Change of the chemical composition I leave out of consideration.
69) R. COLLANDER and H. BÄRLUND — Loc. cit.
70) Compare, untill 1932 the literature by Mc CUTCHEON and B. LUCKÉ.

We shall refrain from a discussion of the objections to the methods mentioned.

The most suitable method seems to me to be the one by which the volume-changes of intact cells of regular shape are measured directly, such as eggs of Arbacia, Asteria, Athocidaris crassispina, Pseudocentrotus depressus and others. In these cases the volume-changes are easily measurable. If the membrane is the only resistance which the permeating water meets, one is justified in saying that the permeability to water can be derived directly from the changes in volume [71]). My only objection to the objects mentioned is that the influence of a conc. range of a certain compound on the permeability to water cannot be measured on the same object, since during the measuring one cannot check whether the cell is still living and which phenomena occur at the possible perish. B. Lucké, M. G. Larrabee and H. K. Hartline [72]) had not yet published their optical method (the great advantage of statistical material therefore did not yet hold). I looked for an object on which the permeability to water could easily be measured, (I am not looking for absolute values; my intention is to fix the changes of the membrane for which I took the changes of the permeability to water as a measure) and whose life could be checked continuously without interrupting the experiment for checking purpose.

Moreover, the object should be of such a nature that on the same object an entire conc. range can be measured. Therefore the material has to endure 50 to 100 times endosmosis and exosmosis of water plus a substance in increasing conc. I believe to have found such an object in Chara ceratophylla [73]).

The life can be continuously checked by the distinctly visible plasma-current; the cell endures scores of times endosmosis and exosmosis of water without plasmolysing, so that the physiology of the cell is not so seriously interfered with as is usually the case in the plasmometrical method. The disadvantage (a disadvantage, indeed, of all unicellular methods) is, that great individuality in the behaviour of the cells requires extensive material and this takes up much time.

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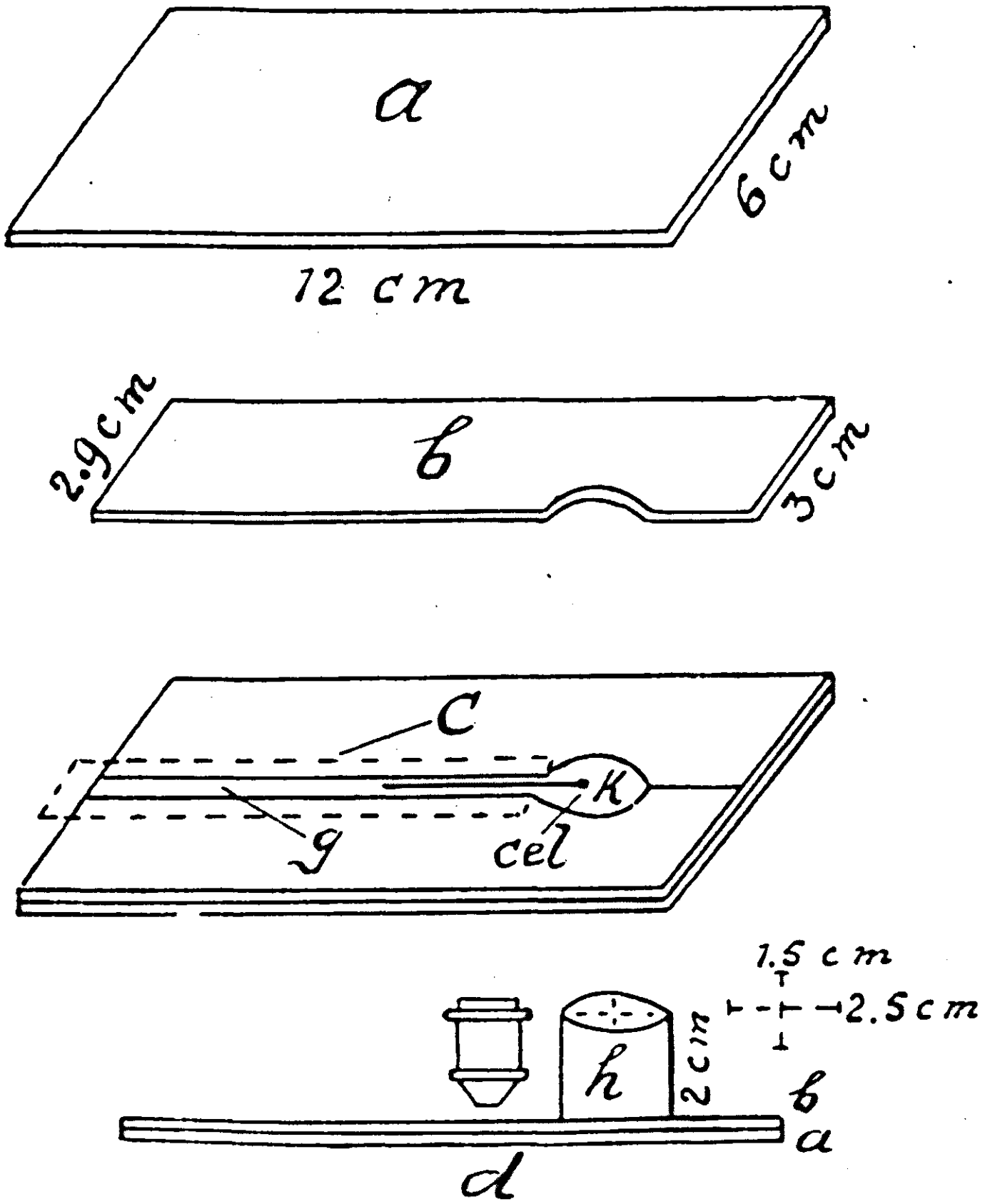
71) In general, permeability is understood to be the quantum of the permeating substance which passes per time-unit through the unit of active cell surface under the driving power of the unit of conc. gradient. The conc. change of H_2O is not easy to measure. Therefore the unit of pressure-gradient is taken here.

72) Journ. Gen. Physiol. 19, 1, 1935.

73) This work was executed at the Botanical Institute, Helsingfors, under the expert guidance of Prof. R. Collander.

B. *Method and Material.*

Figure V



Cells, from Jollasgård and treated as indicated in COLLANDER and BÄRLUND are glued with a mixture of beeswax and yellow wax = 3 to 1 [74]) in the apparatus shown in figure V.

This apparatus consists of a glass plate (a) of 12 × 6 × 0.2 centimeters on which two pieces of glass (b) of the shape illustrated are glued with paraffin. (c). The thus formed drain (g) is closed by a piece of glass (indicated by a dotted line) glued with paraffin, so that a channel of rectangular section is made. Above the cup (k) a piece of oval glass tube (h) is placed, as shown in the illustration (d).

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[74]) The mixture was melted and a drop of the molten glue was put on the stem and the part of the cell adjoining it in such a manner that the drop flowing, when cooled, glues the cell on the glass in the cup. (K).

In placing the apparatus under the microscope, care has to be taken that the top of the cell is clearly visible. Distilled water is now supplied in a constant current [75]). The cell expands and the movement of the top may be followed by an ocular micrometer. If the cell is in balance with the distilled water, the apparatus is moved in such a manner that the top coincides with a mark on the micrometer, as is illustrated in fig. VI. The apparatus can now be fastened to the microscope-table with plasticine. When 0.3

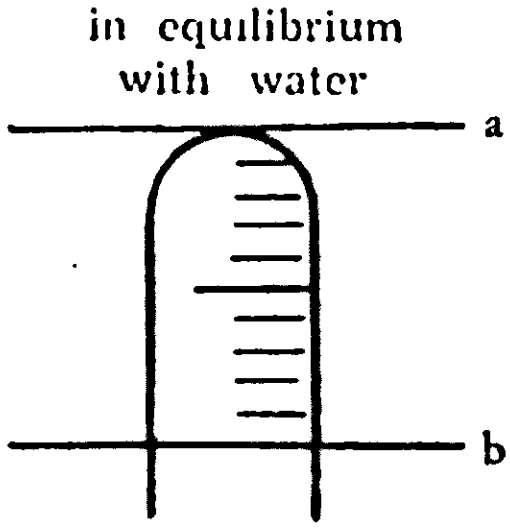

Figure VI

mol. sacch. is supplied in a *constant current*, the cell shrink and the shrinkage can be followed till the cell is again in equilibrium. No matter how many times this endosmosis and exosmosis is repeated, the top of the cell always returns to the respective points on the micrometer. If the longitudinal changes are plotted against the time, mutually identical curves will appear for each exosmosis, just as each endosmosis will give mutually identical curves. These two facts are of importance, as they signify that the measured longitudinal changes — as a function of the not-measured volume-changes of the cell — always take place in the same manner. This again implies that these volume-changes always take place in the same manner too. Irregularities can be the consequence of adding the media too soon, that is to say, before the equilibrium in the previous medium has been completely attained. The supplying of the new medium must also take place always in the same manner, because otherwise the gradient in the osmotic pressure will not be the same at the start, for it always takes some time before the original medium has been entirely replaced by a new one. At first mixture of the media takes place, and only after a while — however, comparitively short — the old m i l i e u is replaced. This can be seen very easily, when a little eosine is added to the liquid supplied. It is also noticeable in the tendency to form streaks if the water pushes back the sugar solution and v.v. After some practice the reading of the longitudinal changes can be followed up to 1/10 of the distance between two marks on the micrometer. In the case of the enlargement used the distance between the two division-marks indicates 10.6 μ

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[75]) For this purpose I made use of buretts placed slantingly on a support. The taps can be placed above the tube (h) in turns, letting the liquid trickle out.

