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Physiology. — *Phosphatide Auto-complex coacervates as Ionic Systems and their Relation to the Protoplasmic Membrane.* By H. G. BUNGENBERG DE JONG and J. BONNER. (Communicated by Prof. H. R. KRUYT).

(Communicated at the meeting of June 29, 1935).

I. *Introduction.*

In the study of complex- and of autocomplex coacervation no urgent need for a consideration of the organic chemical structure of the bio-colloids involved has ever been encountered. Independent of the class of organic compounds to which they belong the colloids which have been studied behave in the same general way and the "electrolyte" or "charged particle" character is the important determinant of their behaviour. The differences which have been met with in the behaviour of the different bio-colloids may all be related directly to the number and the nature of their ionogenic groups. [1]

The auto-complex coacervates of phosphatides stand however quite apart in one respect. The conditions necessary for the initiation of coacervation are normal. [2] Because of the low charge density of the sol particles they require, however, a sufficient "sensitizing" by appropriate substances.

The coacervates have, however, abnormal properties [3] in that:

a. once formed they are difficultly reversible,

b. the vacuoles of the drops exhibit characteristic phenomena,

c. the coacervate drops exhibit a peculiar behavior in an electric field.

The behavior of the phosphatide auto-complex coacervate cannot, then,

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[1] H. G. BUNGENBERG DE JONG, P. VAN DER LINDE and A. DE HAAN, Rec. trav. chim. **54**, 17 (1935).

[2] H. G. BUNGENBERG DE JONG and R. F. WESTERKAMP, Biochem. Z. **248**, 131, 309 (1932).

[3] H. G. BUNGENBERG DE JONG and R. F. WESTERKAMP, Biochem Z. **248**, 335 (1932).

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be understood from the "colloid electrolyte" point view. In this case it has proved useful to consider the chemical properties of the phosphatide molecule and his paper will be devoted to such a treatment of the phosphatide auto-complex systems.

A very simple explanation of the phenomena is possible if we choose as kinetic units the smallest possible ones, namely the ionised phosphatide molecules themselves.

This simplification of our colloid chemical consideration is perhaps justified by the recently discovered separation phenomena in molecularly dispersed electrolyte solutions. [1]) In any event, it leads logically to the picture of a special type of film which may under various circumstances arise upon the surface of the phosphatide coacervate drop. As pointed out in the earlier review of the phenomenon of coacervation [2]), the films in question merit attention from the biologists because of their remarkable faculty of separating, without loss of stability, two miscible aqueous media.

II. *Phosphatide auto-complex coacervates as ionic auto-complex systems.*

An auto-complex coacervate of a phosphatide may vary enormously in fluidity according to the phosphatide (or mixture of phosphatides) used, the nature of the sensitizer and of the electrolytes, and the temperature. The separating mass may thus consist of drops, either liquid, viscous, or even plastic, of irregular lumps, or of flakes.

In what follows, under the name "phosphatide auto-complex coacervate", will be understood the highly fluid isotropic systems.

We will begin then with the assumption, that in the case of phosphatide auto-complex coacervates no greater units than those of molecular dimensions need be considered. It is not, of course, excluded that in phosphatide *sols* we may have to deal with polymolecular kinetic units.

The characteristic irreversibility or near irreversibility of these *coacervates*, makes it clear however that there is no urgent need to assume that sol particles persist as such in the coacervate. The sol micells may indeed be considered as groups of molecules, held together by binding forces of the same sort as those which act between phosphatide molecules in the coacervate.

A further physical-chemical consideration which we must take into account is the fact that the lecithins, kephalins and sphingomyelins possess two ionisable groups, namely an acidic, phosphate group and a basic, choline, or colamine group. We have seen in the preceding paper, that solvated ions are capable of forming ionic liquid complexes and it seems reasonable to assume that the phosphatide coacervate is in reality just such an ionic system.

By virtue of the two ionisable groups ($-PO_4'$ and $-N(CH_3)_3^{\cdot}$ or $-NH_3^{\cdot}$)

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[1]) See preceding paper.
[2]) H. G. Bungenberg de Jong, Protoplasma **15**, 110 (1932).

an effective electrostatic attraction must exist between the phosphatide units themselves. The internal state of this coacervate is, thus, determined again by : "effective electrostatic attraction versus repulsion due to solvation" (see fig. 1) and we shall call it an *"ionic auto-complex system"*.

