CCLIX. PHYSICO-CHEMICAL STUDIES OF LIPOIDS IN THEIR RELATIONS TO SALTS, DRUGS AND PROTEINS¹.

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ALTHOUGH extensive investigations [Remesow, 1930] have been made recently on the physico-chemical behaviour of some lipid sols, explanations are still lacking of some problems interesting to the biologist. Of these problems, the specific therapeutic effect of bromides and the relation of lipoids to proteins have been chosen for a physico-chemical investigation.

In a previous paper [Spiegel-Adolf, 1932], an *in vitro* analogy with the specific effect of bromides on the substance of the central nervous system was demonstrated. While the Hofmeister series of anions gives no clue in this direction, it could be shown that bromides have a stronger depressing effect on the viscosity of lecithin sol than any other salts, except iodides containing traces of free iodine. This seemed to suggest that the specific effect of bromides is not due to the bromide ions, but to free bromine. Pauli [1929] has pointed out the possibility of such a mechanism, and recent work of Lanza [1931] has proved that the addition of iodine to KI does not change the molecular concentration and only very slightly affects the electrolytic dissociation of the latter. Owing to the unsaturated fatty acid content of lecithin a reaction with bromine or iodine is possible. As there is chemical evidence that fatty acids add KI, too, and are then enabled to bind more of other substances, even the behaviour of the compounds of lecithin and protein can be explained in this way.

In order to test this hypothesis, the following series of experiments was made. Though egg-lecithin (Merck) is, according to Remesow [1930], far from pure, it was used, since most work has been done on it, and results permitting comparison with the latter were needed. The lecithin sol was made according to Keeser [1924] by adding small portions of boiling alcoholic solution of lecithin to boiling redistilled water. Some figures concerning the conductivity and c_H of such sols are reported in Table IV. In order to check the possible influence of traces of alcohol, emulsions of lecithin in redistilled water were used in several experiments. Such emulsions [*cf.* Thomas, 1915], are more opaque than a fresh sample of a sol made after Keeser, but are nevertheless stable. The protein used consisted of serum-albumin and pseudoglobulin prepared from horse-serum by $(NH_4)_2SO_4$ fractionation, and purified by reprecipitation, dialysis and electro dialysis.

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It is known that the total refraction of a solution is equal to the sum of the refractions due to the separate solutes, but this is only true if there are no chemical reactions between them. If KBr and KI containing free iodine should act on the lecithin in the way suggested above, it is to be expected that the refractive index of a mixture of lecithin sol with such salts will differ from that of the same sol with KCl, for instance. As only very slight changes were expected, the interferometer of Zeiss was used for the following investigations [cf. Hirsch and Kunze, 1922]. As the lecithin sol that was to be used was far from pure, and the molecular weight of the colloidal particle unknown, no attempt was made to obtain absolute values for the refraction. The 1 % lecithin sol was diluted 20 times; the final concentration of the salts was 0.01 *M*. The interferometric values were measured in the lecithin sol, the salt solution and in a mixture of lecithin sol and salt. Correction for water was made in every case. The results are given in Table I.

Table I. *1/20 lecithin sol—74 I.U., cell 20 mm.*

Neutral salt con- centration	KCl		KBr		KI		KI + I		KCNS	
	obs.	calc.	obs.	calc.	obs.	calc.	obs.	calc.	obs.	calc.
0.01 %	200	199	252	238	347	339	367	349	288	284
Difference	1		14		8		18		4	

In all cases the sum of the refractions of the separate components was lower than the observed value. The effect of bromide however was by far the greatest, only being surpassed by the effect of KI containing 0.02 g. of iodine per 10 cc. of fresh *N* KI. KCl did not significantly alter the refraction, even when the concentration of the salt was raised 10 times. The absolute values of the changes in interferometric units (I.U.) are only small, but while it is possible to get exact readings to ± 1 I.U., there is, according to Marc [1912], less danger, for colloids at least, of being mistaken in the right hand when using dilute solutions; in fact the readings permitted no doubt in this direction. On the other hand, the use of more concentrated solutions would not increase the sensitivity of the method, as the increased opacity would necessitate a shorter cell. The absolute values are well within the ranges that could be expected according to other findings [Hirsch and Kossuth, 1922].