(measured with an object micrometer). The error made in reading the indicator, can, in my experience, be at most 2/10 division-mark, i.e. 2.12 μ. The relative error, however, increases as soon as one wants to trace the longitudinal changes too far. On plotting the longitudinal changes against the time, one gets curves which approach asymptotically the equilibrium-point. A reading in the range between a and b is, owing to the small longitudinal changes, very inaccurate. [See Graph XX on page 781].

If instead of 0.3 mol. sacch. solution and H_2O dist. as milieu for exosmosis and endosmosis, we take 0.3 mol sacch. + x mol. alcohol and H_2O dist. + x mol. alcohol respectively, it will appear that the cell, at least if not a too high alcohol conc. is used, in balance with the milieu has its top at the same points as in the case of 0.3 mol. sacch. ⇄ H_2O dist. Here, too, the longitudinal changes always take place regularly, and as these longitudinal changes are a function of the volume changes, we are justified in saying that these volume changes *progress regularly*. The extent of the longitudinal changes within a certain time, though, is different (bigger or smaller) from that in the case of sacch. ⇄ H_2O. If one plots the longitudinal changes against the time, in the case of sacch. + alcohol ⇄ H_2O + alcohol, the result is a similar curve as has been found for sacch. ⇄ H_2O, but the so-called "Halbwertzeit" — the time necessary to traverse half the process — is different from the case sacch. ⇄ H_2O. The cell behaves in both cases similarly, except that the time necessary for endosmosis and exosmosis is different. The resistance of the cell (cellwall + protoplasm-membrane) seems to change for the passage of water when alcohol is added. As my aim is to prove the existence or non-existence of the changes of the protoplasm-membrane, possible changes in the condition of the cellwall mean a complication. A second complication is the simultaneous permeating of alcohol *and* water, in view of my wish to conclude the changes of the membrane from the changes of the permeability to water. Closer examination reveals that the first-mentioned complication does not exist. To make this clear one can introduce the term "Suction-pressure" of a cell. If a cell is in equilibrium with its milieu, the same quantity of water endosmizes and exosmizes; or in other words the suction-pressure of the medium is the same as the suction-pressure of the cell, and this again is equal to the suction-pressure of the cellsap minus the wall-pressure. Written in symbols it would be $M = A = C—W$, the letters representing the above mentioned terms respectively. If a cell is in equilibrium with distilled water it appears that $A = 0$ and $C = W$. If a cell is in balance with 0.3 mol. sacch., then A = osmotic pressure of 0.3 mol. sacch. = 6.72 atmospheres. During the endosmosis the value of A changes from 6.72 atm. to 0 atm. and during the exosmosis from 0 atm. to 6.72 atm.

The value of M does not change during exosmosis and endosmosis, as one is working with a constant current. A, on the contrary, changes till it is equivalent to M. This is true for every endosmosis and exosmosis, whether the milieu contains alcohol or not. The velocity with which A becomes equal to M, however, changes according to the milieu. As $A = C—W$, the variation of the velocity by which A becomes M may be due to the changed velocities of variation of the quantities C and W, that is, of the osmotic pressure of the cellsap and the wall-pressure. The velocity with which C varies is dependent on the resistance of the protoplasm-membrane and its changes for the passage of

H_2O, *if only water permeates* [76]) and secondly on the wall-pressure.

The permeability of the wall is, according to COLLANDER & BÄRLUND [77]), very large for most substances, so that this factor need not be considered It only concerns changes in the elasticity of the cellwall.

We may assume, first, that the alcohol alters the elasticity of the cell-wall and, therefore, the wall-pressure. If this is altered, then it will also be altered if the cell is *in equilibrium* with the milieu. If, in fact A = M, M being constant, A too is constant and therefore C—W is constant also. W, therefore, can only change if C changes in similar directions, i.e. if W increases or decreases, C must also increase or decrease. This is possible, if the cell in equilibrium takes up a smaller or bigger volume than at first. It comes out in a failure of the cell-point to return to the points on the micrometer [78]). This fact is seen at high alcohol conc., but is immediately followed by an emptying of the cell; it is therefore followed by an exosmosis of osmotically active components. So, the most probable assumption is that the protoplasm-membrane has altered by the addition of alcohol. The change of this membrane only results in a retardation, or possibly in an acceleration of the passage of water. The second complication is reduced by the fact that the concentration of alcohol inside and outside the cell is *nearly* the same at the start of each endosmosis or exosmosis. Exact equivalence in the equilibrium-condition of the concentration of a permeating substance on both sides of the protoplasmic membrane is theoretically not to be expected nor has it been found experimentally [79]). It is not to be expected theoretically, because in equilibrium the pressure in the cell is different from that outside the cell [80]). The two components, alcohol and H_2O, spread inequally over two phases which have a different pressure. This has sometimes been overlooked in permeability research, since in the considerations one always starts from the idea of ideal gases.

If at the beginning of each endosmosis or exosmosis the conc. of the alcohol is nearly equal inside and outside the cell, one cannot expect a great change of this conc. of alcohol, during the endosmosis and exosmosis, as the alcohol permeates nearly as quickly as the water and the volume-changes of the cell are not greater than 1% of the volume at the start. The measured changes in the "Halbwertzeit" are greater than 100%. Even when we assume the "complete impermeability to alcohol" during exosmosis and endosmosis, this change of the "Halbwertzeit" could not be explained by the increase and decrease respectively of the alcohol conc. in the cell. Taking into consideration, however, the dimensions of the Chara cell — the volume of which is so large in relation to the active cell surface — the rise of a local lowering of the alcohol concentration inside the

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[76]) On the basis of thermodynamical and mechanical considerations it is possible that two simultaneously permeating substances, even without change of the membrane, influence each other's permeating velocities in such a manner, that they are no longer in proportion with the pressure-gradient. However, as we have no data for mixtures of alcohol and water, it may suffice to mention this possibility. See SCHREINEMAKER's works.

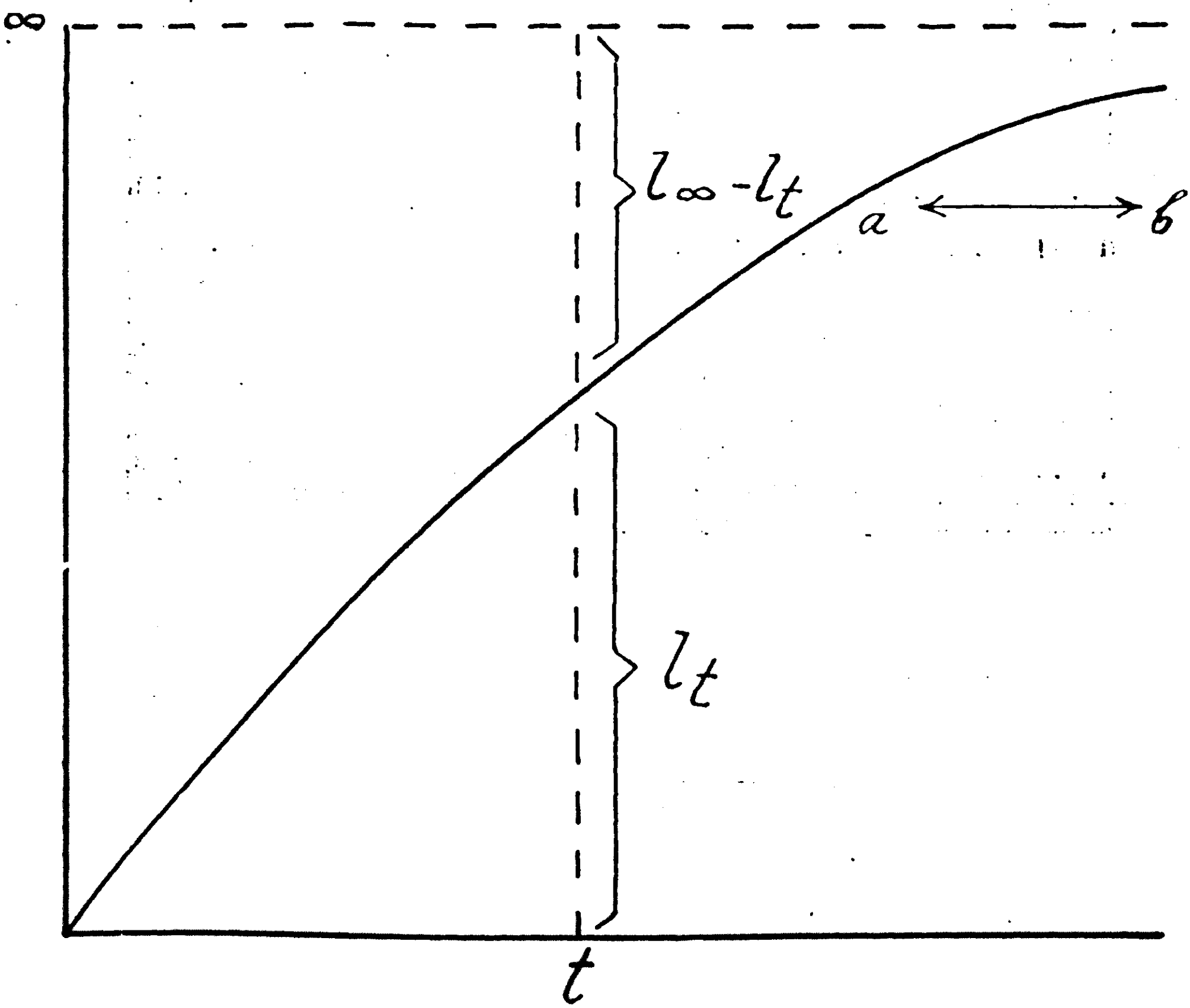
[77]) R. COLLANDER and H. BÄRLUND — Acta Botanica Fennica 11, 1933.