In the case of the highly fluid coacervates here considered, these strongly solvated ions take, in general, positions at random, and the coacervate is thus optically isotropic. The general kinetic movement does not permit of the formation of a permanent, regular patern.

In the physiological range of P_H one encounters, in general, a negative electrophoretic charge. This means that the ionisation of the acidic groups present preponderates over that of the basic groups. Thus, the effective attraction may be far smaller than the maximum possible. In such a case the effective attraction may be increased by the addition of a neutral salt
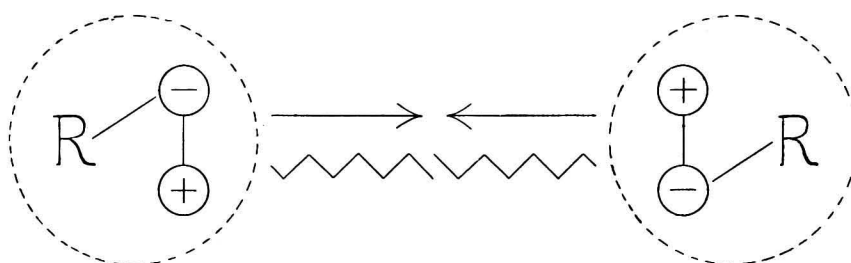

Fig. 1. The phosphatide auto-complex coacervate as an ionic auto-complex system. Dotted circles = hydrated phosphatide ions.
arrows = effective electrostatic attraction
springs = repulsion due to solvation.

whose cation screens more the negative ionised groups than does the anion the positive groups.

Since this screening effect is dependent primarily upon valence, such a salt will be of the type *n*-1, where *n* is equal to or greater than two. Indeed $CaCl_2$ is efficient in increasing the effective attraction, thus causing a diminution of the solvate content.

In the case of NaCl, with two monovalent ions, such a pronounced increase of effective attraction may not be expected. At moderate concentrations it seems that the screening effect of the Cl-ion dominates over that of the Na-ion. This appears for example in the pronounced NaCl—$CaCl_2$ antagonism in mixtures of the two salts.

A great and heterogeneous group of substances may act as "sensitizers" of the aqueous phosphatide sol. Only in the presence of such a "sensitizer" does coacervation with a neutral salt take place. This sensitizing action may be regarded as a diminution of the repulsion between the phosphatide molecules.

The repulsion factor is here of a more complicated nature than is the case with many of the other biocolloids. With these latter, the sensitizing agents thus far discovered are oxygen containing, water soluble non

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electrolytes, such as the lower alcohols and ketones, the phenols, ethyl lactate, chloral hydrate, ethyl urethane, etc. This type of sensitization may be interpreted as a diminution of hydration both of non-ionised groups (as -OH, -CO-NH-) and of ionised groups (-COO`, -SO$_4$`, -PO$_4$` etc). In such cases diminution of colloidal hydration seems to be connected with disturbances which hydrophilic sensitizers cause in the "hydration fields" of hydrophil groups.

These same substances, in moderate concentrations are found to act as sensitizers for phosphatides also, and one may assume that here "sensitizing" is also connected with dehydration of hydrophil groups. Thus far we have not considered the fact that phosphatides are not only hydrophilic, due mainly to their ionogenic groups, but are also „lipophilic" (soluble in ether, petroleum ether etc.), due to their hydrocarbon chains. This fact will now need to be considered, since it is found that substances of a pronounced lipophilic character such as ether, chloroform, heptane, fats, oils, fatty acids and cholesterol, may act as sensitizers. Here the sensitizing action can hardly be connected with hydrophil groups but rather must be upon the lipophil portion of the molecule. It seems that aside from the electrostatic attraction and aside from the repulsion due to hydration, which we have thus far exclusively considered, there exists also an attraction between the hydrocarbon chains. This attraction in the absence of sensitizers is small, but may be greatly increased by the presence of suitable quantities of substances possessing a pronounced lipophil character.

