CLXXXIV. LECITHIN AND CHOLESTEROL IN RELATION TO THE PHYSICAL NATURE OF CELL MEMBRANES¹.

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ALTHOUGH the chemical problems presented by cell membranes are still obscure, work carried out by Clowes [1916] on the reversal of oil-water emulsions by ions, and the application of the results of this work to permeability phenomena in biology, have lent support to the view that cytoplasm consists essentially of an emulsion system containing fatty material and water. Working with olive oil-water systems *in vitro*, Clowes has shown that electronegative ions, such as hydroxyl ion, favour the formation of a system in which water is the continuous phase and oil the disperse phase, whilst electropositive ions, such as calcium ion, favour the reverse type of system. When the aqueous phase consists of a mixture of sodium hydroxide and calcium chloride, the water is the continuous phase provided that the sodium hydroxide is present in excess of the molecular ratio, 4 of NaOH to 1 of CaCl₂, whilst the oil is the continuous phase if the calcium chloride is present in excess of this ratio. The fact that systems in which oil is the continuous phase are much less permeable to water-soluble substances than are systems in which water is the continuous phase, was the basis upon which Clowes formulated his conclusions regarding the permeability of cell membranes, viewed in the light of his own results.

It is with the object of examining the antagonistic action, if any, of the substances lecithin² and cholesterol in relation to permeability that the following work has been carried out. The ultimate object is to attempt to account in some degree for the increased permeability to water-soluble substances attributed to cancer cells.

The general method of procedure has been to investigate the emulsifying powers of these two substances on typical fatty oil-water systems.

¹ This investigation was undertaken on behalf of the Liverpool Cancer Research Committee. Director: Professor W. Blair Bell of the University of Liverpool.

² In general, the lecithin employed was a commercial lecithin which contained pure lecithin, cephalin, sphingomyelin, but no cholesterol.

THE EFFECT OF CHOLESTEROL AND LECITHIN, RESPECTIVELY, ON THE INTERFACIAL TENSION BETWEEN OIL (OLIVE OR COTTONSEED) AND WATER.

In the first place, the effect of cholesterol, dissolved in the oil phase, on the interfacial tension between oil and water, was compared with that of calcium chloride, present in the aqueous phase. The drop pipette method was employed. The results of this comparison are shown in Tables I and II below. The interfacial tensions were calculated from the corresponding drop numbers, the latter quantities being inversely proportional to the interfacial tensions, provided that the densities of the two phases do not sensibly change. The value for the interfacial tension between pure olive oil and water was taken as 18.6 [cf. Quincke, 1870]. The concentration of cholesterol (and, incidentally, of lecithin) in the following work is expressed as a percentage, that is, as the number of grams per 100 cc. of the phase in which it is present (either oil or water).

Table I. *Drop numbers and interfacial tensions of olive and cottonseed oils, respectively, in water in the presence of cholesterol, dissolved in the oil phase.*

% cholesterol	Olive oil		Cottonseed oil	
	Drop number	Interfacial tension dynes/cm.	Drop number	Interfacial tension dynes/cm.
0.0	72	18.6	66	20.3
0.5	—	—	67	20.0
1.0	89	15.0	76	17.6
1.25	—	—	83	16.1
2.0	117	11.4	—	—
2.5	130	10.3	—	—

Table II. *Drop numbers and interfacial tensions of olive and cottonseed oils, respectively, in contact with aqueous solutions containing calcium chloride.*

% calcium chloride	Olive oil		Cottonseed oil	
	Drop number	Interfacial tension dynes/cm.	Drop number	Interfacial tension dynes/cm.
0.0	72	18.6	66	20.3
0.1	73	18.5	—	—
0.25	95	14.1	66	20.3
0.5	180	7.4	69	19.4
2.0	—	—	75	17.9
4.0	—	—	106	12.6

The above results show that cholesterol and calcium chloride have a similar effect on the interfacial tension between oil and water, in that they cause a decrease. In the case of olive oil and water, the interfacial tension is lowered to a greater extent by calcium chloride than by cholesterol, whilst in that of cottonseed oil and water, cholesterol has the larger effect. Presumably this is due to the fact that the cottonseed oil contained less fatty acids than did the olive oil and consequently there was less tendency on the part of the calcium chloride towards the formation of a soap. Donnan [1899] has shown

that substances such as sodium hydroxide have only a very small effect on the interfacial tension between pure oils, containing no fatty acids, and water. The same would be true in the case of calcium hydroxide or a calcium salt, since the effects of sodium and calcium salts on such interfacial tensions depend upon the formation of a soap. The action of cholesterol, on the other hand, does not depend upon the formation of a soap.