[78]) The concentration of alcohol in the cellsap, however, is never so high that it influences the activity of the salts to any appreciable extent.

[79]) R. COLLANDER and H. BÄRLUND on p. 19.

[80]) This is not true in the case of naked protoplasms, the cell-surface of which hardly shows any elasticity.

Graph XX



membrane during the endosmosis (because H_2O permeates quicker than alcohol) has to be taken into account, while the same occurs during exosmosis outside the membrane. Such a local lowering of the alcohol conc. may result in the lengthening of the "Halbwertzeit", because the pressure-gradient is reduced.

Assuming, however, that the free diffusion of the alcohols (methyl, ethyl and propylalcohol) [81]) in the cellsap, respectively m i l i e u takes place quicker, than the rise of the dilution, on account of the water permeating slightly quicker than alcohol, then the changes in the protoplasm-membrane

81) For n. butyl- and n. amylalcohol the conc. used is so small that these considerations become superfluous. Their permeating velocity, also, is too high.

may account for the changes in the "Halbwertzeit". In my opinion, the changes in the "Halbwertzeit" can be used as measure for the changes in resistance which the protoplasm-membrane offers to the passage of H_2O.

It may be observed here, that this choice of the explanatory principle of the phenomena is made, on account of the results obtained by study of the coacervates and directed systems. Compare Chapter II and III.

On closer examination of the curves representing the longitudinal changes as a function of time, the curve is striking as being remarkably regular for a biological process. It is the curve known for processes, which, according to their approach of the equilibrium, proceed regularly slower. Generally, these curves may be indicated by the formula, which is mentioned here in the form important to us $\log (l_\infty - l_t) = -Kt + C$. $(l_\infty - l_t)$ represents at a certain time the distance from the point of balance Graph XX. On plotting the logarithm of this difference against the time, a straightline will be the result, the tangent of which with the abciss, will be represented by K. When $t = 0$, i.e. in the beginning of endosmosis or exosmosis, $\log (l_\infty - l_0)$ becomes C. In this formula C signifies the possibility of expansion or contraction of the cell, and is a characteristic of each cell. This formula is only applicable, if C, i.e. as micrometer-reading the distance between equilibrium-points A and B, is constant. (See figure VII). For non-poisonous alcohol conc., this C as a matter of fact is constant, as are the K's calculated by experimental data. As appears from the above mentioned, the application may be justified.

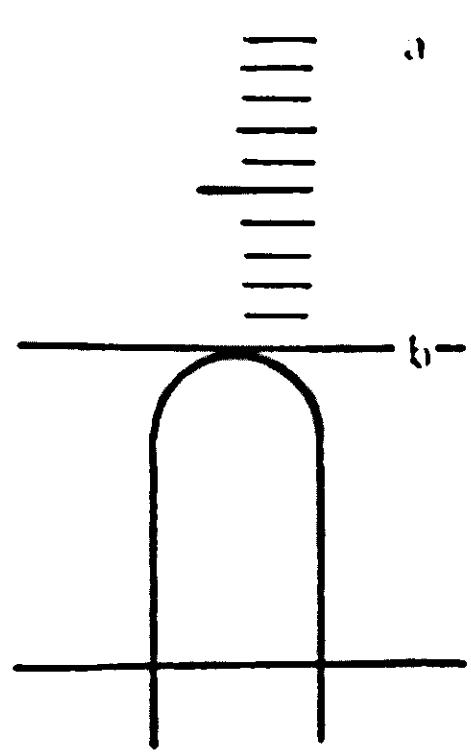


Figure VII

If the process progresses differently when an alcohol is added, this is noticeable in the calculated K (= tangent of the straight lines). It is unnecessary to say that these K's are not permeability-constants. They only indicate how quickly the "constant" course of the process progresses; they are measures for the resistance which the permeation of water meets in both directions.

The following has to be paid attention to in measuring:

a. The cell-top has to be cleaned carefully, as otherwise a vague limit will make the measuring inaccurate. This cleansing has to be done carefully with wet filter paper, while the cell is held between thumb and forefinger, because it can hardly stand mechanical influences.

b. The cell-top must have a symmetrical shape, because during endosmosis or exosmosis a small displacement of the longitudinal axis of the cell to the left or to the right may cause wrong readings. (See figure VIII). The shape of the cell top influences the readings, which can be prevented

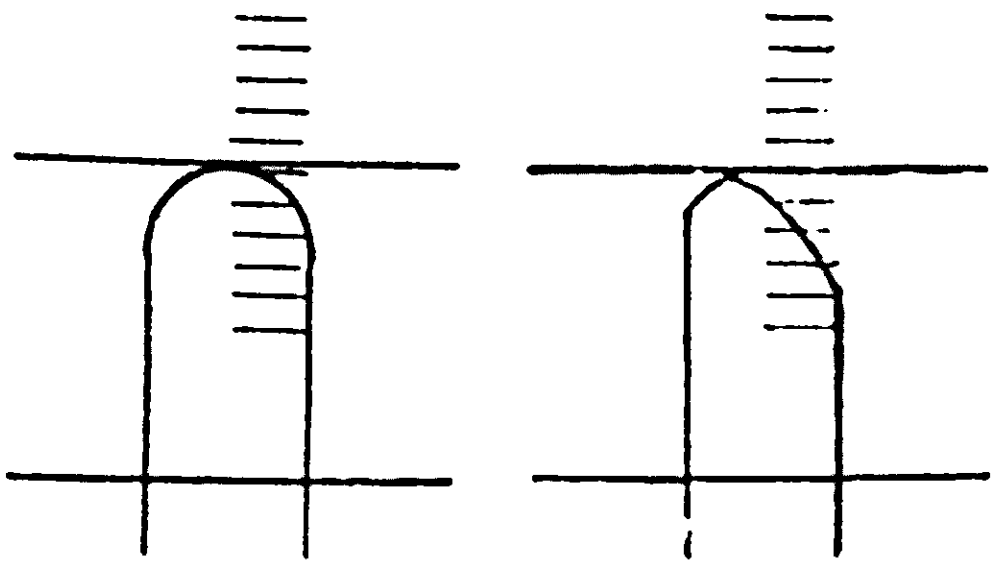


Figure VIII

c. by taking straight and not too long cells. Moreover, care must be taken in gluing solidly.

d. The cell must not be too thick, as it otherwise causes retardation in the longitudinal changes, because the total quantity of permeating water per time-unit is dependent on the quotient of $\frac{\text{active cell surface}}{\text{cellular volume.}}$

e. The cell must have a fast protoplasm-current in order that it may serve as an indicator of vitality. If the protoplasm-current of a certain cell nearly stops with a certain alcohol conc., no further experiments should be made with such a cell.

C. *Description of the experimental results.*

§ 1. Experiments without alcohols.

Prior to the experiments we determined the maximal shrinkage and expansion tolerated by the cell as well as the "Halbwertzeit" of exosmosis and endosmosis in different concentrations of saccharose.

When distinct plasmolysis was obtained in 0.5 mol. saccharose it was found that this phenomenon was concommittant with death, inasmuch as, in Chara ceratophylla, the protoplasm adheres firmly to the wall and may not be separated from it without injury.

The exosmosis should be studied therefore in sub-plasmolitic concentrations limited by 0.45 mol. saccharose. In 0.4 mol. saccharose exosmosis may be brought about 3—10 consecutive times. The cell than will shrink in an irregular way. 0.3 mol. saccharose solution is endured well by the cell. In distilled water many cells will show normal endosmosis provided that the cell — when it has reached equilibrium — is taken from this milieu

immediately, since otherwise the cell will show abnormal behaviour. Convulsive contractions and slow expansions successively may be observed in that case. It seems as if the pressure on the protoplasmic membrane is so high in this case that a temporary lesion appears, which seriously interferes with the vital functions. If such cells are placed again in water containing electrolytes, they usually die within a few hours. During this process a normal protoplasmic current was observed. The expansions often lead to bursting. If a cell does not burst at the first endosmosis, experience shows that it will be able to endure 50—70 consecutive endosmoses and exosmoses in alternating treatments with water and 0.3 mol. saccharose solution. The "Halbwertzeit" remains constant during the entire process. If such a cell be transferred into water containing electrolytes, it is able to live for days.

The accuracy of our measurements depends on the total change in cell-length. If this change is great, the velocity of change is also appreciable. This total change in length depends upon the difference in osmotic pressure of the exosmotic and endosmotic milieu (in our experiments 0.30 mol. saccharose solution and distilled water resp.). The values of this gradient for various solutions are given below.

Osmotic pressure gradient in atmospheres:

I 6.72 (0.30 mol. saccharose and distilled water as exosmotic and endosmotic milieu resp.).

II 4.48 (0.20 mol. saccharose and distilled water as exosmotic and endosmotic milieu resp.).

III 2.24 (0.10 mol. saccharose and distilled water as exosmotic and endosmotic milieu resp.).

The "Halbwertzeite" and the quotient $\frac{\text{"Halbwertzeit" of endosmosis}}{\text{"Halbwertzeit" of exosmosis}}$ are given in table IV.

From this table it may be seen that endosmosis always requires less time than exosmosis. The quotient between the endosmotic and exosmotic "Halbwertzeite" appears to be constant for different cells at a given difference in osmotic pressure of the endosmotic and exosmotic milieu, independent of the absolute values of these "Halbwertzeite". Table V gives the quotient of the constant K's calculated from the equation given on pag 782.

The quotient limits to unity when the difference between endosmotic- and exosmotic pressure values decreases.