The complex nature of the "repulsion factor", which, as we have seen, is the result of the presence of both hydrophilic and lipophilic groups in the phosphatide molecule, is not fully expressed by the simple scheme of fig. 1. Even the expression "repulsion factor" is not completely appropriate. There is not, of course, a simple algebraic summation of the interactions of hydrophilic and lipophilic groups since these groups are separated in the molecule.

The characteristic slight, or complete lack of, reversibility which is typical of the auto-complex coacervate is a direct indication of the actual existance of an independent attraction due to lipophilic groups. It might be added that further peculiarities of phosphatide coacervates (which are to be described in the following section) can only be understood if we assume a polar character for the phosphatide molecule as a whole.

It will be, therefore, not only convenient, but actually necessary to distinguish from now on *three separate major factors* which regulate the interaction of phosphatide ions. The scheme given in fig. 2 will suffice for considerations of the phenomena thus far described, i.e. influence of electrolytes or of non-electrolytes upon solvate content of liquid, non double refracting coacervates.

More detail, than that given in fig. 2 of the relative position of ionised, hydrophilic and lipophilic groups within the ion would here not be of

any particular advantage, since the ions have random orientations which respect to one another. In the next section, however, we will discuss phenomena, in which this relative position is of the greatest importance.
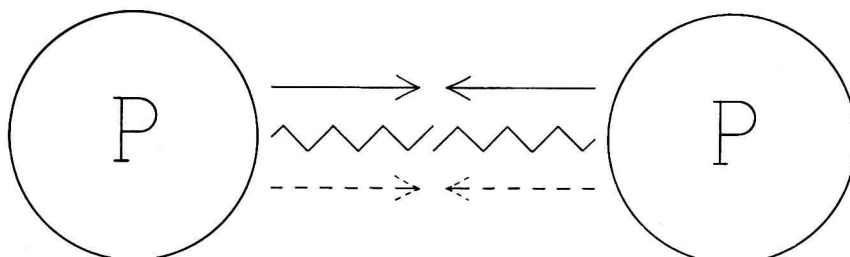

Fig. 2. Diagrammatic representation of the three principal factors which determine the internal state of the phosphatide auto-complex coacervate.
P = phosphatide ion,
solid arrows = effective electrostatic attraction,
springs = repulsion due to hydration,
dotted arrows = attraction due to lipophilic groups.

III. *Single and double films of oriented phosphatide ions.*

It will now be shown that the peculiar phenomena concerned with the vacuole of phosphatide coacervate drops become understandable if we assume that the boundary, dispersion medium-coacervate, consists of a layer of oriented phosphatide ions. In the case of the fluid coacervates here considered the phosphatide molecules within the drop have of course random positions with respect to one another.

At first sight it might seem that the polar groups of the surface molecules should be directed towards the dispersion medium, the fatty acid chains, thus, towards the interior of the coacervate drop. A closer consideration, however, shows that just the reverse is more probable. The phosphatide coacervate has just formed from the sol by virtue of a sufficiently great electrostatic attraction between the phosphatide units. The attraction between positive and negative groups, to which the formation and the continued existance of the coacervate is due, causes the coacervate to be more polar than the surrounding dispersion medium.

As a starting point for further discussion we shall choose therefore the orientation shown in fig. 3 in which the two circles represent the ionisable groups (black dots — phosphate groups, circles — choline or colamine groups) and the two heavy parallel lines the hydrocarbon chains. For our present purposes we may neglect the further details of the structural formula.

With this assumed orientation it is possible to explain some of the peculiarities of these auto-complex coacervates as regards "wetting" of foreign surfaces in the presence of their own suspension liquid.