As was mentioned in the previous paper, the swelling of lecithin sol is lowered to very different degrees by chloride and bromide. It seems that a part of the lowering effect on the viscosity common to both salts is due to electrical discharge of the lecithin sol, hydration being in close connection with the electrical charge. But the greater effect of bromide may be due to colloidal changes consequent on chemical reaction. In order to decide whether the observed relations between viscosity and refraction hold for other colloids, interferometric measurements were made on protein solutions in the same way as on the lecithin sol, and it was found that the two salts had practically

the same effect on the viscosity and the refraction of both serum-albumin and pseudoglobulin.

The experiments which are described in this paper confirm those previously reported, in demonstrating the specific effect of bromides. Naturally they are not sufficient to decide whether bromides act in the same way on the brain, or if they do, that this physico-chemical behaviour is the reason for their specific effect.

Winterstein [1926], considering the results of Hansteen Cranmer confirmed by Biedermann, believes that the lipoids actually present in the cells are quite different from those obtained by extraction. On the other hand, Handovsky and Wagner [1911] observed no reaction between lecithin sol and drugs in their experiments on viscosity. In order to investigate the possibility of an additional parallelism between viscosity and refraction, further experiments were made on these lines. As far as possible, the kind and concentration of the drugs used were the same as those employed by Handovsky and Wagner. Experiments were also made with sodium-luminal, the clinical use of which is similar to that of bromides. Finally, a convulsion-producing poison, strychnine nitrate, was tried in the same way. The results are summarised in Table II.

Table II. *1/20 lecithin sol—74 I.U.*

1% lecithin sol diluted %	Final concentration	Interferometric units		Difference	Cell mm.
		Measured	Calculated		
1/20	0.1 % sodium-luminal*	288	271	17	20
1/20	0.15 % chloral hydrate	271	255	16	20
1/10	Sulfonal, half saturation	102	103	-1	5
1/10	Sulfonal, 9/10 saturation	137	137	0	5
1/10	Trional, half saturation	146	146	0	5
1/10	Trional, 9/10 saturation	208	211	-3	5
1/10	0.05 % urethane	302	302	0	5
1/10	0.01 % strychnine nitrate	98	98	0	5

* NaOH of the same p_H as the sodium-luminal solution has no effect on the refraction of the lecithin sol.

The figures suggest a reaction between lecithin sol and sodium-luminal, or chloral hydrate, though they may also mean that the dispersion of the lecithin is increased. The negative results with sulfonal, trional and urethane agree with the findings of Handovsky and Wagner. The opposite effect exercised by the highest concentration of trional on lecithin sol, is explained by a decrease of the dispersion of the latter. Strychnine nitrate had no effect on the refractive power of lecithin sol.

The reaction between lecithin and proteins depends largely upon the c_H of the solution, the presence of neutral salts, and the age of the lecithin sol. The first point has been extensively investigated by Rona and Deutsch [1926], but underestimation of the important rôle of salt concentration in the relation between lecithin sol and proteins seems to be the reason for contradictory opinions in this matter.

It was noted previously [Spiegel-Adolf, 1932] that electrolyte-free solutions of serum-albumin and pseudoglobulin flocculate lecithin-sol made according to the method of Keeser [1924]. In good accord with results found by Pauli and Weiss [1928] in their investigations on colloid dyes, pseudoglobulin has a stronger flocculating effect than serum-albumin. More than 50 times as much of either protein is necessary for flocculation of 24 hours-old lecithin sol than for a week-old sol. Table III records the results of some experiments of this kind.

Table III.

Concentration of protein %		× = flocculation.								
		1.5	0.75	0.28	0.14	0.045	0.028	0.014	0.005	0.003
Fresh lecithin sol	{Serum-albumin	× × ×	0	0	0	—	0	0	0	—
	{Pseudoglobulin	× ×	× ×	×	0	—	0	0	0	—
Old lecithin sol	{Serum-albumin	× × ×	× × ×	× × ×	× × ×	0	0	0	0	—
	{Pseudoglobulin	× × ×	× × ×	× × ×	× × ×	—	× ×	× ×	×	0

According to Freundlich [1922], the ageing of lecithin sol consists of changes by which the latter partly loses its hydrophile properties and grows more hydrophobe. In good accord with this, the amounts of protein necessary for the flocculation of aged lecithin sol are within the range of the corresponding values for gold sol [Spiegel-Adolf, 1927].