From Clowes' work, it is known that the presence of calcium chloride favours the water-dispersed-in-oil type of emulsion. It does not follow from the above interfacial tension measurements alone that cholesterol favours this type. However, it is fairly generally recognised [cf. Hughes and King, 1923] that any substance, soluble in a fat phase, which lowers the interfacial tension in a fatty oil-water system will favour an emulsion of the water-dispersed-in-oil type. Cholesterol is such a substance and hence one would expect it to form a water-dispersed-in-oil emulsion. That such is the case has been shown by Seifriz [1923] and this has been confirmed during the present work.

An attempt was also made to study the effect of lecithin on the interfacial tension between oil and water, olive oil only in this case being employed. Lecithin is practically insoluble in olive oil and in water, but disperses readily in the latter.

In the first place, olive oil was saturated with lecithin, and the interfacial tension between such a solution and water was measured by the drop pipette method. When the sample of lecithin was entirely free from cholesterol, there was no definite effect on the interfacial tension, owing presumably to the negligibly small solubility of such lecithin in olive oil. When a sample of lecithin containing cholesterol was employed, the increase in the drop number of the olive oil in water was quite marked and corresponded to a decrease in the interfacial tension between the oil and water of 10 %. From the results obtained for the interfacial tensions of olive oil in water, in the presence of cholesterol, it may be shown that the amount of cholesterol, dissolved in the oil, which would cause such a decrease, is 0.5 %. But the total dissolved lecithin was much less than 0.5 % and therefore the amount of cholesterol must have been less than 0.005 %. Hence we infer that the lecithin, in so far as it is dissolved in the oil, whether in the form of a lecithin-cholesterol complex or not, behaves in a similar manner to cholesterol, in lowering the interfacial tension between olive oil and water. These measurements do not give any information regarding the type of emulsion formed between water and oil when lecithin is used as emulsifying agent. However, previous work [*vide* Bhatnagar, 1921; Seifriz, 1923] has shown that lecithin as emulsifying agent, favours an oil-dispersed-in-water system.

In studying the effects of the presence of both lecithin and cholesterol on the interfacial tension, the lecithin, as already mentioned, was dispersed, not dissolved, in the aqueous phase; the cholesterol, on the other hand, was dissolved in the oil phase.

The general result obtained was that, although these substances give rise

to different types of emulsion systems, their combined effect on the interfacial tension between oil and water is additive. The following table gives the results of such measurements.

Table III. *Drop numbers and interfacial tensions of olive oil in water in the presence of a mixture of lecithin and cholesterol.*

% cholesterol in the oil phase	% lecithin in aqueous phase	Drop number	Interfacial tension dynes/cm.
0.0	0.0	72½	18.6
0.0	1.0	91	14.8
0.5	1.0	95	14.2
1.0	1.0	105½	12.8
1.5	1.0	121	11.1

THE ANTAGONISTIC EFFECTS OF LECITHIN AND CHOLESTEROL ON OIL-WATER SYSTEMS.

In order to ascertain whether lecithin (cholesterol-free) and cholesterol itself, exert antagonistic effects when employed together as emulsifying agents in oil-water systems, it was of importance to examine the stability and nature of the emulsions formed by the individual substances. In all of the following work, the types of the emulsions were determined by means of the Briggs drop method, that is, by adding a drop of the emulsion to water and so discovering whether oil or water was the continuous phase. Such results were also confirmed in most cases by staining one phase and examining microscopically. Attention was paid to the ease with which the emulsions were formed in the respective cases.

When equal volumes of a 1 % aqueous lecithin dispersion and olive oil were shaken, complete emulsification occurred after about half-a-dozen shakes. Such an emulsion was of the oil-dispersed-in-water type and was fairly stable, a cream only separating out after several hours.