In contrast with other coacervates (simple, complex or auto-complex) the drops of phosphatide coacervates accumulate carbon particles only

on their surface, but do not take these particles into their interior.
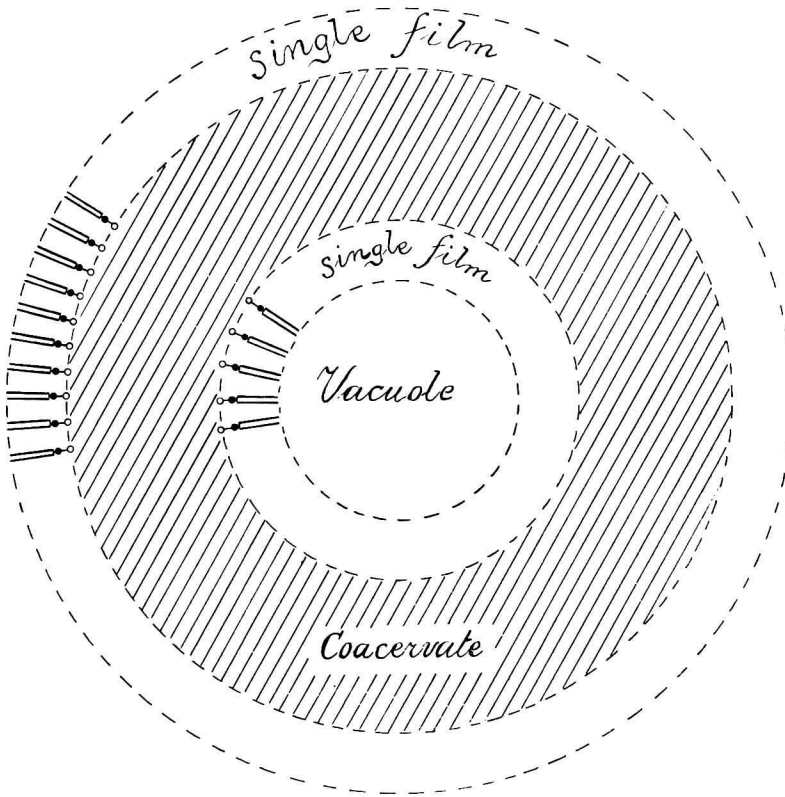

Fig. 3. Orientation of phosphatide molecules at the boundary coacervate-dispersion medium. (All other constituents of the coacervate, as water, sensitizer, electrolytes, are not represented).

A similar difference is met with in the behaviour of the phosphatide coacervate drop toward glass. Whereas all other coacervates wet glass
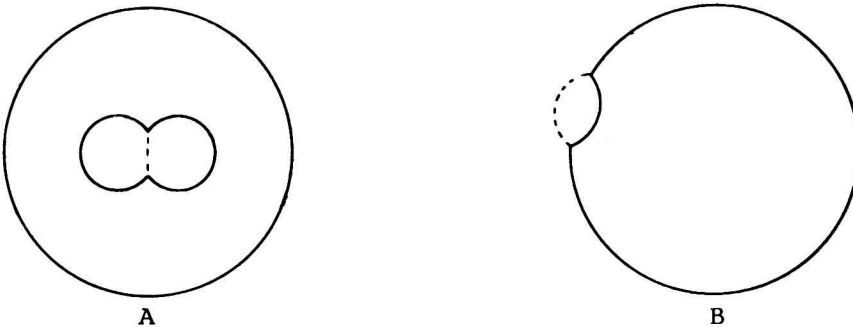

Fig. 4. The meeting of coacervate drops or their vacuoles.
A = Two vacuoles flatten against each other on meeting but do not fuse.
B = Vacuole attached to drop surface. The protruding double film (dotted) is barely visible (cross section).

ıapidly so that drops upon the surface of a slide rapidly flatten and loose their sharp contour, drops of phosphatides may lie several hours upon a glass surface without loosing their spherical form. The behaviour of phosphatide coacervate drops with respect to carbon as well as with respect to glass is closely similar to the behaviour exhibited by paraffine drops with respect to these substances. This is in itself a strong indication that the surface of the phosphatide coacervate is "paraffine like", i.e. that the hydrocarbon chains are directed outwards.

