RESEARCHES ON PLANT GROWTH REGULATORS

XV. THE INFLUENCE OF FATTY ACIDS ON SOAPCOACERVATES

by

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I. INTRODUCTION

In the course of time indications became more and more numerous that on the outside of protoplasm there is a layer which regulates the permeability or rather the intrability of the protoplasm for dissolved substances. Especially the experiments of CHAMBERS and his collaborators really leave no possibility for other opinions. A solution of sodium chloride, when brought into the interior of an Amoeba is absolutely harmless, but on the outside of the cell the salt has a toxic effect. With a solution of calcium chloride it is exactly the other way about: an Amoeba, brought into such a solution, does not experience the slightest damage, but if the $CaCl_2$-solution is injected into the Amoeba an irreversible flocculation results (CHAMBERS AND REZNIKOFF, 1926). Therefore, the protoplasm of these Amoeba is evidently covered with a protective outer layer.

Indications of such a regulating protoplasmic membrane can also be found with other organisms. It does not seem too presumptuous to postulate the presence of a protoplasmic membrane in all living cells. This does not mean that this membrane might be observed with a microscope and it must certainly not be confused with the cell-walls formed by living protoplasm as *e.g.*, the cellulose-wall of plant cells. The membrane is part of the protoplasm which only owes its particular properties to its position (the border cell/medium). It has often been observed that when the protoplasm is damaged (the contents of which are usually miscible with water) a new membrane is formed spontaneously. This can be traced with certain dyes, which diffuse freely through the protoplasm but are checked by the protoplasmic membrane (NAEGELI).

There is but little direct information about these protoplasmic membranes. As the membrane will determine the permeability of the cell, experiments on the permeability may give indications regarding the structure of the protoplasmic membrane.

It is a difficult task to select the most satisfactory solution from the many given on the question of permeability. In our opinion all theories on permeability may be reduced to two principles:

a. The permeating substance is soluble in the protoplasmic membrane.

b. The permeating substance passes through pores in the membrane.

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** Influence of organic compounds on oleate and phosphatide coacervates VIII, The lipoid theory (OVERTON) is centered around the first principle and the filter theory (M. TRAUBE, RUHLAND) ascribes the permeability to the second mechanism. It seems worth while to show that the many other theories do not differ in essentials from these two theories.

1. The micellar theory (NAEGELI-PFEFFER) is essentially a filter theory.

Here it is important that the pores can be changed by external influences. The real filter theory does not harbour this possibility.

2. In our opinion the adsorption theory (J. TRAUBE) cannot be considered to be a permeability theory *sensu stricto*. Usually two factors that play a part in the permeating of substances are involved, *viz.* the permeability through the protoplasmic membrane and the instigating force — *i.e.*, the difference in the concentration of the substance on both sides of the membrane. If a compound is adsorbed at the protoplasmic surface it need not be concluded that the permeability for that compound is abnormally high, only that the instigating force is greater (at least if the solubility of the substance in the protoplasm is larger than in water). For a positive adsorption means a higher concentration and therefore a seemingly greater permeability. TRAUBE himself points out that the adsorption theory is essentially a support of the lipoid theory and that it does not exist by itself.

3. Electrostatic influences — to which MICHAELIS draws the attention — can increase or decrease the permeability (depending upon whether the compound and the membrane have an opposite or a similar electric charge). In this case we must speak of influences upon the resistance.

4. A consideration of the so-called colloid-chemical theory of HANSTEEN CRANNER immediately shows that here we have to do with a variation of the lipoid theory. HANSTEEN CRANNER only indicates that the membrane would consist of two phases: phosphatides soluble in water and insoluble phosphatides.

5. Besides these there are a few theories that try to combine the two principles. CLOWES compares the protoplasmic membrane with an emulsified colloid. Under the influence of certain electrolytes it is possible for an emulsion of oil in water to change into one of water in oil. According to CLOWES something similar would also take place in the protoplasmic membrane.

According to the mosaic theory (NATHANSON) the protoplasmic membrane is composed of two kinds of structures. The first will follow principle *a*, the other principle *b*. In a more modern setting this theory is called the lipoid-filter theory (COLLANDER).

Reviewing these possibilities we can combine them all in a few rules:

1. Some molecules permeate as one would expect according to their solubility in lipoids, while the permeation of other molecules is mainly determined by their size.

2. The biological objects are very different, all possible forms between principles *a* and *b* do exist.

It is evident that now our ideas concerning the protoplasmic membrane must fit in with these data. Hence we may conclude that a membrane of a thick lipoid layer (thickness of many molecules), as well as a membrane without lipoid, are not very probable. In the first case substances that are insoluble in lipoid (even water) would not be able to permeate, in the second case one would not understand why non-polar substances permeate so quickly.

Then it appears that in molecular dimensions (a mono-, di-, or paucimolecular layer of lipoid molecules) the principles *a* and *b* can be united without the necessity of accepting a heterogeneous structure of the protoplasmic membrane as in the mosaic theory. In many cases it seems admissible that besides lipoids there are also proteins in the membrane. As there is little reason for these substances to accumulate on the border cell/medium under influence of the boundary tension, another reason must be found for this. We here think of complex relations (Bungenberg de Jong, 1938, 1936): electrostatic attraction forces which are probably the strongest stabilising factors of the membrane. In connection with these considerations especially amphoteric phosphatides must be assumed to occur as constituents of the protoplasmic membrane (Bungenberg de Jong and Bonner, 1935). Fig. 1 gives a very schematic picture of a membrane, conceived in this manner.

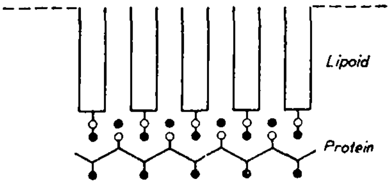

Fig. 1. Diagram of the structure of the protoplasmic membrane (the black spots represent positive groups, open circles are groups with a negative charge). This membrane is stable because of the strong complex relations in the polar part of the membrane. The component parts are: lecithin, protein and a cation (usually Ca).

Here we must note that the most important points of this theory of the protoplasmic membrane — which we should like to call the complex theory, because the complex-relations play such an important part — are the following:

1. The regulating part proper of the protoplasmic membrane with respect to uncharged molecules consists of hydrocarbon chains in positions parallel to each other (Fig. 1, upper part).

Already some time ago Bungenberg de Jong and Saubert (1937) put forward the idea that in this membrane cholesterol would be present as a "condensing substance", *e.g.*, in the way as shown in Fig. 2.

This diagram shows how the filter theory and the lipoid theory are mutually compatible in molecular dimensions: place *a* represents a pore in the lipoid-membrane. Care must be taken not to consider these diagrams from a static point of view only. The thermal movement of the paraffin chains will give the membrane an ever changing aspect. The chance that at some given moment a pore of small diameter will occur is great (even in a quite homogeneous membrane), and though the chance for wider pores decreases continuously, it does remain. This seems to be a plausible explanation for the fact that with an enlargement of the molecular volume of the permeating substance, the permeability for that substance decreases considerably. We do not consider it necessary to presume that there are many small pores, few large and very few very large pores. If the protoplasmic membrane is looked upon as a dynamic system then a homogeneous molecular layer of lipoids must also show this effect. It is evident that the permeability is very strongly affected by the nature of the paraffin chains (many or few unsaturated chains) and the amount and nature of the condensing substance (*e.g.*, cholesterol).