However, when a solution of 1 % cholesterol in olive oil was shaken with an equal volume of water, complete emulsification was not attained with the same ease. Intermittent shaking was resorted to, that is, the mixture was allowed to stand for about half-a-minute between each half-a-dozen shakes. After thirty-five to forty shakes, complete emulsification occurred and the emulsion was of the water-dispersed-in-oil type.

On shaking a 0.02 % aqueous cholesterol dispersion¹, with an equal volume of pure olive oil, complete emulsification occurred after about fifty shakes, the emulsion being of the water-dispersed-in-oil type. This observation agrees with that of Seifriz [1923], who employed equal volumes of a 0.01 % aqueous cholesterol dispersion and olive oil.

The emulsions formed through the emulsifying agency of cholesterol were not as stable as those formed in the case of lecithin. Breaking down commenced immediately the emulsions were allowed to stand and after half-an-hour, practically complete separation of the two phases had occurred.

In view of the totally different types of emulsion formed by lecithin and

¹ Prepared by adding 1 cc. of a 2 % solution of cholesterol in alcohol to 99 cc. of water.

cholesterol, respectively, especially in respect of stability, caution had to be exercised in the later determinations of the type of emulsion which resulted when mixtures of lecithin and cholesterol were employed as emulsifying agents. For example, in practically every case in which the two emulsifying agents were present, the emulsion formed after the first dozen shakes was of the oil-dispersed-in-water type. In those cases in which reversal of phase did actually occur, such reversal was only attained after a great deal of intermittent shaking. In one case, reversal took place after about six hours' shaking, but at the end of this time the emulsion which, after the first few shakes, was of the oil-dispersed-in-water type, undoubtedly had become reversed to the water-dispersed-in-oil type.

The reason for the above phenomenon probably lies in the differences in the ease with which emulsification is brought about by lecithin and cholesterol, respectively.

In Table IV appear the results of experiments carried out on the above lines, employing mixtures of lecithin and cholesterol as emulsifying agents, both of these substances being originally present in the aqueous phase. Concentrations are expressed as percentages, as before.

Table IV. *The effect of mixtures of lecithin and cholesterol on emulsion systems.*

Vol. of oil phase cc.	Vol. of aqueous phase cc.	% lecithin in aqueous phase	% cholesterol in aqueous phase	Ratio lec./chol.	Continuous phase
5	6.0	0.33	0.017	19.4	Water
5	5.5	0.18	0.018	10.0	"
5	5.4	0.148	0.0185	8.0	(Indefinite)
5	5.3	0.113	0.019	6.0	Oil
5	5.2	0.077	0.019	4.1	"
5	5.1	0.04	0.02	2.0	"

From the results shown in Table IV, for the case in which lecithin and cholesterol were both present in the aqueous phase, it is evident that the point of inversion of the emulsion occurs when the ratio of the concentration of lecithin to that of cholesterol, expressed in g. per 100 cc. aqueous solution, is about 8:1.

When, however, the cholesterol was originally present in the oil phase and the lecithin in the aqueous phase, relatively less lecithin was required at the point of inversion of the emulsions, as is shown by the results given in Table V.

Table V. *The effect of mixtures of lecithin, present in the aqueous phase, and cholesterol, in the oil phase, on emulsion systems.*

Vol. of oil phase cc.	Vol. of aqueous phase cc.	% lecithin in aqueous phase	% cholesterol in oil phase	Lec./chol. ratio	Continuous phase
5	5	0.08	1.0	0.08	Oil
5	5	0.2	1.0	0.2	"
5	5	0.4	1.0	0.4	"
5	5	1.0	1.0	1.0	(Indefinite)
5	5	2.0	1.0	2.0	Water

From the results shown in Table V, it is concluded that the ratio of the concentration of lecithin to that of cholesterol at the inversion point is between 1:1 and 2:1, the cholesterol being originally present in the oil phase. In the case in which cholesterol was originally present in the aqueous phase (in the dispersed form), the ratio of the concentrations of lecithin to cholesterol at the inversion point was 8:1¹.