TABLE IV

Concentration of saccharose of resp. exosmosis- and endosmosis milieu	Quotient $\frac{\text{Halbwertzeit endosmosis}}{\text{Halbwertzeit exosmosis}}$			
	Cell I	Cell II	Cell III	Cell IV
0.30 ⇄ 0.00	$\frac{48.5}{70.7}=0.686$	$\frac{1.4}{1.9}=0.737$	$\frac{30.5}{41.0}=0.744$	$\frac{31.7}{42.7}=0.742$
0.20 ⇄ 0.00	$\frac{44.4}{55.5}=0.800$	$\frac{1.4}{1.7}=0.824$	$\frac{26.5}{32.0}=0.828$	$\frac{27.6}{33.7}=0.820$
0.10 ⇄ 0.00	$\frac{41.4}{38.4}=1.08$	$\frac{13.0}{13.0}=1.00$	$\frac{24.0}{28.0}=0.875$	$\frac{26.1}{27.7}=0.940$

§ 2. Differential permeability.

Experiments upon this so-called differential permeability for water are scanty. A survey of the literature yielded the following:

1. Arbacia eggs — Mc Cutcheon and B. Lucke, Physiological Reviews 12, 1, 1932.
2. Caudina Chilensis — Koizumi — Sci. Reports Taihoku Imp. Univ., Ser. 4, Biol., Vol. 7, p. 259, 1932.
3. frog's skin — on this matter quite a polemic has developed.
4. Phascolosoma gouldi E. F. Adolph — Journ. of Cell. and Comp. Physiol. 9, 117, 1937.

It should be of general occurrence, however, as similar phenomena have been repeatedly observed in plasmolytic experiments, the velocity of deplasmolysis being greater than the velocity of plasmolysis. Höfler found a ratio of ± 3. Levitt, Scarth and Gibbs (Protoplasma 26, 237, 1936) found a ratio of ± 2.5 (for complete plasmolysis). This ratio limits to unity when the "degree of plasmolysis" is smaller than, or equals 2/5. If it equals 1, the deplasmolysis-velocity increases strongly. A sharp increase of deplasmolysis-velocity was observed also by de Haan.

For *Chara ceratophylla* a difference in endosmosis- and exosmosis-velocity was always found, when the difference in osmotic pressure of the media exceeded ± 2.5 atmospheres, as Table IV indicates. For a difference in pressure of 6.72 atmospheres a greater velocity of endosmosis was always found, no matter if one worked with or without alcohols. This difference cannot be explained by the elasticity of the cell wall, since the tension of the cell wall increases with the expansion of the cell and therefore counteracts the endosmosis, while the exosmosis is assisted by the wall-tension.

It may be predicted therefore, that the difference between endosmotic and exosmotic velocity would still be greater, if the action of the cell-wall could be excluded. The differential permeability cannot be accounted for by the specific action of the saccharose and the distilled water inasmuch as this phenomenon also occurs in other media such as water containing electrolytes. From the equation $\log (l_\infty - l_t) = -Kt + C$, a constant K both for endosmosis and for exosmosis may be obtained. The resistance of the membrane remains therefore constant during the experiments. This shows that the difference in resistance of the membrane to the passage of water in both directions is also constant. In order to explain this phenomenon for Chara ceratophylla one could think of the difference in osmotic pressure gradient at beginning endosmosis and exosmosis.

Let us assume again suction pressure of the cell = suction pressure of the cell sap minus wall pressure, or in symbols $A = C - W$, or written in another way $C = A + W$. If the cell is in equilibrium with a sugar solution at osmotic pressure = x atmospheres, the formula may be written $C_1 = x + W_1$, as in equilibrium $A = x$. If the cell is in equilibrium with distilled water, $C_2 = 0 + W_2$ holds. At beginning endosmosis the osmotic pressure gradient of cell sap and milieu is $C_1 - 0 = x + W_1$. At beginning exosmosis the osmotic pressure gradient is $x - C_2 = x - W_2$. During endosmosis resp. exosmosis the values $x + W_1$ resp. $x - W_2$ become equal to zero (in equilibrium). The wall pressure W_2 is always greater than W_1, so that $x + W_1 > x - W_2$.

If x is reduced, also the difference between W_1 and W_2 is reduced, as the longitudinal changes of the cell and, consequently, the differences in wall-tension are reduced. Therefore, $\frac{x + W_1}{x - W_2} \rightarrow 1$, in accordance with the experimental results.

Inasmuch as we cannot determine the magnitude of W_1 and W_2, the endeavour to apply the equation in detail proves abortive. We might assume, however, on the basis of the above considerations an asymmatrical structure of the membrane.

Especially the constancy of the ratio $\frac{\text{K endosmosis}}{\text{K exosmosis}}$ points in this direction (see Table V), for if all Chara cells would show a similar structure of the membrane, a constant quotient could be accounted for. The membrane should show variable anisotropy (on cross section), the amount of this anisotropy increasing

TABLE V

Quotient $\frac{\text{K endosmosis}}{\text{K exosmosis}}$ for a concentration of saccharose of resp. exosmosis- and endosmosis milieu 0.30 ⇄ 0.00

1	$\frac{972}{757} = 1.28$	13	$-\frac{914}{662} = 1.38$
2	$-\frac{984}{680} = 1.45$	14	$\frac{902}{534} = 1.69$
3	$\frac{710}{527} = 1.35$	15	$-\frac{689}{462} = 1.49$
4	$\frac{1269}{902} = 1.41$	16	$\frac{182}{111} = 1.64$
5	$\frac{1374}{926} = 1.48$	17	$\frac{1030}{623} = 1.65$
6	$\frac{737}{548} = 1.34$	18	$\frac{990}{584} = 1.70$
7	$\frac{2304}{1103} = 2.09$	19	$\frac{577}{363} = 1.57$
8	$-\frac{690}{450} = 1.53$	20	$-\frac{814}{589} = 1.38$
9	$-\frac{757}{505} = 1.50$	21	$-\frac{862}{637} = 1.35$
10	$-\frac{1929}{787} = 2.45$	22	$-\frac{609}{423} = 1.44$
11	$-\frac{1483}{929} = 1.60$	23	$-\frac{708}{569} = 1.24$
12	$\frac{1102}{676} = 1.63$	24	$-\frac{825}{584} = 1.41$

with the difference in osmotic value between endosmotic and exosmotic milieu and which anisotropy approaches to zero when the two above mentioned, osmotic values coincide. The difficulty remains to test this assumption.

§ 3. Curious phenomena.

Before starting the description of the experiments with alcohols, a few curious phenomena should be described. *a*. When a cell is left too long in an alcohol, it will in the subsequent endosmotic milieu show a failure to return to its original length. The cell empties itself immediately, often preceded by the convulsive movements described above. These phenomena are all indicative of approaching death. It seems the more curious,

because rotation of the protoplasm may persist up to the moment that the cell content flows out through the ruptured membrane. *b.* This protopalsm-current may cease for a moment when the cell is brought from its natural brackish milieu into distilled water, to reappear after about 10 seconds. c. Only the higher alcohol concentrations show a deceleration of the current, low concentrations being not appreciably effective. It seems, however, that the plasmatic velocity is a function of the water permeability of the membrane.

§ 4. Experiments with alcohols.

a. By applying a complete concentration range of a certain alcohol to one and the same cell, the sequence of concentration is varied in order to be able to measure a possible after-effect. After treatment with an alcohol the velocity of exosmosis and endosmosis was tested in the usual way by means of saccharose solution and distilled water. Comparison always was made with cells which did not undergo previous treatment with alcohol (the blanks). Fair agreement between the two series was obtained when after the alcohol treatment the cells were "washed" by repeated endosmosis and exosmosis in various electrolytes concentrations, followed by a period of rest of about 15 minutes in a medium approaching in its composition the natural milieu of the Alga. The agreement between the two series was satisfactory, using the lower concentrations of alcohols. After use of high concentrations of alcohols often a narcotic after-effect might have obscured the result.

b. Measurements were performed at room temperature which, in the Laboratory at Helsingfors show the daily fluctuation of about 0.5° C. In order to prevent bursting we attempted to reduce the stock of assimilates by placing the cells in the dark for 12 hours in artificial nutrient solutions — prior to the experiments.

c. In the experiments with the alcohols two conditions have to be imposed;

I. After exosmosis and endosmosis with or without application of alcohol the cell has to return to its original length, for only in that case we might apply the equation $\log (l_\infty - l_t) = -Kt + C$.

II. During the entire experiment the protoplasm-current should not stop; measurements during which this cessation occurs, were discarded.