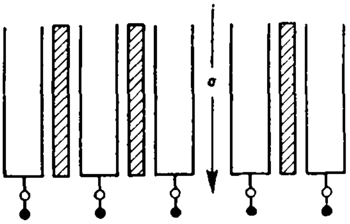

Fig. 2. Cholesterol can be present as a condensing factor between lecithin-molecules. Whenever the cholesterol is missing we have to do with a "pore" in the membrane (a). According to Bungenberg de Jong and Saubert, 1937.

2. The permeation of charged compounds (*viz.*, ions) will greatly depend on the momentary situation of the electric system of the membrane (Fig. 1, lower part).

Winkler's experiments (1940) on the membrane of the pig's erythrocytes give fine support to the idea that the membrane is a tricomplex system. WINKLER is able to conclude from the ionic spectrum of the erythrocytes that phosphatides play an important part. The membrane proves to be a tricomplex of phosphatide, stromatin and a cation. It will be possible to replace the cation in the protoplasmic membrane by another cation, which may give rise to great changes in the permeability (BOOIJ, 1940).

Of course our diagram (Fig. 1) is very schematic. It is not impossible that the phosphatide molecules are turned towards the interior or that there are several layers of parallely arranged molecules present. It is of special interest, however, that the complex relations are of vital importance for the structure of the membrane. Without Coulomb forces between the polar groups of the membrane components it is impossible to imagine how on the boundary protoplasm/medium a layer of lipoid molecules in parallel arrangement would be formed.

Certain properties of the protoplasmic membrane can be studied on coacervates of lecithin (BUNGENBERG DE JONG, 1937). But such coacervates are rather unmanageable, and if only the properties of the non-polar part of the membrane need be studied then one can make very good use of soap coacervates, since very probably the molecules in concentrated soap solutions are arranged in a parallel manner (HESS, 1941; STAUFF, 1941). Therefore this model will be especially useful for examining non-electrolytes or molecules with a large non-polar part. Such an investigation was made by BUNGENBERG DE JONG and collaborators (1938). In this regard it is important that the organic non-electrolytes — as to their reaction on oleate coacervates — can be placed in a certain sequence and that practically the same sequence is encountered in tracing the inhibiting influence of these compounds upon the germination of pollen of sweet pea (BOOIJ, 1940).

The natural and synthetic compounds acting as plant growth substances all consist of molecules with a considerable non-polar part. Besides this non-polar part they also contain a carboxyl group. VELDSTRA (1944) deduced from experiments on the relations between the structure and the activity of the plant growth substances that compounds of this structure may possibly influence the permeability of plant cells, in that sense that they would further the permability by a turgescent or opening influence on the membrane at a low concentration (physiologically stimulating), whereas they would probably have a condensing effect in higher concentrations (physiologically inhibiting).

To test this hypothesis experiments were made regarding the influence of plant growth substances on systems which, to a certain extent, can be considered to be models of the protoplasmic membrane. This was done firstly by examining the interaction between the compounds mentioned and mono-molecular layers of lecithin (HAVINGA, VELDSTRA, 1948) and secondly, as is described in this and in the following paper, by examining the influence of growth substances on coacervates, especially on oleate coacervates. According to the hypothesis a pronounced interaction with the lecithin film and an opening effect on the above-mentioned coacervate-systems might be expected. Of course we must not forget that these are only models and that the real relations are far more complicated. All the same, clear indications can be obtained by these experiments.

Preliminary experiments by one of us (BUNGENBERG DE JONG) showed that indeed α-naphthaleneacetic acid has a turgescent action on soap coacervates, as the coacervation is annulled by adding a small quantity of α-naphthaleneacetic acid. The p_H of these soap coacervates is high, so in this case the anion, and not the undissociated molecule, is active*.

Many compounds had already been examined in connection with their action on oleate coacervates (BUNGENBERG DE JONG *et al.*, 1938; ROSENTHAL, 1939), but practically all these belonged to the non-electrolytes.

Before extending our experiments to the synthetic plant growth substances, we thought it would be worth while to examine previously more simple anions. For this we chose the homologous series of normal fatty acids. When the action of this series is known, the effect of the growth substances can be compared with it.

2. EXPERIMENTS WITH FATTY ACIDS (ANIONS)

BUNGENBERG DE JONG *et al.* (1938) examined a large number of organic compounds as to their influence on oleate coacervates and were able to deduce the following general rules. We must, however, emphasize the fact that these rules have only been found for non-electrolytes.

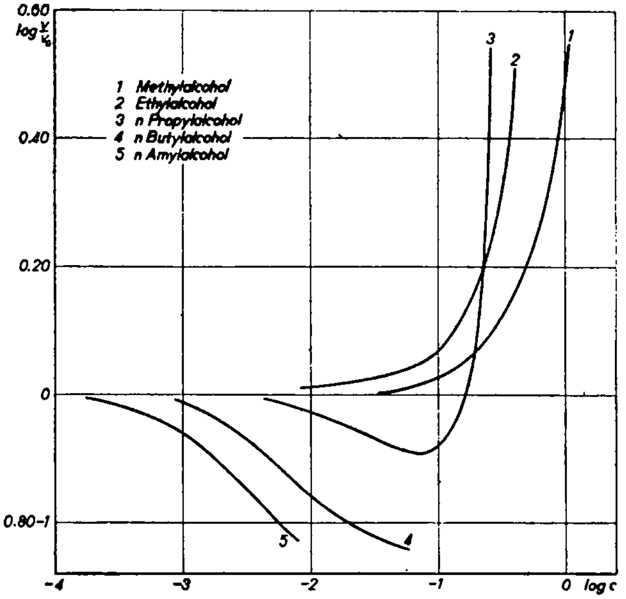

Fig. 3. Action of the normal alcohols on the volume of an oleate coacervate (BUNGENBERG DE JONG, BOOIJ AND SAUBERT, 1937).

1. In a homologous series of compounds with a polar group the minimal concentration displaying activity decreases with an increasing number of carbon atoms.
2. In such a series the reaction is generally inverted at a given chain-length, the lower terms having a turgescent, the higher a condensing effect. Fig. 3 shows this for the series of the normal alcohols.

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* Botanical experiments gave rise to the conviction that only undissociated molecules would be physiologically active (cf. STRUGGER, 1932; BONNER, 1938 and VAN SANTEN, 1940). In one of the following articles we shall discuss this contradiction more in detail.

3. The influence on oleate coacervates of compounds with a hydrophilic group is the result of the antagonistic action of two factors:

   a. a condensing influence of the hydrocarbon chain,

   b. an opening influence of the polar group.

4. The condensing effect of the hydrocarbon chain increases with its length. With an equal number of carbon atoms the influence decreases on branching of the chain, on ring-closure and on the substitution of a saturated six-membered ring by an aromatic nucleus.