There seem to exist no systematic investigations of the physico-chemical changes during ageing of lecithin sol, though there are various hints in this direction [Porges and Neubauer, 1908; Rona and Deutsch, 1926]. For the better understanding of what has been said above and what is to follow, some observations are recorded in Table IV.

Table IV.

Age of lecithin sol (Keeser)	Conductivity rec. Ohm	Viscosity 1/5 sec.	c_H
24 hours	1.69×10^{-5}	1405	1.37×10^{-4}
8 days	1.84×10^{-5}	1380	—
12 days	—	—	1.77×10^{-4}
18 days	2.10×10^{-5}	—	1.95×10^{-4}
30 days	2.33×10^{-5}	—	—
Lecithin sol water emulsion 24 hours	—	—	1.95×10^{-4}

The above experiments were performed on lecithin sols kept exclusively in bottles of pyrex glass under optimum conditions for stability. Nevertheless, the 1 % solutions, which were first translucent and of a reddish-yellow hue, became more opaque after a few days and, in the course of time, much of their colour faded. A sample kept for a year was nearly white. If infection by moulds were avoided, the sol seemed to be fairly stable. As long as no precipitation had occurred, no changes in the refractive index of the colloid solution could be detected, and measurements of the c_H using the hydrogen electrode showed only small changes. Measurements made on the same sample immediately after preparation, and a week later, indicated a slight increase of the acidity.

After 18 days, a c_H was reached corresponding to that of a fresh watery emulsion of lecithin. The secondary increase of c_H was accompanied by a similar change of the conductivity, while the viscosity showed a decrease from the initial value.

Porges and Neubauer [1908] have already shown that the flocculation of lecithin sol by proteins is inhibited by the presence of neutral salts. This is not a property peculiar to the lecithin sol, but is observed with negative sols such as colloid solutions of gold, mastic and cholesterol. In this way, it is possible to obtain stable solutions containing known amounts of lecithin, protein, and salts. In a previous communication [1932], the viscosity of such solutions under the influence of chlorides and bromides has been investigated. Handovsky and Wagner [1911] had used similar solutions in order to ascertain whether there were any reason for assuming the formation of a compound between protein and lecithin sol, but obtained negative results. Later on, Arnd and Hafner [1926] found that the refractive power of a pseudoglobulin solution was markedly lowered by the addition of lecithin sol, which they accepted as proof of formation of a lipoid-protein compound. In order to explain the discrepancy between these opinions, experiments were started, using interferometric measurements. At the same time, the influence of different salts was tested. Some results are reported in Tables V and VI.

Table V. *Lecithin sol and protein (final concentration 0.1 %).*

NaCl concentration N	Serum-albumin		Pseudoglobulin	
	Observed	Calculated	Observed	Calculated
0.025	162	160	137	146
0.05	—	—	222	220
0.1	398	396	366	365
0.2	669	668	—	—

Table VI. *Pseudoglobulin and lecithin sol (final concentration 0.1 %).*

0.1 N final concentration	KCl		KBr		KI		KCNS		(NH ₄) ₂ SO ₄	
	Obs.	Calc.	Obs.	Calc.	Obs.	Calc.	Obs.	Calc.	Obs.	Calc.
Sol 7 days	408	413	527	524	779	776	130*	130*	88*	86*
Sol 14 days	—	—	95*	111*	—	—	—	—	—	—
Fresh 1 day	—	—	528	525	—	—	—	—	—	—

* All measurements were made in a 5 mm. cell except those marked with asterisks, for which a 1 mm. cell was used.

The figures suggest the following interpretation. As long as the salt concentration is sufficient to avoid the faintest signs of flocculation, no changes of the refractive indices are observed. Different salts seem to behave in the same way. If the salt concentration is lowered to a point at which the solution becomes unstable, then the interferometric value of the solution is lower than the sum of the corresponding values for the separate components. The results are the same whether the instability be due to an initial low salt concentration, or whether through the ageing of the colloid, the salt concentration becomes

insufficient. These findings explain the observations of Handovsky and Wagner as well as those of Arnd and Hafner [1926], though they do not necessarily confirm the conclusions of the latter authors. The decrease in refraction must be explained primarily by a decrease of soluble material.