Methyl-, ethyl-, n. propyl- and n. butyl alcohol were tested in the maximal concentration range. Inasmuch as on one indivi-

TABLE VI

Methylalcohol

Alcohol conc. in mol./l.	Cell 1		Cell 2		Cell 3		Cell 4		Cell 5		Cell 6		Cell 7		Cell 8		Cell 9		Cell 10		Average $\frac{K_1}{K_2}$
	K_1	K_2	K_1	K_2	K_1	K_2	K_1	K_2	K_1	K_2	K_1	K_2	K_1	K_2	K_1	K_2	K_1	K_2	K_1	K_2	
0.00	0.0044[5]	0.0037[3]	0.0097[2]	0.0075[7]	0.0098[4]	0.0068[2]	0.0071	0.0052[7]	0.0127	0.0090[2]	0.0137	0.0092[6]	0.0060[2]	0.0036[9]							1.398
0.10	0.0040[6]	0.0034	0.0099[3]	0.0071[7]	0.0089[8]	0.0059[1]	—	—	0.0111	0.0088[8]	—	—	—	—							
0.20	0.0043[9]	0.0033[6]	0.0101	0.0068[3]	0.0070[5]	0.0053[4]	0.0066[3]	0.0053[6]	0.0099	0.0078[1]	0.0126	0.0084[8]	0.0038[4]	0.0034[3]							
0.40	0.0040	0.0032[5]	0.0092[8]	0.0067	0.0064[3]	0.0048[2]	0.0057[6]	0.0045[5]	0.0096[2]	0.0070	0.0114	0.0075[5]	0.0031[2]	0.0028[9]							
1.00	0.0034[6]	0.0028[8]	0.0071[5]	0.0052[7]	0.0055[4]	0.0047	0.0044[9]	0.0036[3]	0.0092	0.0063[9]	0.0080[5]	0.0054[9]	0.0025	0.0024[3]							
2.00	—	—	0.0037[4]	0.0032[2]	0.0038[5]	0.0032[2]	0.0030[6]	0.0022[5]	—	—	0.0061[3]	0.0050[5]	0.0019[5]	0.0018[1]							
0.00	0.0046[9]	0.0035[5]	0.0095[6]	0.0070[5]	0.0097	0.0069[5]	0.0068	0.0049[9]	—	—	0.0136	0.0091[6]	—	—							

Ethylalcohol

Alcohol conc. in mol./l.	Cell 1		Cell 2		Cell 3		Cell 4		Cell 5		Cell 6		Cell 7		Cell 8		Cell 9		Cell 10		Average $\frac{K_1}{K_2}$
	K_1	K_2	K_1	K_2	K_1	K_2	K_1	K_2	K_1	K_2	K_1	K_2	K_1	K_2	K_1	K_2	K_1	K_2	K_1	K_2	
0.00	0.0054[9]	0.0051[9]	0.0057[7]	0.0036[3]	0.0081[4]	0.0058[9]	0.0086[2]	0.0063[7]	0.0060[9]	0.0042[3]	0.0082[5]	0.0058[4]	0.0070[8]	0.0056[9]	0.0084[3]	0.0063					1.352
0.07	0.0056[2]	0.0054[6]	—	—	—	—	0.0090[4]	0.0072[6]	—	—	—	—	—	—	—	—					
0.14	0.0059[6]	0.0057[2]	0.0059[7]	0.0037[2]	0.0083[4]	0.0062[8]	0.0090[4]	0.0074	0.0063[2]	0.0044[4]	0.0090[1]	0.0059[3]	—	—	0.0086[2]	0.0070[3]					
0.28	—	—	0.0061[3]	0.0038	0.0086[1]	0.0070	0.0090[5]	0.0080[1]	0.0060[1]	0.0039[3]	0.0072	0.0051[1]	0.0068	0.0056[2]	0.0088	0.0072					
0.7	0.0063	0.0060[2]	0.0066	0.0043[6]	0.0089[5]	0.0075	0.0083[8]	0.0082[5]	0.0056[3]	0.0037	0.0061[2]	0.0043[1]	0.0063[1]	0.0055[3]	0.0088[2]	0.0073[4]					
1.05	0.0064	0.0062	—	—	0.0088	0.0071	—	—	0.0049[7]	0.0033[6]	0.0059	0.0039[1]	0.0061[1]	0.0055	0.0087	0.0069					
1.40	0.0060[5]	0.0058	0.0069	0.0045[2]	0.0087	0.0066[3]	0.0079	0.0076[3]	0.0051	0.0032[2]	0.0048[5]	0.0036[7]	0.0065[7]	0.0053[9]	0.0085[5]	0.0064[3]					
0.00	0.0053[5]	0.0050	0.0059[1]	0.0036[3]	0.0081[6]	0.0059[5]	—	—	0.0060[2]	0.0047	0.0085	0.0051[2]	—	—	—	—					

n. Propylalcohol

Alcohol conc. in mol./l.	Cell 1		Cell 2		Cell 3		Cell 4		Cell 5		Cell 6		Cell 7		Cell 8		Cell 9		Cell 10		Average $\frac{K_1}{K_2}$
	K_1	K_2	K_1	K_2	K_1	K_2	K_1	K_2	K_1	K_2	K_1	K_2	K_1	K_2	K_1	K_2	K_1	K_2	K_1	K_2	
0.00	0.0217	0.0107	0.0089[5]	0.0033[9]	0.0091[4]	0.0066[2]	0.0090[2]	0.0053[4]	0.0068[9]	0.0046[2]	0.0183	0.0111	0.0160	0.0088	0.0103	0.0062[3]	0.0099	0.0058[4]	0.0123	0.0088	1.710
0.106	0.0200	0.0097[6]	0.0080[3]	0.0035[6]	0.0085	0.0063[4]	0.0087[9]	0.0055[1]	0.0059[3]	0.0044[4]	0.0162	0.0089[8]	0.0149	0.0080[3]	—	—	0.0097[6]	0.0057[4]	0.0116	0.0080[5]	
0.212	0.0151	0.0078[9]	0.0077[6]	0.0034[2]	0.0080[1]	0.0058[8]	0.0081[6]	0.0050[5]	0.0056[5]	0.0041[9]	0.0120	0.0071[6]	0.0130	0.0074[9]	0.0088	0.0053[8]	0.0095[8]	0.0055	0.0099[5]	0.0071[2]	
0.424	0.0105	0.0052	—	—	0.0076[3]	0.0054[1]	0.0068[3]	0.0045[3]	0.0054[2]	0.0038[3]	—	—	0.0110	0.0060[5]	0.0066	0.0049[6]	0.0085[8]	0.0052	0.0089[7]	0.0065[3]	
0.00	0.0215	0.0109	—	—	0.0093[2]	0.0070[1]	0.0090[5]	0.0055[2]	0.0065[1]	0.0040[9]	—	—	0.161	0.0086[1]	0.0101	0.0065[4]	—	—	0.0125	0.0089[8]	

n. Butylalcohol

Alcohol conc. in mol./l.	Cell 1		Cell 2		Cell 3		Cell 4		Cell 5		Cell 6		Cell 7		Cell 8		Cell 9		Cell 10		Average $\frac{K_1}{K_2}$
	K_1	K_2	K_1	K_2	K_1	K_2	K_1	K_2	K_1	K_2	K_1	K_2	K_1	K_2	K_1	K_2	K_1	K_2	K_1	K_2	
0.00	0.0230	0.0110	0.0159	0.0078[6]	0.0069	0.0045	0.0233	0.0165	0.0075[7]	0.0050[5]	0.0115	0.0091[7]	0.0193	0.0078[7]	0.0148	0.0092[8]	0.0110	0.0067[6]	0.0073[7]	0.0054[8]	1.655
0.026	0.0231	0.0115	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	
0.052	0.0214	0.0109	0.0138	0.0073[1]	0.0065[7]	0.0044[8]	0.0215	0.0129	0.0075	0.0048[7]	0.0117	0.0080[2]	0.0188	0.0075[2]	0.0139	0.0082[7]	0.0101	0.0058[2]	0.0070[4]	0.0055[1]	
0.104	0.0164	0.0093[3]	0.0120	0.0068[7]	0.0060	0.0043[1]	0.0185	0.0132	0.0074[8]	0.0049[6]	0.0117	0.00707	0.0160	0.0097[1]	0.0120	0.0074[3]	0.0099[1]	0.0057	0.0070[6]	0.0063[2]	
0.156	—	—	—	—	0.0058[2]	0.0043[3]	0.0171	0.0121	0.0062[1]	0.0051[1]	0.0109	0.0062[3]	0.0142	0.0095[9]	0.0110	0.0067[6]	—	—	0.0050[8]	0.0051[3]	
0.00	0.0225	0.0107	—	—	0.0069	0.0044[8]	0.0230	0.0168	0.00787	0.0049[5]	—	—	0.0189	0.0076[5]	0.0147	0.0094[1]	—	—	0.0074	0.0055[8]	

dual cell a concentration range of an alcohol was used in such measurements which took from 6 to 8 hours, only few cells survived — both concentration and time effect of the alcohol being active.

Especially in the highest concentrations of alcohols tested, the protoplasmic movement may decrease after some time and stop entirely, which phenomenon is concommittant with a decrease in water permeability. The value K was only calculated for those cases in which this plasmatic velocity shows no steep decline. The influence of an alcohol was determined on 10 cells each of which has to undergo 6—10 subsequent endosmoses and exosmoses. Therefore, using 4 concentrations and its corresponding blanks, a cell should be able to shrink and expand 6×6 to 6×10 i.e. 36 to 60 consecutive times.

§ 5. Calculations.

Table VI gives the values of K for endosmosis and exosmosis calculated from $\log (l_\infty - l_t) = -Kt + C$. Column 1 indicates the alcohol concentrations in mol. per litre and the next columns give the corresponding K's for endosmosis (K_1) and for exosmosis (K_2). Each column indicates the values measured on one cell.

The constants of different cells are not directly comparable, as the size of the K is dependent upon the quotient $\frac{\text{active cell surface}}{\text{cell volume}}$.

This quotient co-determines the velocity of the changes in volume and consequently the velocity of the longitudinal changes. The greater the quotient, the faster the longitudinal change will be, if we assume an equal resistance of the membrane to the passage of water. This quotient, however, is eliminated if the K's measured in alcoholic milieu are divided by the corresponding K's measured without alcohol in the milieu, i.e. the blanks. $\frac{K\ (w)ater\ plus\ (a)lcohol}{K\ (w)ater}$ obtained in this way both for endosmosis and exosmosis yields the relative change of K at increasing alcohol concentrations.

These values are given in Table VII

$\left(\frac{Kw + a}{Kw}\right)_1$ and $\left(\frac{Kw + a}{Kw}\right)_2$ mean the relative resistance to the permeation of water/alcohol mixtures resp. for endosmosis and exosmosis. For a concentration of alcohol $= 0$, the value $\frac{Kw + a}{Kw}$

$= 1.000$. If the alcohol concentration increases, the value of this quotient increases or decreases at increase resp. decrease of the Kw + a. The quotients $\frac{Kw + a}{Kw}$, both for endosmosis and exosmosis of different cells, are comparable. The average value over all cells is represented in column 12 of table VII for both endosmosis and exosmosis. If these values are plotted against the log of the alcohol concentration, the curves for endosmosis and exosmosis for a certain alcohol run parallel. Ethyl alcohol does not yield smooth curves, but the broken curves for exosmosis and endosmosis still run parallel.