5. Introduction of a halogen into an aliphatic chain results in a stronger condensing effect.

6. The OH-, ether-, ketone-, ester-oxygen-groups may be mentioned as promoting a turgescent effect.

7. By mutual comparison of the action of polar groups it appears that with equal numbers of carbon atoms *e.g.*, urethanes have a stronger effect than primary alcohols and that ketones act more strongly than secondary alcohols.

Resuming it may be concluded that the action of a compound is determined by the structure of the hydrocarbon chain (number and distribution of the carbon atoms and their type of binding) and by the nature, number and location of the polar groups.

The experimental method was as follows (BUNGENBERG DE JONG *et al.*, 1937): A gradually increasing quantity of KCl is added to an oleate solution and as a result two

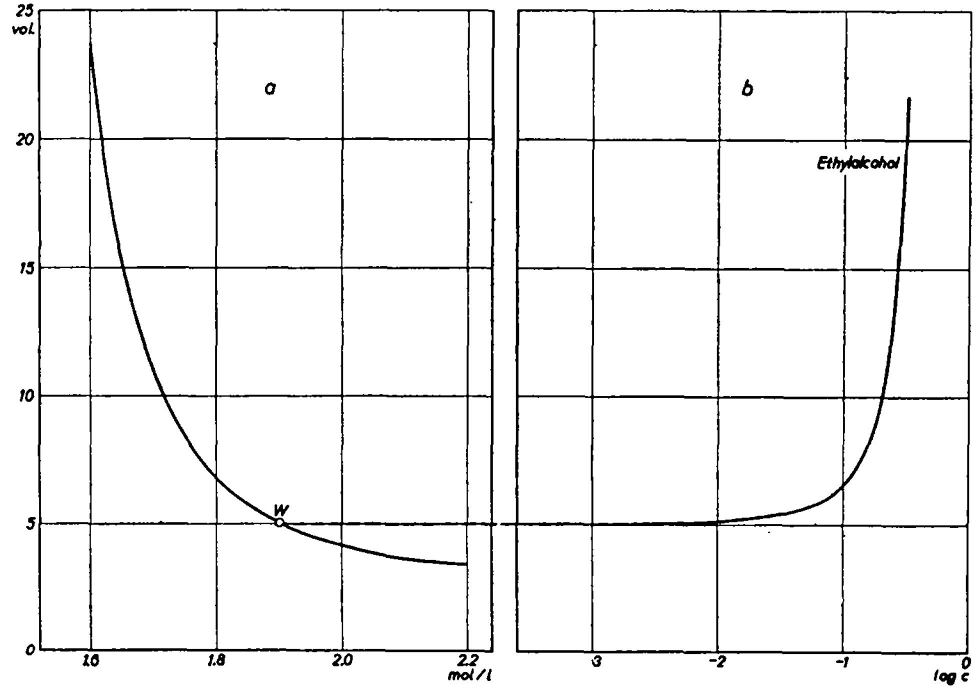

Fig. 4. Experimental method of BUNGENBERG DE JONG *et al.*, concerning the influence of different compounds on oleate coacervates.

a. KCl causes the formation of a coacervate in an oleate solution, the volume of which decreases as the concentration of KCl increases.

b. On this curve we choose a convenient working-point (w) and add (with a constant KCl concentration) increasing quantities of the compound to be examined (*e.g.*, ethyl-alcohol).

liquid phases begin to separate at a certain concentration. The top layer contains practically all the soap. This layer becomes thinner as the concentration of KCl increases (see Fig. 4a).

We now choose a convenient working-point on this curve and add, keeping the KCl concentration constant, an increasing quantity of the compound to be examined (*e.g.*, ethylalcohol, see Fig. 4b). If the top layer (which is relatively rich in colloid) increases in volume -- meaning that this layer gradually takes up more and more water -- we have to do with a turgescent effect. The influence of a condensing substance will appear by a decrease of the coacervate layer.

This method is not suitable for examining the fatty acid anions. As is known, the solubility of soap rapidly becomes low. Most fatty acids, however, are well soluble in an oleate-solution to which an excess of KOH has been added. We can then compare the KCl curve before and after the addition of the fatty acid and the shifting of the curve tells us something about the action of the added substance.

So our method takes the following form: A 2% solution of sodium oleate is prepared (with a strongly sensitised soap it is advisable to add some KOH, see BUNGENBERG DE JONG *et al.*). To 500 ml of this solution are added 50 ml of 2 N KOH (this serves as a blank). The desired quantity of fatty is weighed and to this *e.g.*, 100 ml of the blank are added. From this solution, with the aid of the blank, different solutions are prepared, so that a series of oleate solutions is obtained in which the added compound is present in different concentrations. In wide test-tubes the following mixtures are made, which are shaken thoroughly:

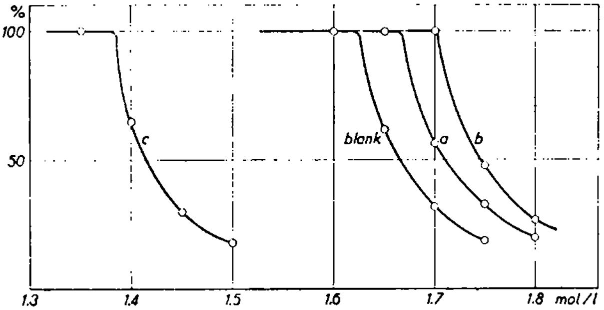

Fig. 5. After determining the KCl-curve for the separation of the oleate (blank) the influence of palmitate is measured (a = 0.625 m mol; b = 1.25 m mol). The graph shows the volume of the coacervate expressed as per cent of the volume of the whole liquid. The shifting of the KCl-line (taken at 50%) is a measure for the turgescent effect of the added compound. For comparison the influence of a condensing substance is also given (c = benzylalcohol, 5 m mol).

5 ml of oleate (with or without added substance)
x ml of 3.8 N KCl
(14 - x) ml of H_2O

The test-tubes are placed in a thermostat* and the next day the height of the coacervate layer is read. We plot the percentage of the layer with respect to the total column against the KCl concentration (Fig. 5).

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* When we started these experiments it was not yet possible (on account of gas rationing) to work at a constant temperature. We therefore performed the experiments at room-temperature (which generally varied between 12° and 16° C) and to avoid sudden changes of temperature the tubes were placed in a large volume of water. The influence of the temperature on coacervation is, however, rather appreciable and as soon as it was possible we worked in a thermostat.

The shifting of the KCl-curve to higher concentrations means that the added palmitate ion has a turgescent effect. Evidently more KCl is necessary to attain the same degree of coacervation. For comparison the curve for the addition of a condensing substance (benzylalcohol) is also given.

In this way the homologous series of normal fatty acids* was examined (see Table I).