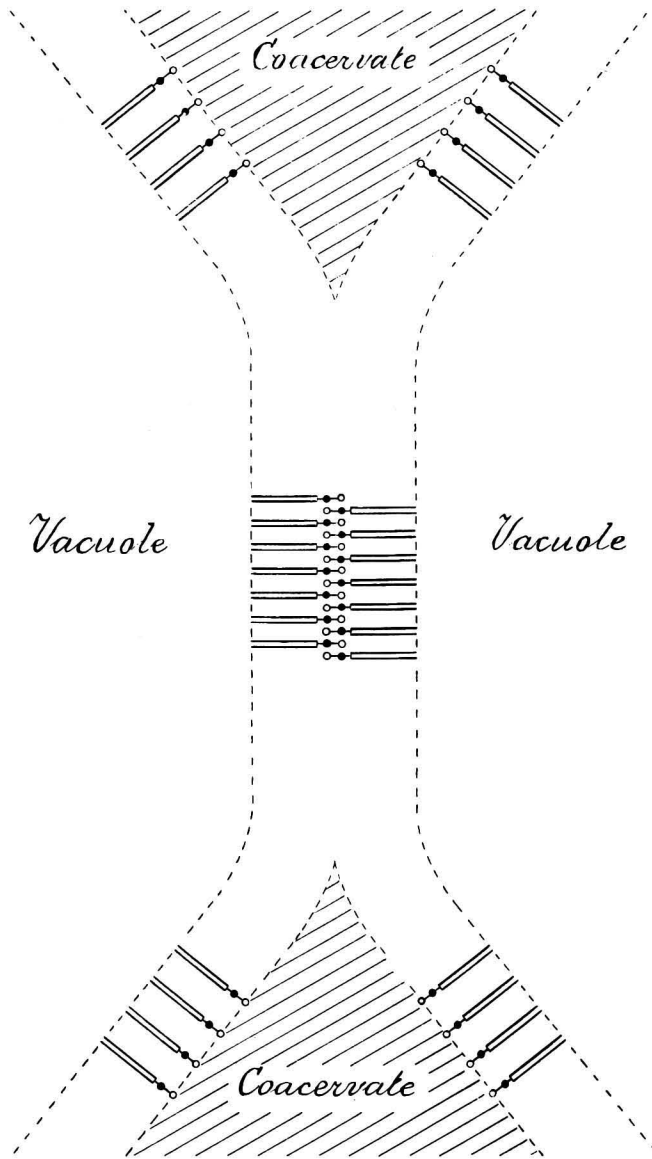Since the vacuole content is dispersion medium, the molecules of phos-

Fig. 5. The formation of "double film" at the contact between two vacuoles.

phatide in the film surrounding a vacuole should be orientated in the same way as that at the boundary, dispersion medium coacervate drop (see fig. 3).

For a number of reasons it seems profitable to assume that when the ionized polar groups of two such oriented films (consisting of single layers of phosphatide ions) meet, a highly stable "double film" is formed. This assumption makes understandable, for example, the peculiar behaviour of vacuoles in the phosphatide auto-complex drop. In the case of all of the other coacervates which have been studied two vacuoles fuse upon meeting. In the case of the phosphatide auto-complex, two *vacuoles* do not fuse, i.e. the stable "double film" is formed and prevents fusion (see fig. 4A and fig. 5). If two *drops* meet, however they fuse, since they present hydrocarbon chains to each other and the "double film" is not formed.

We shall now consider those vacuoles which have come in contact with the interior of the coacervate drop's surface film. In all of the other (sufficiently fluid) coacervates which have been studied, this uniting of vacuole with outer membrane results in the discharge of the vacuole content (which is dispersion medium) into the surrounding liquid, together with a subsequent rounding out of the drop surface.

In the case of the phosphatide coacervate drop, however, the vacuole contents are not discharged but the vacuole as a whole remains attached to the surface of the drop, as shown in fig. 4B. An explanation similar to that given above for the formation of "articulated" vacuoles may be made. In this case, also, the two films meet with their "dipole" ends and a stable double film is formed. If many small vacuoles are attached in this way, the circumference of the drop appears "facetted", and the drop surface as a whole is dotted with them.

IV. *The double film of oriented phosphatide ions as intergral constituent of the protoplasmic membrane.*

In the preceding review [1]) it was pointed out that phosphatide auto-complex coacervates would seem to be of importance in relation to the problem of the protoplasmic membrane. The arguments brought forward for this point of view can be divided into two principal groups:

1. analogies between properties of the coacervates and properties of biological membranes, for example, the effects of certain electrolytes and non electrolytes, upon the one hand upon the solvate content of the coacervate, upon the other hand, upon "permeability";

2. those arguments which relate to the difficult reversibility of the coacervates, and in particular to the occurrence of films which may separate, without loss of stability, two miscible aqueous media.