Neglecting the difference in value of endosmosis and exosmosis, a representation may be obtained of the influence of an alcohol on the permeability to alcohol/water mixtures by taking the average of the values $\left(\frac{Kw+a}{Kw}\right)_1$ and $\left(\frac{Kw+a}{Kw}\right)_2$ and by plotting the logarithms of these averages against the log of the alcohol concentration.

Graph XXI

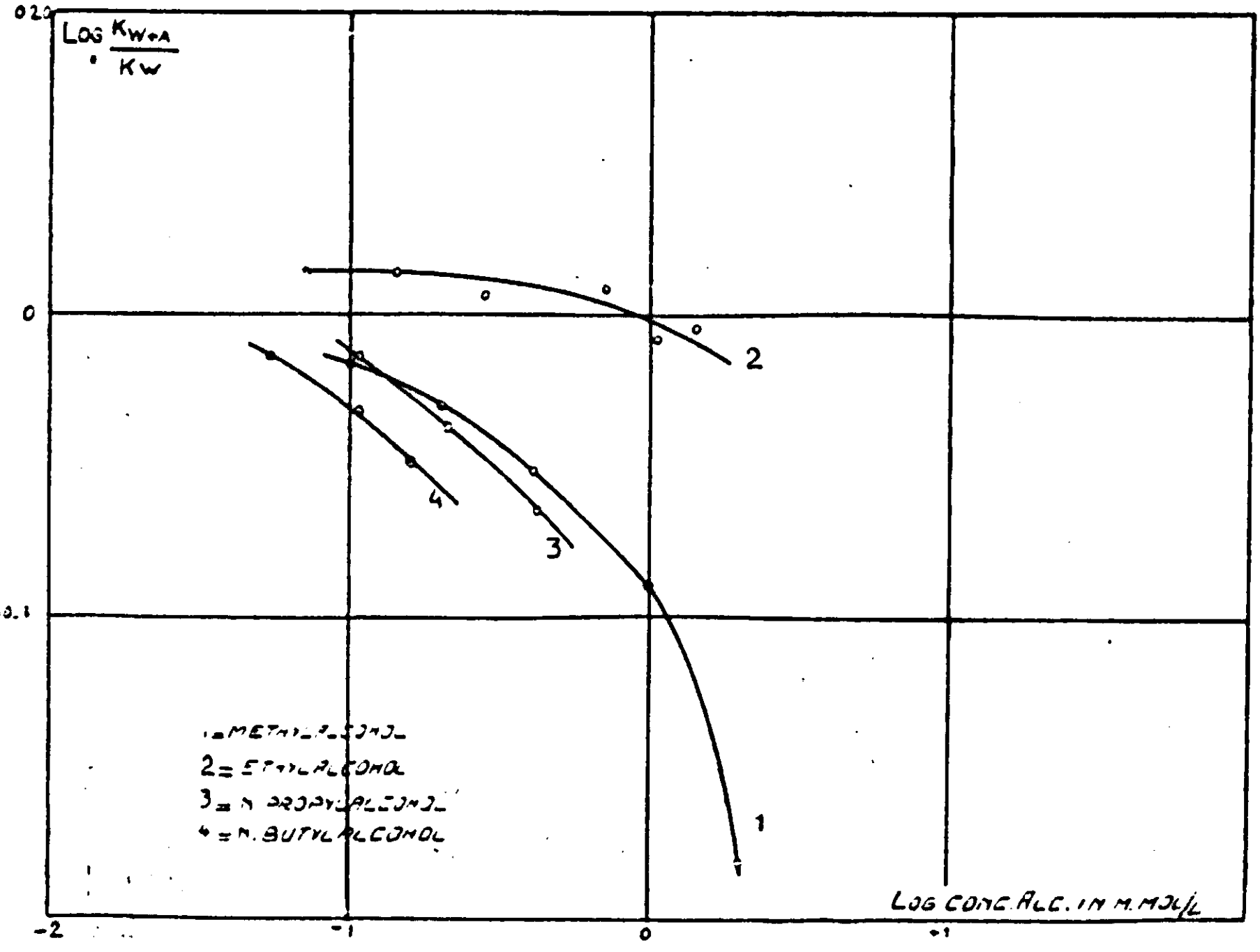


TABLE VII

Methylalcohol

Alcohol conc. in mol./l.	Cell 1		Cell 2		Cell 3		Cell 4		Cell 5		Cell 6		Cell 7		Cell 8		Cell 9		Cell 10		Average $\left(\frac{Kw+a}{Kw}\right)$		$\frac{(Kw+a)_1}{(Kw+a)_2}$
	$\left(\frac{Kw+a}{Kw}\right)_1$	$\left(\frac{Kw+a}{Kw}\right)_2$	$\left(\frac{Kw+a}{Kw}\right)_1$	$\left(\frac{Kw+a}{Kw}\right)_2$	$\left(\frac{Kw+a}{Kw}\right)_1$	$\left(\frac{Kw+a}{Kw}\right)_2$	$\left(\frac{Kw+a}{Kw}\right)_1$	$\left(\frac{Kw+a}{Kw}\right)_2$	$\left(\frac{Kw+a}{Kw}\right)_1$	$\left(\frac{Kw+a}{Kw}\right)_2$	$\left(\frac{Kw+a}{Kw}\right)_1$	$\left(\frac{Kw+a}{Kw}\right)_2$	$\left(\frac{Kw+a}{Kw}\right)_1$	$\left(\frac{Kw+a}{Kw}\right)_2$	$\left(\frac{Kw+a}{Kw}\right)_1$	$\left(\frac{Kw+a}{Kw}\right)_2$	$\left(\frac{Kw+a}{Kw}\right)_1$	$\left(\frac{Kw+a}{Kw}\right)_2$	$\left(\frac{Kw+a}{Kw}\right)_1$	$\left(\frac{Kw+a}{Kw}\right)_2$	$\left(\frac{Kw+a}{Kw}\right)_1$	$\left(\frac{Kw+a}{Kw}\right)_2$	
0.10	0.917	0.912	1.022	0.947	0.912	0.869	—	—	0.874	0.985	—	—	—	—							0.931	0.928	1.402
0.20	0.986	0.901	1.042	0.902	0.716	0.786	0.934	0.864	0.780	0.866	0.920	0.916	0.638	0.930							0.859	0.881	1.363
0.40	0.899	0.871	0.953	0.885	0.653	0.709	0.811	1.021	0.757	0.776	0.832	0.815	0.518	0.783							0.775	0.807	1.342
1.00	0.777	0.772	0.736	0.696	0.563	0.691	0.632	0.689	0.724	0.708	0.588	0.593	0.415	0.659							0.634	0.687	1.290
2.00	—	—	0.385	0.425	0.391	0.474	0.431	0.427	—	—	0.447	0.545	0.324	0.491							0.396	0.472	1.173

Ethylalcohol

Alcohol conc. in mol./l.	Cell 1		Cell 2		Cell 3		Cell 4		Cell 5		Cell 6		Cell 7		Cell 8		Cell 9		Cell 10		Average $\left(\frac{Kw+a}{Kw}\right)$		$\frac{(Kw+a)_1}{(Kw+a)_2}$
	$\left(\frac{Kw+a}{Kw}\right)_1$	$\left(\frac{Kw+a}{Kw}\right)_2$	$\left(\frac{Kw+a}{Kw}\right)_1$	$\left(\frac{Kw+a}{Kw}\right)_2$	$\left(\frac{Kw+a}{Kw}\right)_1$	$\left(\frac{Kw+a}{Kw}\right)_2$	$\left(\frac{Kw+a}{Kw}\right)_1$	$\left(\frac{Kw+a}{Kw}\right)_2$	$\left(\frac{Kw+a}{Kw}\right)_1$	$\left(\frac{Kw+a}{Kw}\right)_2$	$\left(\frac{Kw+a}{Kw}\right)_1$	$\left(\frac{Kw+a}{Kw}\right)_2$	$\left(\frac{Kw+a}{Kw}\right)_1$	$\left(\frac{Kw+a}{Kw}\right)_2$	$\left(\frac{Kw+a}{Kw}\right)_1$	$\left(\frac{Kw+a}{Kw}\right)_2$	$\left(\frac{Kw+a}{Kw}\right)_1$	$\left(\frac{Kw+a}{Kw}\right)_2$	$\left(\frac{Kw+a}{Kw}\right)_1$	$\left(\frac{Kw+a}{Kw}\right)_2$	$\left(\frac{Kw+a}{Kw}\right)_1$	$\left(\frac{Kw+a}{Kw}\right)_2$	
0.07	1.023	1.052	—	—	—	—	1.049	1.143	—	—	—	—	—	—	—	—					1.036	1.098	1.276
0.14	1.085	1.102	1.035	1.025	1.025	1.066	1.049	1.162	1.038	1.050	1.092	1.015	—	—	1.022	1.116					1.049	1.077	1.317
0.28	—	—	1.066	1.047	1.058	1.189	1.050	1.258	0.987	0.929	0.873	0.875	0.960	0.987	1.044	1.143					1.005	1.061	1.280
0.7	1.147	1.160	1.144	1.201	1.100	1.274	0.972	1.295	0.924	0.875	0.742	0.738	0.891	0.972	1.046	1.165					0.996	1.085	1.241
1.05	1.165	1.195	—	—	1.082	1.209	—	—	0.816	0.794	0.715	0.669	0.863	0.966	1.032	1.095					0.946	0.988	1.294
1.40	1.102	1.118	1.196	1.245	1.069	1.126	0.916	1.198	0.837	0.761	0.588	0.628	0.928	0.947	1.014	1.020					0.956	1.005	1.286