TABLE I

CONCENTRATION OF ADDED FATTY ACID ANION IN MMOL

Number of C-atoms	0.15^{5}	0.31	0.62^{5}	1.25	2.5
8	—	—	—	0.05^{5}	0.12^{5}
9	—	—	—	0.17^{5}	0.41
10	—	—	—	0.39	0.91
11	—	—	—	0.44	0.90
12	—	—	—	0.37^{5}	0.73
13	—	—	—	0.27^{5}	0.51
14	—	—	—	0.18	0.32
15	—	—	—	0.09	0.17
16	—	—	0.04^{5}	0.08	0.12
18	—	—	0.06^{5}	0.11^{5}	0.11
20	0.05^{5}	0.10	0.20^{5}	0.22^{5}	—
22	0.06	0.10	0.21	0.22^{5}	—

Shifting of the KCl-line (in normality) under influence of different fatty acid anions (temperature 12–16° C). The substrate is an oleate coacervate.

On examining the higher terms difficulties are encountered owing to the insolubility of these soaps, as shown in Fig. 6. Generally the shifting of the KCl-curve in low concentrations (the point at a height of 50% is considered) is proportional to the concentration of the added compound.

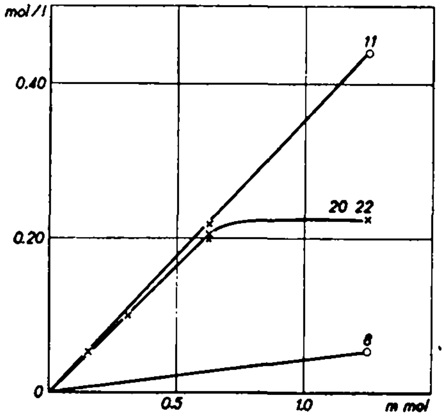

Fig. 6. The shifting of the KCl-curve (in mol/l) for the lower members of the homologous series of fatty acid anions is practically proportional to the concentration (compare undecanoate(11) and caprylate(8). With the higher members trouble is encountered in the insolubility above a certain concentration (see arachidate(20) and behenate(22).

In order to compare the effects of the different fatty acid anions we must of course remain below the concentration at which the curves begin to flatten. Hence we plot the shifting of the KCl concentration, caused by adding 0.5 m mol of anion, against the number of carbonatoms (see Fig. 7).

The activity-curve of the homologous series of the fatty acid anions with 8 to 22 C-atoms shows an unexpected and remarkable course. At first sight there does not seem to be any reason whatsoever that the turgescent effect should first increase with the lengthening of the chain, then decrease and finally increase again. What we expected theoretically — owing to the simple constitution of the added compounds and of the oleate — was an effect (either turgescent or condensing), becoming more marked with the lengthening

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* We are very much indebted to Prof. Dr P. E. Verkade, Delft, who was so kind as to place a large number of fatty acids of high purity at our disposal.

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of the carbon chains. From former experiments (BUNGENBERG DE JONG *et al.*, 1937) we already knew that the long-chain alcohols have a strong condensing effect. Replacing the OH-group by a carboxyl-group (in a dissociated state) causes a strong turgescent effect. Obviously it is our first task to explain the remarkable activity curve of the fatty acid anions.

3. THEORETICAL BACKGROUND OF THE EXPERIMENTS

Trying to explain Fig. 7 we come to consider the following possibilities:

1. Though not easily explainable on theoretical grounds, it might be possible that at certain lenghts of the hydrocarbon chains a stronger effect occurs, expressing itself in a periodicity. Then we would conclude from Fig. 7 that in the neighbourhood of valeric acid there will be another minimum. Under the conditions sketched in Fig. 7 the lower terms show no effect whatsoever in low concentrations so that in this case another method ought to be applied.

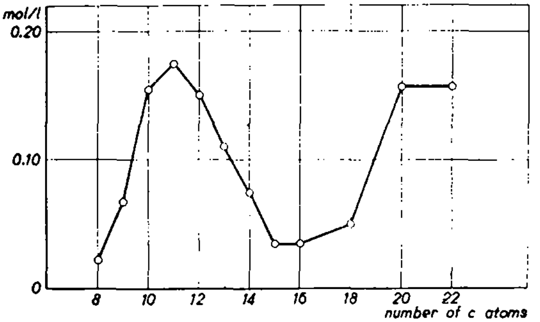

Fig. 7. Shifting of the KCl-curve (in mol/l) under influence of 0.5 m mol. of added fatty acid anion (the substrate is an oleate coacervate, temperature 12–16° C). The influence is evidently strongly dependent on the number of carbon atoms of the added compound.

It would certainly also be worth while to examine acids longer then behenic acid, but few of these acids are as yet known and obtainable in the pure state.

2. It is known that many soaps are comparatively poorly soluble and crystallise from their solutions. The solubility is much greater in oleate solution than in water. If we now presume that the higher members of the homologous series form small crystalline micells when KCl is added, independent of the oleate coacervate, then we can understand the decrease of the activity of these higher terms. These micells must, however, be very small indeed, as the coacervates are absolutely clear. Moreover the increase of activity of arachidic and behenic acid remains incomprehensible. In any case an increase of the temperature should have a considerable influence because it checks the forming of crystalline micells.

3. There are indications that in soap solutions micells are present, in which the soap molecules (or ions) are arranged in a parallel manner (HESS *et al.*, 1941). It does not seem improbable that there is also a similar arrangement in soap coacervates. Considering such an oleate coacervate, in which the oleate molecules are somehow arranged parallel to one another, it does not matter for the experiment whether or not we add a small extra quantity of oleate. Molecules of a different length (shorter as well as longer ones) do not fit into the orderly micell; the order is disturbed and we observe this as the "turgescent effect" of these non-fitting molecules. That is how this conception can explain the minimum at 15 to 16 C-atoms. We must remember that the effective length of the hydrocarbon chain of the oleate molecule is certainly less than 18 C-atoms because oleic acid possesses the cis-configuration.

First of all we turn our attention to the lower members of the homologous series. As already mentioned these anions show no activity in low concentrations. Only with caproate can we demonstrate a slight turgescent effect with the above-mentioned method. If we want to test the lower terms we will have to change our method. The method to be followed is clear, instead of KCl we use KOH as a means of causing the coacervation. We also do not dissolve the acid to be examined in the oleate solution, but in 5 N KOH.

Mixtures, treated in the same way as in the previous method, (p. 248), are prepared as follows:

5 ml of oleate 2%
5 ml of a solution of fatty acid in 5 N KOH
x ml 5 N KOH
(10 - x) ml of H_2O

The lower terms of the series then appear not to have a turgescent, but a condensing effect (Fig. 8).

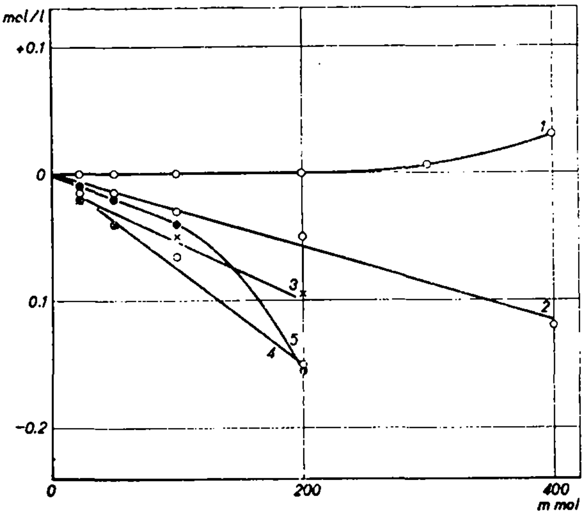

Fig. 8. The lower terms of the series of fatty acid anions only act in high concentrations. Formate(1) opens weakly, the other salts: acetate(2), propionate(3), butyrate(4) and valerate(5) condense slightly.