Bearing in mind that, in the body, lecithin and cholesterol are most probably present in that phase in which they are more readily soluble, namely, aqueous and fatty phases, respectively, it is presumed that the results quoted in Table V will be more applicable to the permeability phenomena exhibited by cell membranes, than will those which appear in Table IV.

DISCUSSION OF RESULTS.

As previously mentioned, the foregoing measurements were carried out with the intention of attempting to account for the increased permeability of cancerous tissues, compared with that of normal tissues. Assuming that cytoplasm consists, even if only partly, of an emulsion formed between fats and water, it is evident that the conclusions arrived at by means of experiments carried out *in vitro*, such as those described, should be applicable to the definition of the state of such cytoplasm as a function of, for example, its lecithin and cholesterol content. Whatever other rôle these two substances may have, such as the regulation of oxidation processes in the cells, it is of primary importance to consider their part in the problem of the permeability of cell membranes, since changes in the permeability of an emulsion system such as is represented by cell membranes will most certainly affect the normal metabolism of the cell by modifying the direction and extent of ferment action. Thus it is conceivable that changes in permeability may stand in intimate relation to various abnormal states of the tissues, for example, neoplasia. If the lecithin-cholesterol ratio were found to be higher in cancer cells than in normal cells, it would be expected, on the basis of the above results, that cancer cells would be more permeable to water-soluble substances than would normal cells. A few results obtained by Bullock and Cramer [1913] on the lecithin and cholesterol content of malignant tumours, indicate that the lecithin-cholesterol ratio is higher in rapidly growing tumours than in slowly growing tumours. Hence in the light of the results detailed in the present paper, one would expect that rapidly growing tumours would be more permeable to water-soluble substances than would slowly growing tumours. It has actually been shown by Waterman [1922] that cancerous tissues, freshly removed from the body, have a higher electrical conductivity and permeability, than normal tissues under the same conditions.

¹ It has been suggested by Professor Ramsden that the differences in inversion points, indicated by Tables IV and V, respectively, may possibly be due to two different kinds of complex formed between lecithin and cholesterol, the constitution of the complex depending on the conditions which attend its formation.

It is not suggested that the lecithin-cholesterol ratio is the only important factor in determining the permeability of cancer tissues. It has been found that rapidly growing cancer cells are deficient in calcium and rich in potassium [Clowes and Frisbie, 1905]. This latter condition would also point to increased permeability of such cells.

Mayer and Schaeffer [1914] have already suggested that the state of protoplasmic membranes is dependent upon the relative amounts of lecithin and cholesterol present, but they ascribe the effect of these substances to an alteration in the degree of dispersion of the fats contained in cytoplasm.

SUMMARY.

1. The effect of lecithin, cholesterol, and mixtures of these two substances on the interfacial tension between oil and water has been studied. Both substances cause a lowering of the interfacial tension, and in the case of mixtures of the two substances, the effect is additive, in spite of the fact that lecithin and cholesterol favour different types of emulsion.

2. Emulsification experiments on olive oil-water systems have been carried out, in order to examine the antagonistic effect, if any, of lecithin and cholesterol on such systems. In agreement with the work of Seifriz [1923], lecithin is found to favour an oil-dispersed-in-water type of emulsion, whilst cholesterol favours the reverse type. When mixtures of these substances were present in the aqueous phase, antagonistic effects on the type of emulsion were observed. The inversion point of the emulsion occurred when the ratio of the concentrations (expressed in g.) of lecithin to cholesterol was about 8:1, both substances being present initially in the aqueous phase. When cholesterol was present in the oil phase to begin with, and the lecithin was in the aqueous phase, the inversion ratio was found to be between 1:1 and 2:1. Probably the latter condition, namely, that in which the lecithin and cholesterol are dissolved in different phases, approximates more closely to the conditions found in cell membranes. Consequently the latter result, which states that inversion takes place when the ratio of lecithin to cholesterol is between 1:1 and 2:1, is probably the more significant result in connection with permeability phenomena, exhibited by cell membranes¹.

¹ The above ratio has been found by Lange and Lawaczek [1923] to be that at which the rate of oxygen intake is a maximum in the case of the system—lecithin, cholesterol, ferric chloride, water, *in vitro*.

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