At the time of the earlier review nothing was known of the nature of these last films and hence no positive statements concerning a direct

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[1]) H. G. BUNGENBERG DE JONG, loc. cit.

relation between the properties of *a.* such films, *b.* of coacervates and *c.* of biological membranes could be made.

The conception, developed earlier in this paper, that single phosphatide ions constitute the kinetic units of the interior of the coacervate drop as well as of its surface films, makes it now possible to state in more precise terms the significance of phosphatide auto-complex coacervates for biology.

Since the remarkable films mentioned in section III must be considered as double films of oriented phosphatide ions, such double films may be considered as bimolecular, oriented "coacervate layers". Beside the phosphatide ions, all of the other constituents which participate in the phosphatide auto-complex coacervate will be present to some extent in the film.

As to the location of the non phosphatide constituents of the double film, it will be clear that water molecules, as well as ions, will be found principally in the hydrophilic middle zone, but that sensitizers of a typical lipophilic character will be found in the two adjacent lipophilic zones (shaded areas in fig. 6). Lipophilic sensitizers will thus fill up, to a certain extent, the area of "water pores" left open in the membrane.
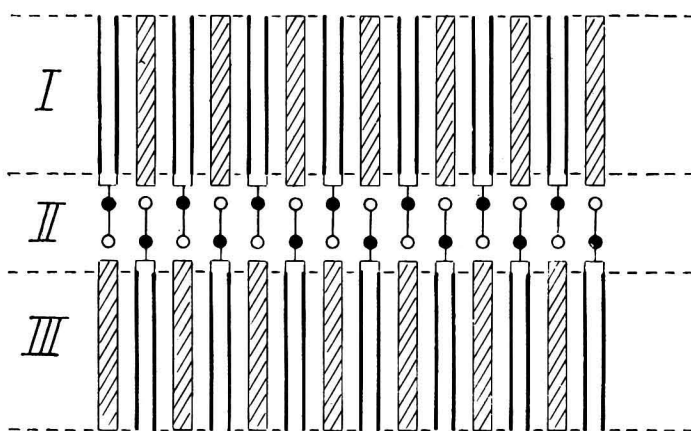

Fig. 6. Diagrammatic representation of double film consisting of oriented phosphatide ions.
I and III lipophilic zones.
II hydrophilic zone containing ionised groups.
Shaded areas = sensitizer molecules (cholesterol, triolein, etc.)

Electrolytes or non electrolytes, through their action upon the three mayor factors mentioned in Section II, may alter the molecular packing and hence, the area of this film. This will of course result in changes of permeability.

The "analogies" included under 1. above may perhaps now be more properly restricted to analogies between such oriented bimolecular coacervate films and the protoplasmic membrane.

This leads to the working hypothesis, that the special properties of the protoplasmic membrane, and perhaps of other membranes within the cell, depend upon the occurrence in them of one or more double layers of phosphatide ions. [1])

Summary.

1. Phosphatide auto-complex coacervates may be regarded as *fluid, ionic, auto-complex systems*. The interaction of the phosphatide ions in the coacervate is determined by *three* major factors:
    a. effective electrostatic attraction due to oppositely charged ionised groups;
    b. repulsion due to hydration;
    c. attraction due to lipophilic groups.

2. From theoretical as well as from experimental considerations it is concluded that the phosphatide ions at the surface of the coacervate are oriented with their polar groups inwards, their hydrocarbon chains, thus, outwards.

3. For a number of reasons it seems profitable to assume that when the ionized polar groups of two such oriented films (consisting of single layers of phosphatide ions) meet. a highly stable "double film" is formed. This assumption makes understandable, for example, the peculiar behaviour of vacuoles in the phosphatide auto-complex drop.

4. It is suggested, as a working hypothesis, that the special properties of the protoplasmic membrane depend upon one or more double layers of oriented phosphatide ions similar to the "double films" of the phosphatide auto-complex coacervate.

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[1]) A more detailed discussion of the peculiar properties of the coacervates and their importance for the problem of the protoplasmic membrane, will be undertaken in a paper to be published shortly in Protoplasma.