Only formic acid — a compound which differs in many respects from the other members — has a very weak turgescent effect. The lower fatty acid anions only being active at high concentrations, it seems possible that this effect has an origin (salt effect), different from the action of the higher terms. We must of course still verify whether the higher terms show the same reactions in the KOH-method as in the KCl-method. This indeed proves to be the case; the maximum of the activity-curve remains present at undecanoic acid. The shape of the curve is absolutely comparable with that of Fig. 7. There is no question of a minimum in effect at valeric acid — as might have been expected should a certain periodicity occur in the homologous series. Therefore we can now drop the idea of a periodicity that cannot hold good theoretically and practically.

The second possibility was that the higher terms of the series of fatty acid anions should form independent micells. A drawback of this idea has already been mentioned: the coacervate layers remain quite clear, so that we might presume that the formed micells are very small. And moreover the strong turgescent effect of arachidate and behenate cannot be explained by this supposition. On second thoughts we can already deduce from Fig. 6 an explanation of the problem of independent micells. As long as there are no independent micells the effects of the added compound must be more or less proportional to the concentration. The moment independent micells are formed this proportion disappears and the curve will run parallel to the abscissa. The curves for arachidate and behenate indeed show this course. Therefore we must compare the effects of the homologous series at low concentrations.

The formation of independent crystalline micells is, from its very nature, very dependent on the temperature. An increase of temperature to 40° proves to have practically no effect on the shape of the activity curve of Fig. 7, however. Therefore we must conclude that this curve is indeed real and that formation of micells does not play an important rôle. The curves for arachidate and behenate remain linear over a longer stretch (Fig. 9) because at a higher temperature the formation of independent micells takes place with a higher concentration.

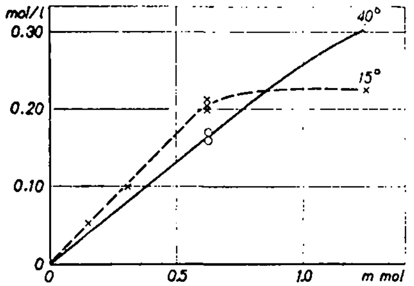

Fig. 9. The solubility of arachidate and behenate is larger at a higher temperature (40° C) than at a temperature of 15 °C; in the first case the effects of these compounds remain proportional to the concentration for a longer period.

So only a third possibility is left: a certain order of oleate molecules exists in the oleate coacervate and this order is disturbed by longer or shorter molecules. In this way we can explain the minimum in the activity curve at 15 to 16 carbon atoms. The increasing effect in the area between caproate and undecanoate still remains unexplained, however.

We may anticipate that in these experiments part of the added substance will be adsorbed by the oleate micells (we call this C_M), while the rest will remain in the equilibrium-liquid (C_E). We can now imagine that the lower members of the homologous series of fatty acid anions will mainly be accumulated in the equilibrium liquid ($C_E > C_M$), while on the other hand the higher ones will mostly be found inside the micells ($C_M > C_E$). As they are not taken up by the micells, the lower members must have hardly any influence on the order of the micells. The area between caproate and undecanoate would be the transition range between the lower and the higher members. The effect increases with a lengthening of the carbon chain because more and more of the compound is adsorbed by the oleate micells.

We can verify this idea by making experiments at different oleate concentrations. We examine, by means of the method described, the shifting of the KCl-curve under influence of stearate, starting from 1%, 2% and 4% standard solutions of sodium oleate. Table II shows the shiftings found.

TABLE II

Concentration of stearate	Shifting of the KCl-curve (in mol/l) with oleate of:		
	1%	2%	4%
0.31 m mol	0.06 N	0.02^5 N	0.01 N
0.62^5 m mol	0.10 N	0.05 N	0.02 N
1.25 m mol	0.15^5 N	0.09 N	0.05 N

If we assume the experimental error not to exceed 0.01 N, we can draw the following conclusions. The shifting of the KCl-curve (turgescent effect) is proportional to the concentration of the stearate. The only deviation from this rule is found at 1.25 m mol. stearate when the oleate concentration is low. This must be ascribed to the fact that as the oleate concentration decreases, the solubility of the stearate also decreases. The most important point, however, is that the effect is inversely proportional to the oleate concentration. If the amount of oleate is twice as little, the effect of the stearate is twice as large. We can now find graphically which stearate concentrations are necessary to cause a shifting of 0.05 N KCl. If we plot these figures against the oleate concentration of the standard solutions we see immediately that the curve goes through zero (Fig. 10). This means that all the added stearate has been adsorbed into the oleate micells.

In the same way we examine nonanoate. Table III shows the shifting of the KCl-curve under influence of nonanoate at different oleate concentrations.

TABLE III

Concentration of nonanoate	Shifting of the KCl-curve (in mol/l) for oleate solutions of:		
	1%	2%	4%
0.31 m mol	0.04 N	0.03^{5} N	0.03 N
0.62^{5} m mol	0.09 N	0.08^{5} N	0.07 N
1.25 m mol	0.19 N	0.17^{5} N	0.15 N

It is remarkable that here we find no proportion whatsoever between the effect and the inverse of the oleate concentration. The calculation of the equilibrium becomes complicated because the blank lines for 1, 2 and 4% standard solutions of oleate run differently. Therefore it is better to compare the shifting of log C_{KCl} under influence of the added nonanoate (Table IV).

TABLE IV

Concentration of nonanoate	Shifting of log C_{KCl} for oleate solutions of:		
	1%	2%	4%
0.31 m mol	.0113	.0097	.0079
0.62^{5} m mol	.0237	.0244 (?)	.0182
1.25 m mol	.0508	.0475	.0382

If we now determine graphically the nonanoate concentrations that cause a shifting

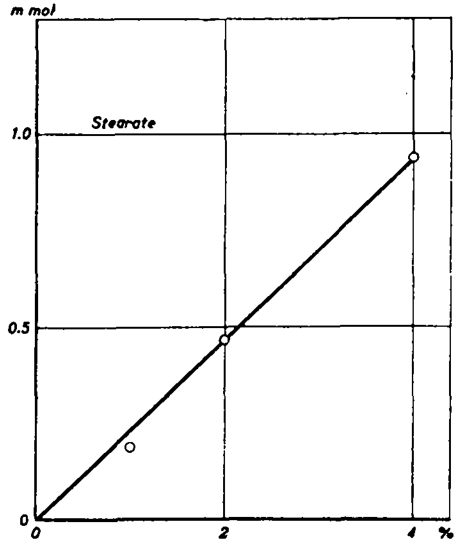

Fig. 10. The quantity of stearate required to cause a shifting in the KCl-curve of 0.05 mol/l (plotted along the abscissa) varies for different concentrations of the oleate solution (standard: 1, 2 and 4%). The line connecting these points goes through zero, so evidently all the stearate is adsorbed into the oleate micells.