n. Propylalcohol

Alcohol conc. in mol./l.	Cell 1		Cell 2		Cell 3		Cell 4		Cell 5		Cell 6		Cell 7		Cell 8		Cell 9		Cell 10		Average $\left(\frac{Kw+a}{Kw}\right)$		$\frac{(Kw+a)_1}{(Kw+a)_2}$
	$\left(\frac{Kw+a}{Kw}\right)_1$	$\left(\frac{Kw+a}{Kw}\right)_2$	$\left(\frac{Kw+a}{Kw}\right)_1$	$\left(\frac{Kw+a}{Kw}\right)_2$	$\left(\frac{Kw+a}{Kw}\right)_1$	$\left(\frac{Kw+a}{Kw}\right)_2$	$\left(\frac{Kw+a}{Kw}\right)_1$	$\left(\frac{Kw+a}{Kw}\right)_2$	$\left(\frac{Kw+a}{Kw}\right)_1$	$\left(\frac{Kw+a}{Kw}\right)_2$	$\left(\frac{Kw+a}{Kw}\right)_1$	$\left(\frac{Kw+a}{Kw}\right)_2$	$\left(\frac{Kw+a}{Kw}\right)_1$	$\left(\frac{Kw+a}{Kw}\right)_2$	$\left(\frac{Kw+a}{Kw}\right)_1$	$\left(\frac{Kw+a}{Kw}\right)_2$	$\left(\frac{Kw+a}{Kw}\right)_1$	$\left(\frac{Kw+a}{Kw}\right)_2$	$\left(\frac{Kw+a}{Kw}\right)_1$	$\left(\frac{Kw+a}{Kw}\right)_2$	$\left(\frac{Kw+a}{Kw}\right)_1$	$\left(\frac{Kw+a}{Kw}\right)_2$	
0.106	0.922	0.912	0.897	1.050	0.930	0.958	0.975	1.032	0.860	0.961	0.885	0.809	0.931	0.913	—	—	0.986	0.983	0.943	0.915	0.925	0.948	1.669
0.212	0.696	0.737	0.867	1.009	0.876	0.888	0.905	0.946	0.820	0.907	0.656	0.645	0.813	0.851	0.854	0.863	0.968	0.942	0.809	0.809	0.826	0.860	1.642
0.424	0.484	0.486	—	—	0.835	0.817	0.757	0.848	0.786	0.829	—	—	0.688	0.688	0.641	0.796	0.867	0.890	0.729	0.742	0.723	0.762	1.623

n. Butylalcohol

Alcohol conc. in mol./l.	Cell 1		Cell 2		Cell 3		Cell 4		Cell 5		Cell 6		Cell 7		Cell 8		Cell 9		Cell 10		Average $\left(\frac{Kw+a}{Kw}\right)$		$\frac{(Kw+a)_1}{(Kw+a)_2}$
	$\left(\frac{Kw+a}{Kw}\right)_1$	$\left(\frac{Kw+a}{Kw}\right)_2$	$\left(\frac{Kw+a}{Kw}\right)_1$	$\left(\frac{Kw+a}{Kw}\right)_2$	$\left(\frac{Kw+a}{Kw}\right)_1$	$\left(\frac{Kw+a}{Kw}\right)_2$	$\left(\frac{Kw+a}{Kw}\right)_1$	$\left(\frac{Kw+a}{Kw}\right)_2$	$\left(\frac{Kw+a}{Kw}\right)_1$	$\left(\frac{Kw+a}{Kw}\right)_2$	$\left(\frac{Kw+a}{Kw}\right)_1$	$\left(\frac{Kw+a}{Kw}\right)_2$	$\left(\frac{Kw+a}{Kw}\right)_1$	$\left(\frac{Kw+a}{Kw}\right)_2$	$\left(\frac{Kw+a}{Kw}\right)_1$	$\left(\frac{Kw+a}{Kw}\right)_2$	$\left(\frac{Kw+a}{Kw}\right)_1$	$\left(\frac{Kw+a}{Kw}\right)_2$	$\left(\frac{Kw+a}{Kw}\right)_1$	$\left(\frac{Kw+a}{Kw}\right)_2$	$\left(\frac{Kw+a}{Kw}\right)_1$	$\left(\frac{Kw+a}{Kw}\right)_2$	
0.026	1.017	1.045	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	1.017	1.045	1.610
0.052	0.930	0.991	0.890	0.934	0.952	0.995	0.923	0.782	0.991	0.964	1.017	0.875	0.974	0.956	0.939	0.891	0.918	0.861	0.955	1.006	0.949	0.926	1.696
0.104	0.713	0.848	0.774	0.874	0.869	0.958	0.794	0.806	0.988	0.982	1.017	0.771	0.829	1.234	0.811	0.800	0.901	0.843	0.958	1.153	0.865	0.860	1.544
0.156	—	—	—	—	0.843	0.962	0.738	0.752	0.820	1.012	0.948	0.680	0.736	1.219	0.743	0.728	—	—	0.689	0.936	0.788	0.812	1.453

Table VIII indicates these average values and includes the complete material, treated statistically. In the "General discussion" these values will be considered further. Graph XXI depicts the results.

If the quotient between $\left(\frac{Kw + a}{Kw}\right)_1$ and $\left(\frac{Kw + a}{Kw}\right)_2$ is determined, we obtain $\frac{\left(\frac{Kw + a}{Kw}\right)_1}{\left(\frac{Kw + a}{Kw}\right)_2} = \frac{(Kw+a)_1}{(Kw+a)_2} \times \frac{Kw_2}{Kw_1}$ ..... (1)

$\frac{Kw_2}{Kw_1}$ is constant — see in § 1, $\frac{Kw_1}{Kw_2}$ is also constant — and if we multiply (1) by the reciprocal of this quotient (last Column of table VI), we obtain the value $\frac{(Kw + a)_1}{(Kw + a)_2}$ ..... (2.)

These values are found in the last column of table VII. They indicate the quotient of the endosmosis and exosmosis K for alcohol/water mixtures. It appears from the values plotted in graph XXII (against the log of the alcohol concentration), that the quotient is influenced by alcohols in increasing concentration. This influence consists in equalization of the values $(Kw + a)_1$ and $(Kw + a)_2$. Alcohols, therefore, decrease the quotient $\frac{K_1}{K_2}$ For water without alcohol — i.e. $\frac{(Kw)_1}{(Kw)_2}$ — this value is indicated in graph XXII by a dotted line. This level constitutes our baseline. From the graph it appears that all these levels are not equal. They are calculated as averages from the blanks corresponding to the experiments made with one and the same alcohol.

This average $\frac{(Kw)_1}{(Kw)_2}$ is nearly equal for the experiments with methyl- and with ethyl alcohol. The values are resp. 1.398 and 1.352. For the experiments with n. propyl- and n. butyl-alcohol the values are 1.710 and 1.655 respectively.

Both the two latter and the two former values approach one another. It should be kept in mind, however, that the material used in the experiments with methyl- and with ethyl alcohol were collected at a different locality and in another season than the material used in the experiments with n. propyl- and n. butyl-alcohol. From the graph it may possibly be concluded that ethyl-alcohol exerts its influence on $\frac{(Kw + a)_1}{(Kw + a)_2}$ in a small degree,

TABLE VIII

Methylalcohol

Log alcohol concentration	Average $\frac{Kw + a}{Kw}$	Log average $\frac{Kw + a}{Kw}$
0.000—1	0.930	0.969—1
0.301—1	0.870	0.940—1
0.602—1	0.791	0.898—1
0.000	0.661	0.820—1
0.301	0.434	0.638—1

Ethylalcohol

Log alcohol concentration	Average $\frac{Kw + a}{Kw}$	Log average $\frac{Kw + a}{Kw}$
0.845—2	1.067	0.028
0.146—1	1.063	0.027
0.447—1	1.033	0.014
0.845—1	1.041	0.017
0.021	0.967	0.985—1
0.146	0.980	0.991—1