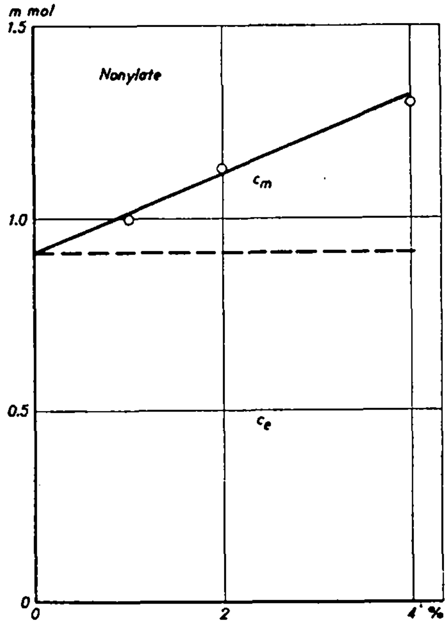

Fig. 11. The situation for nonanoate differs from that for stearate. The line cuts the ordinate at 0.91 m mol of nonaoate so that this must be the equilibrium-concentration of this compound (see also Fig. 10).

of 0.04 in log C_{KCl} we find a line that does not run through zero (Fig. 11). For nonanoate there is evidently a high equilibrium-concentration (C_E). The quantity (C_M) adsorbed by the micells is of course proportional to the number of micells available (*i.e.* the concentration of the oleate).

In a certain case — a shifting of the log C_{KCl} of 0.04 and for an oleate solution of 2% — C_M is 19% of the quantity of the added nonanoate.

The same experiment, performed with undecanoate, shows that for this compound the quantity adsorbed by the oleate-micells is much greater (Fig. 12). If we take the same circumstances as in the case of nonanoate — shifting of log C_{KCl} by 0.04 and for an oleate standard solution of 2% — then C_M is 83%.

An experiment with behenate affirms that of the higher terms 100% is adsorbed into the oleate micells (Fig. 13).

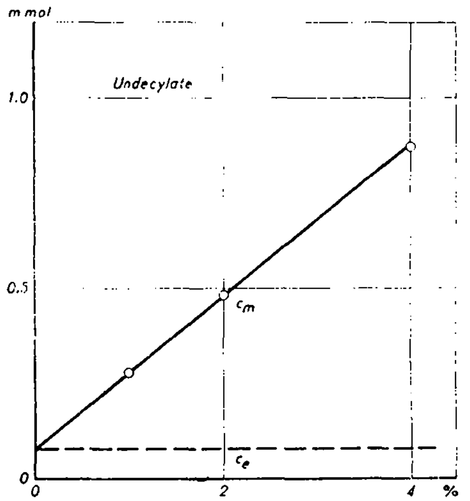

Fig. 12. For undecanoate the equilibrium concentration is lower than for nonanoate (compare Fig. 10 and 11).

It is evident that the figures for the lower members are only of a comparative value; they only apply to certain conditions. Nevertheless, the conclusion which may be drawn from these experiments is clear: the lower members of the homologous series have no effect or only a weak one in the lower concentrations, because they are not, or only slightly adsorbed into the oleate micells. It has already been explained why the curve for the adsorption (Fig. 13) does not correspond with the activity-curve (Fig. 7). It is of course logical to test this first for a stearate coacervate. In principle experiments with stearate coacervates are completely comparable with those on oleate coacervates. The only important difference is that we use K_2CO_3 instead of KCl and that the temperature must be chosen high (60° C). If our theory is right then the minimum in the activity-curve must be situated at 18 carbon atoms and the experiment confirms this prediction completely (see Table V and Fig. 14).

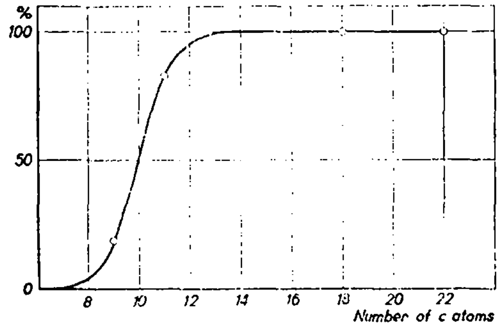

Fig. 13. We can deduce from Figs. 10, 11 and 12 which percentage of the added fatty acid anion is absorbed into the micells under certain circumstances (standard of the oleate solution = 2%, shifting in log C_{KCl} = 0.04).

The depth of the minimum in the activity-curve depends on the degree of conformity between the substrate and the added compound. As a matter of fact the minimum is deepest when the added ion corresponds with the substrate (see, in Fig. 14, the effect of stearate on a stearate coacervate).

An experiment on the influence of the same series of fatty acid anions on a substrate of a compound with a totally different structure (*viz.*, on a coacervate of desoxycholate) now becomes very interesting.

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TABLE V

Number of C-atoms of fatty acid	Shifting of the K_2CO_3 curve in (mol/l) by fatty acid anions	
	Conc. 1.25 m mol	Conc. 2.50 m mol
6	0.00^5	0.01^5
8	0.08^5	0.26^5
9	0.24	0.59
10	0.30^5	0.64
11	0.28	0.55
12	0.21	0.42^5
13	0.16	0.30
14	0.08^5	0.17^5
15	0.03	0.06^5
16	0.00^5	0.01
18	− 0.01	-0.02^5
20	0.09^5	0.18
22	0.07^5	0.14^5

Shifting of the K_2CO_3-curve (in mol/l) under the influence of fatty acid anions of different length (temp. 60° C). The substrate is a stearate coacervate.

If 1 ml of a 10% solution of desoxycholate is mixed with a concentrated solution of NaCl or KOH a coacervate is formed. The water content of this coacervate, however, is low, so that volume measurements do not offer many possibilities. With an increase of temperature the coacervate suddenly disappears and this criterion is useful in our experiments. We bring about 5 ml of the mixture to be measured into a wide test-tube, stirring the liquid with a thermometer. Besides this, glass beakers filled with water of ± 60° C and ± 10° C respectively are kept at hand. First of all we examine the influence of KOH on the temperature of separation. This point can be fixed with an accuracy of about 0.2° C, many times running. The mixtures are of the following composition:

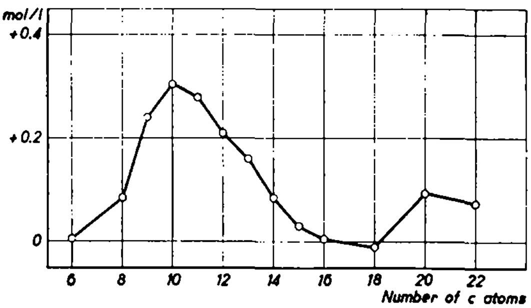

Fig. 14. The activity-curve of the fatty acid anions on a stearate coacervate shows a minimum at 18 carbon atoms, in contrast to an oleate coacervate for which this minimum is found at 15 to 16 C-atoms (see Fig. 7)

1 ml of sodium desoxycholate 10%
x ml of 5 N KOH
(39 − x) ml of H_2O

Upon increase of the KOH concentration the temperature of separation rises rapidly (Fig. 15).

We now choose a convenient working point on this curve (about 51° C) and prepare a supply of a mixture that possesses this temperature of separation. From this supply 5 ml — at a temperature higher than 51° C of course — are brought by means of a pipette into wide test-tubes, which already contain the compounds to be examined. The substance is weighed by means of a torsion balance. The higher fatty acid anions have such strong effects that this weighing-method cannot be used. In that case we dissolve a small quantity of fatty acid in 5 ml of ligroin. Of this solution we bring 0.25, 0.5 and 1 ml into the test-tubes with a pipette, and evaporate the solvent.

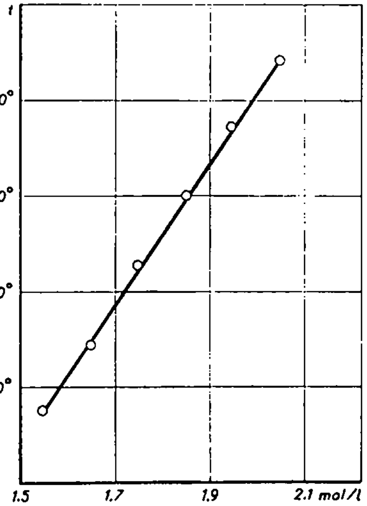

Fig. 15. Addition of KOH to a solution of desoxycholate causes a turbidity (coacervate). With higher concentrations of KOH the temperature at which this coacervate disappears is also higher.

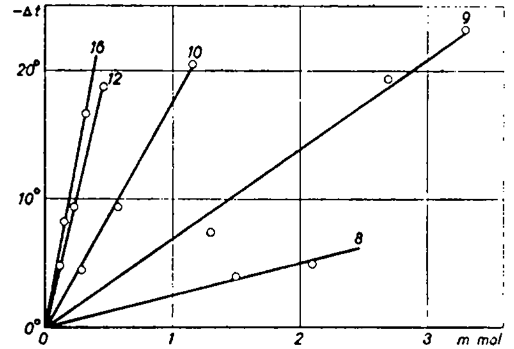

Fig. 16. Decline of the temperature of separation ($-\Delta t$) of a desoxycholate coacervate under influence of fatty acid anions with a different number of C-atoms (8 = octanoate, 9 = nonanoate, etc.).

As in the oleate coacervate the fatty acid anions have a turgescent effect, which is observed by a decline of the temperature of separation. If we plot this decrease ($-\Delta t$) against the concentration of the added substance the effect can be read immediately (Fig. 16).

If we now compare the lowering of the temperature caused by a certain concentration of fatty acid anions, the result is again an activity curve, but this time it does not show a definite minimum (Fig. 17). We do, however, notice that with the C_{13} and C_{14} fatty acid anions an irregularity appears in the curve.

It is known that also in solutions of sodium desoxycholate micells occur (McBain and Brady, 1943) and again we can imagine that the fatty acid anions disturb the order of these micells. Hence their turgescent effect on the desoxycholate. If the thickness of these micells is about equal to twice the length of the molecules of tridecanoic acid or of tetradecanoic acid, then we can expect that their ions will be slightly less active than the higher fatty acid anions.

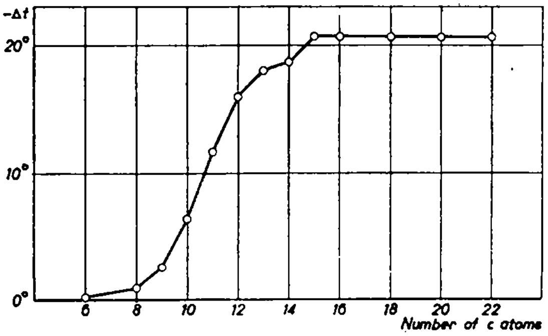

Fig. 17. Activity-curve of the fatty acid anions with a desoxycholate coacervate. There is no obvious minimum in the turgescent effect (as in Fig. 7 and 14).

We can also again determine the quantity of the added substance that is adsorbed into the micells (C_M). The method is the same as was applied with the oleate coacervates; we compare the effect of the added compounds at different desoxycholate concentrations. The resulting curve (Fig. 18) exhibits a great conformity with the activity-curve, although the irregularity at 13 and 14 carbon atoms has disappeared.

Attempts to determine the activity-curve with other substrates have failed. We tried to examine the influence of fatty acid anions on the separation of phenol and water, aniline and water, cresols and water. The results obtained are not reproducible, as these compounds are oxidised in air. Experiments with a coacervate of dehydrocholate also gave results which were too erratic to be of use.

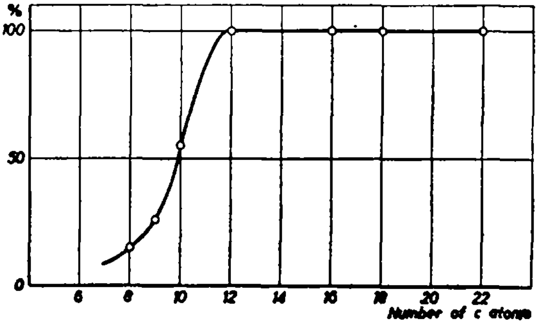

Fig. 18. Percentages of the added fatty acid anions that are adsorbed into the desoxycholate micelles.

CONCLUSION

If we study the effect of a homologous series of fatty acid anions on an oleate coacervate, we observe a minimum in the activity-curve. Generally all higher fatty acid anions have a turgescent (opening) effect on such a coacervate, this in contrast to the higher alcohols. Particularly important in connection with the minimum in the activity-curve are the views on the conformity in structure of the substrate and the agent. If these are identical then practically no effect is to be expected (see *e.g.*, in Fig. 14 the effect of stearate on a stearate coacervate). If there is a great difference in length between the molecules of the agent and of the substrate, then the activity is high (*e.g.*, undecanoate as agent and an oleate coacervate as substrate, Fig. 7). Of course an agent can also be inactive because it is not adsorbed into the micells of the substrate. This is the case with the lower fatty acids (compare the rise in the curve in Fig. 7 and Fig. 13). In the desoxycholate molecule there is little conformity with the fatty acid anions. Therefore the activity curve (Fig. 17) only shows a slight irregularity, does not have a definite minimum and strongly resembles the curve representing the adsorption of the fatty acid anions into the micelles (Fig. 18).

It must not be considered improbable that such a turgescent effect may offer an explanation for certain physiological or pharmacological reactions. The remarkable feature is that one single molecule can disturb the order of a large number of molecules (see also SCHEIBE, 1939). We may reasonably presume that in many organisms there exists a certain order of lipophilic molecules. In this connection we may call to mind the protoplasmic membrane, the nerves, the grana; indeed, we may perhaps say that within organisms containing lipids a certain order of these molecules may be encountered (see for example the work of BEAR, PALMER AND SCHMITT, 1941).

Other compounds may then have a disturbing effect on organs that are characterized by such an order of molecules. It now becomes very interesting to examine more in detail the conditions for the occurrence of this disturbing activity. According to the starting-point of these investigations this problem will be studied first by trying to answer the question whether the action of the synthetic plant growth substances is due to an effect on the protoplasmic membrane in the sense that the permeability for water (and for water-soluble compounds) increases. From the points of view given it follows that it is logical to attack this problem by examining the effects of growth substances and related compounds on oleate coacervates.