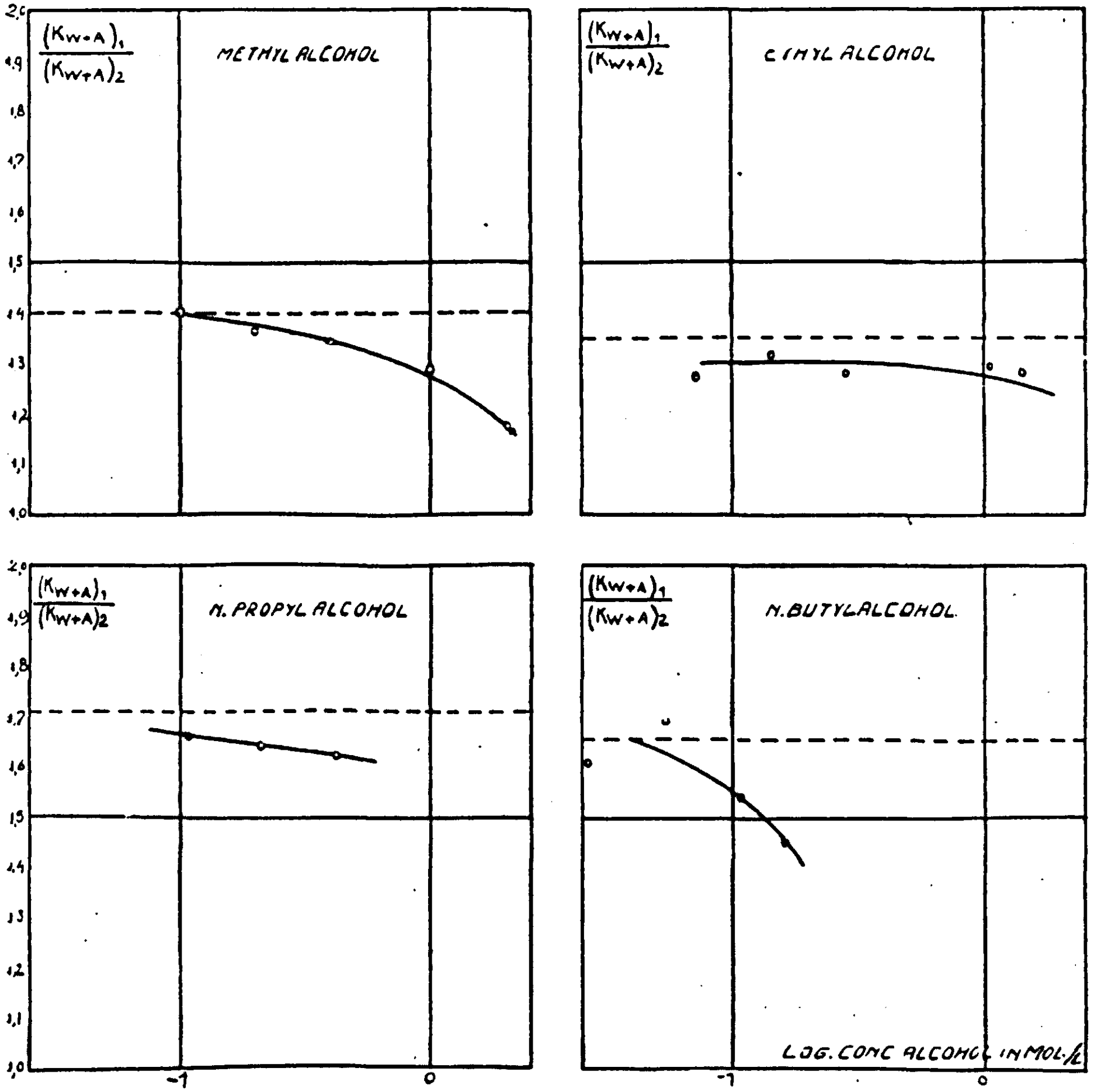
n. Propylalcohol

Log alcohol concentration	Average $\frac{Kw + a}{Kw}$	Log average $\frac{Kw + a}{Kw}$
0.025—1	0.937	0.972—1
0.326—1	0.843	0.926—1
0.627—1	0.743	0.871—1

n. Butylalcohol

Log alcohol concentration	Average $\frac{Kw + a}{Kw}$	Log average $\frac{Kw + a}{Kw}$
0.716—2	0.938	0.972—1
0.017—1	0.863	0.936—1
0.193—1	0.800	0.903—1

Graph XXII



n. propyl alcohol in a higher degree and n. butyl alcohol in a still more pronounced fashion. The place of methyl alcohol is not well defined. Perhaps it acts a little more feebly than n. butyl alcohol, the concentration range of the latter being situated within such narrow limits that the graph does not allow us to determine at which concentration of n. butyl alcohol the value $\frac{(Kw + a)_1}{(Kw + a)_2}$ becomes equal to 1.

If the alcohols are arranged in a series according to their

action on $\frac{(Kw + a)_1}{(Kw + a)_2}$, this series will in all probability be as follows:

n. butyl-, methyl- > n. propyl > ethyl alcohol.

This represents the sequence of the homologous series, methyl alcohol exepted. It is worth mentioning that this series is the series of the permeation-velocity of the alcohols itself. The same sequence is seen in graph XXI, p. 790.

§ 6. Permeation-velocity of the alcohols.

The permeation velocity of the alcohols appeared to be dependent upon the concentration for methyl and ethyl alcohol, if at least the longitudinal changes of the cell may be taken as criteria. Essentially the same criterion has been taken as in the plasmometric method. Methyl- and ethyl alcohol may permeate in higher concentrations (not regularly observed) more rapidly than water, which is concluded from the fact that a cell in equilibrium in distilled water or in distilled water plus x mol. alcohol at first expands and then shrinks on addition of distilled water plus x_1 mol. alcohol ($x_1 > x$). This observation also accounts for the phenomenon that cells in a 2—4 mol. alcoholic solution burst more frequently than in distilled water without alcohol [82]). This increased permeation of the alcohol occurs for methyl alcohol only at concentrations of 2 to 3 mol. and higher. With some cells it already took place at 1 mol alcohol. For ethyl alcohol the concentrations at which the alcohol permeates more rapidly are situated, between 1 to 3 mol. In ethyl alcohol, however, the phenomenon does not occur so often as in methyl alcohol. Therefore, with ethyl alcohol shrinkage is usually obtained, that is to say that water keeps permeating more rapidly than the alcohol. In n. propyl alcohol the phenomenon was never observed; neither in n. butyl alcohol.

GENERAL DISCUSSION.

As indicated in the General Introduction, it was endeavoured to consider the results of the biological work in the light of certain experiments upon colloid-systems. As many such attempts have been made, it should be clearly understood that it has not been the intention of this investigation to directly compare the living with the inanimate. In the colloid-models we

82) On account of the fast penetration of the alcohol the cell expands in such an active way that the wall gives way. The higher the concentration of alcohol, the more cells burst per unit-time.

only see the range of possibilities that nature offers, possibilities which may help us to understand vital phenomena, both, in the nature of its factors and in the way in which these factors are correlated. Inasmuch as phosphatid-systems may be nearest to the protoplasmic membrane both in structure and in composition (ch. IV, A, p. 765) the influence of alcohols on these systems (Chapter II) has been compared with the influence of alcohols upon the protoplasmic membrane of the living cells of Chara ceratophylla.

On account of the preceeding considerations we see some agreement in graph IV, page 732 (phosphatid coacervates) and graph XXI, page 790 (algal cells).

In both graphs the curves of methyl alcohol descend; the ascending part of the curve of methyl alcohol of graph IV would disappaer if one had to do with a system of higher charge-density.

The curves of ethylalcohol at first ascend in both graphs and then descend.

The curves of n. butylalcohol have the same shape in both graphs.

The alcohols, however, exert their influence upon the living membrane at much lower concentrations than they do upon the phosphatid-coacervates (ratio 1 : 10). Another difference between the model and the living membrane appears from the action of n. propylalcohol. This substance decreases the permeability of the living protoplast which means, according to our theory that it causes a condensation of the membrane, while this alcohol exerts an opening up action on the phosphatid coacervate. This diversity may be ascribed to the difference in charge-density as (chapter II) in phosphatid systems of high charge-density the addition of n. propylalcohol causes the system to condense (graph III, p. 729). The condensation in this case may be explained by the influence of the alcohol on the dielectric medium.

The assumption of a membrane with high charge-density needs not alter the shape of the curves for methyl- and ethyl alcohol. It might be possible, that methylalcohol produces a stronger shrinkage, i.e. a decrease of the permeability, on account of the increase of the charge-density of the membrane-components. This might also be the case with ethylalcohol, which would cause the permeability to decrease already in lower concentrations.

At very low concentrations, n. propylalcohol may increase the permeability of the protoplasm-membrane in a similar way as

it increases the volume of the coacervate.

Table VI shows, that the lowest concentration of ethylalcohol was 0.07 mol/l, and of n. propylalcohol 0.106 mol/l. It is not to be excluded that a proportionally lower concentration of n. propylalcohol (0.02 mol/l) would increase this permeability. The values represented in graph XXI are not contrary to this supposition. Further experiments might elucidate this matter.

The influence of n. butylalcohol on the Alga cannot be predicted from graph IV.

The influence of alcohols on the dielectric medium is significant at concentrations 10 times as high as those in which they exert an influence upon the permeability. The alcohols may exert a perceptible influence upon the system, if this system be near its zero charge. The values found in the literature for the surface charge of the protoplast need not be those of the zone which regulates the permeation of substances (DE HAAN, 1935 WAKKIE 1936). Therefore the above assumption (the membrane being near its zero charge) need not to be in conflict with the values found in literature.

If we want to give a provisional explanation of the influence of alcohols on the permeability of the protoplasm membrane, we have to take into account:

a. the action of these alcohols on the carbon chains of the membrane-components;

b. the action of these alcohols on the dielectric condition of the system;

c. the structure of the system (amorphous or oriented).

From the fact that the results of the biological research are compared with the behaviour of phosphatid-coacervates, one should not conclude that the protoplasmic membrane is an amorphous system.

The sensitized amorphous models dealt with in Chapter II as well as the oleate systems which consist of liquid crystals both show that with the decrease in distance between the components of such a system, these distances may be increased by the addition of alcohols which show a condensing action upon the unsensitized coacervates. A reasoning by analogy therefore would lead one to expect that alcohols should show an opening up action on a protoplasmic membrane composed of directed components. For the oriented systems dealt with in Chapter III the charge factor, however, is of small importance to the density of the molecular packing, as these systems are far removed from their zero charge. Only further research upon orien-

ted systems in which the electric charge might exert a great influence, will yield the necessary data to prove this case. A symmary of this work is given in the general introduction, the introduction in Chapter II and the discussion in Chapter III.

I avail myself of this opportunity to tender my best thanks to Prof. H. G. Bungenberg de Jong for the way in which he guided me in the problem of colloid models and for the favour he granted me to work in his Laboratory. Also to Prof. R. Collander I express my gratitude for the hospitality extended to me in his Laboratory at Helsingfors during two successive summers. He introduced me into the problem of the permeability of plant cells.