SUMMARY

1. The complex theory of permeability (BUNGENBERG DE JONG) affords a possibility to unite the lipoid theory and the filter theory. If the protoplasmic membrane is conceived as a tricomplex system, composed of phosphatides, proteins and cations, the differences between these well-known theories vanish, when considered in molecular dimensions.

2. Oleate coacervates may serve very well as a model for the non-polar part of the protoplasmic membrane. The influence of many on this model organic compounds has been determined previously; these investigations were now extended by experiments on the influence of fatty acid anions.

3. The influence of fatty acid anions proved to be strongly dependent on the length of the carbon chain, very remarkable differences being found (Fig. 7).

4. In general the fatty acid anions demonstrate a turgescent effect (the water content, and so the volume, of the coacervate augments) at low concentrations. This influence becomes perceptible with six carbon atoms in the molecule and, in the homologous series, strongly increases up to the anion with eleven carbon atoms. After a minimal activity at fifteen C-atoms it rises again.

5. The first increase of activity can be explained by the distribution of the added fatty acid anions between the equilibrium liquid and the soap micells; with a growing number of C-atoms increasing amounts are adsorbed by these micells. On that occasion the added fatty acid anion exerts a disturbing action upon the orderly soap micells.

6. This disturbing action is minimal if the added fatty acid anion fits into the order of the oleate micells (in the case of 15 carbon atoms).

7. With another substrate (e.g. stearate coacervates) the minimum appears at a different point (with 18 C-atoms).

8. If there is but little similarity between the substrate and the added fatty acid anion (as in the case of sodium desoxycholate coacervate), a pronounced minimum is not observed any longer.

9. This obvious difference in activity of compounds from a homologous series, dependent on structural relations between "substrate" and "agent", may be of primary importance to the general problem of the influence of chemical structure on physiological action.

RÉSUMÉ

1. La théorie des complexes de la perméabilité (BUNGENBERG DE JONG) fournit une possibilité de combiner la théorie des lipoïdes et la théorie du filtre. Si la membrane protoplasmique est en effet considérée comme un système tricomplexe, constitué par des phosphatides, des protéines et des cations, les différences entre les deux théories disparaissent si on se place à l'échelle des dimensions moléculaires.

2. Des coacervats d'oléate sont un bon modèle pour la partie non polaire de la membrane protoplasmique. L'influence de nombreuses substances organiques sur ce modèle a été déterminée précédemment. Ces recherches ont été étendues à l'influence des anions d'acides gras.

3. L'influence des anions d'acides gras s'est montrée sous la dépendance directe de la longueur de la chaîne carbonée (Fig. 7).

4. En général, les anions d'acides gras manifestent une action turgescente à des concentrations faibles. Cette action devient perceptible à partir de 6 atomes de carbones dans la molécule et, pour les homologues, s'accroît fortement jusqu'à 11 atomes de carbone. Après un minimum pour 15 atomes, cette action croît de nouveau.

5. Le premier accroissement d'activité peut être expliqué par la répartition des anions d'acide gras entre le liquide en équilibre et les micelles de savon. Lorsque le nombre d'atomes de carbone s'accroît, une quantité plus grande des anions d'acide gras est adsorbée par les micelles, ce qui provoque un désordre dans les micelles de savon.

6. Un tel désordre est réduit au minimum lorsque l'anion d'acide gras est tel qu'il puisse participer à l'ordre des micelles d'oléate (c'est le cas pour 15 atomes de carbone).

7. Avec un autre substrat (p. ex. des coacervates de stéarate), le minimum se manifeste pour un nombre d'atomes de carbone différent(18).

8. S'il n'existe que peu d'analogie entre le substrat et l'anion d'acide gras ajouté (comme dans le cas d'un coacervat de désoxycholate de sodium), on n'observe pratiquement plus de minimum.

9. Une différence aussi marquée dans l'activité de substances appartenant à des séries analogues, différences dépendant des relations de structure entre "substrat" et "agent", peut être d'importance fondamentale dans le problème général des relations existant entre la structure chimique et l'action physiologique.

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ZUSAMMENFASSUNG

1. Die Komplextheorie der Permeabilität (Bungenberg De Jong) ergibt die Möglichkeit, die Lipoid- und Filtertheorie zu vereinigen. Wenn die Protoplasmamembran als ein trikomplexes System aufgefasst wird, das aus Phosphatiden, Eiweiss und Kationen zusammengesetzt ist, fallen die Unterschiede zwischen obengenannten wohlbekannten Theorien weg, wenn man sie in molekularen Dimensionen betrachtet.

2. Oleatkoazervate können sehr gut als Modell für den unpolaren Teil der Protoplasmamembran dienen. Der Einfluss vieler organischer Verbindungen auf dieses Modell ist bereits früher bestimmt worden; diese Untersuchungen wurden jetzt durch Versuche über den Einfluss von Fettsäureanionen erweitert.

3. Hierbei wurde bewiesen, dass der Einfluss der Fettsäureanionen in starkem Masse von der Länge der Kohlenstoffkette abhängt, wobei sehr bemerkenswerte Unterschiede festgestellt wurden (Abb. 7).

4. Im allgemeinen zeigen die Fettsäureanionen bei niedrigen Konzentrationen einen Turgeszenzeffekt (der Wassergehalt, und dadurch ebenso das Volumen, des Koazervats nimmt zu). Dieser Einfluss wird bei sechs Kohlenstoffatomen im Molekül deutlich wahrnehmbar und steigt in der homologen Reihe erheblich bis zum Anion mit elf Kohlenstoffatomen. Nach einem Aktivitätsminimum bei fünfzehn Kohlenstoffatomen steigt er wieder.

5. Der erste Aktivitätsanstieg kann durch die Verteilung der zugefügten Fettsäureanionen zwischen der Gleichgewichtsflüssigkeit und den Seifenmicellen erklärt werden; bei steigender Anzahl C-Atome werden zunehmende Mengen von diesen Micellen adsorbiert. Hierbei übt das zugefügte Fettsäureanion eine Störwirkung auf die geordneten Seifenmicellen aus.

6. Diese Störwirkung ist minimal, wenn das zugefügte Fettsäureanion in die Ordnung der Oleatmicellen passt (im Falle von fünfzehn Kohlenstoffatomen).

7. Bei einem anderen Substrat (z.B. Stearatkoazervaten) tritt das Minimum bei einem anderen Punkt auf (bei 18 C-Atomen).

8. Wenn nur eine sehr geringe Ähnlichkeit zwischen dem Substrat und dem zugefügten Fettsäureanion besteht (wie im Falle des Natriumdesoxycholatkoazervats), tritt ein ausgesprochenes Minimum nicht mehr auf.

9. Diese deutlichen Unterschiede in der Aktivität von Verbindungen aus einer homologen Reihe, die von strukturellen Beziehungen zwischen "Substrat" und "Agens" abhängen, sind möglicherweise von grösster Bedeutung für das allgemeine Problem des Einflusses der chemischen Struktur auf die physiologische Wirkung.

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