The first row of figures in Table V indicates a difference between the behaviours of serum-albumin and pseudoglobulin towards lecithin at the same low salt concentration. While the latter is not sufficient to avoid flocculation of the pseudoglobulin-salt-lecithin mixture, it is still able to guarantee the stability of a solution in which pseudoglobulin is replaced by serum-albumin. Although there is some similarity in this respect to the behaviour of the same salt-protein mixtures toward gold sol, it is not possible to speak, in the case of lecithin sol, of the protective power of the proteins. The same amount of neutral salt which inhibits the flocculating effect of proteins, in both cases precipitates the gold sol, but leaves the lecithin sol apparently unchanged. In earlier experiments on gold sol, evidence has been found for assuming that a compound of proteins and neutral salt would be able to protect the gold sol from the flocculating effect of further salt addition. Experiments of a similar kind were started with lecithin sol, only, instead of salt addition, pseudoglobulin was tried for testing the protective power of a serum-albumin-neutral salt compound. Only in cases in which there was a certain gap between the salt concentration able to inhibit the flocculating effect of serum-albumin and that having the same influence on pseudoglobulin, are such experiments possible. For this purpose, only lecithin sol of a certain age could be used. In recently prepared samples, the differences are too slight; in very old sols, sometimes the protective power of salts, even against albumin, vanishes. Only lecithin sols 14–21 days after the preparation according to Keeser, fresh emulsions of lecithin in distilled water, or lecithin sols derived from an older, perhaps oxidised sample of lecithin, proved to be of use for the experiments in question. The results of two series are summarised in Table VII.

These results seem to show that under certain conditions, flocculating concentrations of pseudoglobulin do not show this effect in the presence of serum-albumin and neutral salt. Though the latter at the same concentration is, in itself, not sufficient to prevent the flocculation effect of pseudoglobulin, its presence is necessary for giving the serum-albumin protective power. Non-flocculating concentration of the latter in an electrolyte-free medium is unable to inhibit the precipitation of the lecithin sol by pseudoglobulin. The negative result of interferometric investigation made in order to trace some reaction between electrolyte-free serum-albumin and pseudoglobulin was in accordance with these findings. For this reason, the above-mentioned influence of the neutral salt-serum-albumin compound on pseudoglobulin cannot be directly compared with observations made by Pauli and Singer [1932], who found that ovalbumin prevented, to some extent, the flocculation of certain colloid dyes by serum-albumin in the absence of salt.

Next to the salt concentration, the protective power depends upon the

Table VII. *Part I.*

Lecithin sol 19 days old	× =flocculation.		+ =opacity.	
	NaCl final concentration <i>N</i>	Serum- albumin %	Pseudoglobulin %	Result
1/4	0.05	0	0	0
1/4	0	0.25	0	× × ×
1/4	0	0	0.25	× × ×
1/4	0.05	0.25-0.5	0	0
1/4	0.05	0	0.25-0.5	× × ×
1/4	0.05	0.25	0.25	0
1/4	0.05	0.2	0.3	0
1/4	0.05	0.15	0.35	× ×
1/4	0.05	0.1	0.4	× × +

Table VII. *Part II.*

Lecithin sol aged by boiling	KCl final concentration <i>N</i>	Serum- albumin %	Pseudoglobulin %	Result
1/4	0.1	0	0	0
1/4	0	0.75	0	× × ×
1/4	0	0	0.75	× × ×
1/4	0.1	0	0.7-1.4	×
1/4	0.1	0.75-1.4	0	0
1/4	0.1	0.75	0.7	0
1/4	0.1	0.6	0.84	0
1/4	0.1	0.45	1.0	0
1/4	0.1	0.3	1.1	× + +
1/4	0.1	0.15	1.25	× × ×

ratio of serum-albumin to pseudoglobulin. If the latter falls below 2/3-1/2, precipitation starts. It seems therefore that the behaviour of the total protein mixture toward the lecithin sol depends on the relation of its components. The similarity of this behaviour to that of serum under various pathological conditions is somewhat striking. There, too, the so-called albumin/globulin quotient [Hofmann, 1883] seems to determine, in many respects, the properties of the serum and other biological fluids. The reactions of spinal fluid toward gold sol seem in particular, according to Schmitt [1927] to depend largely on this property. An increase of globulin has been described in nearly all luetical changes. The above-mentioned results may be of some value for a further understanding of luetical reactions using the flocculation methods.

SUMMARY.

1. Confirming prior experiments on viscosity, interferometric measurements seem to give evidence for a supposed reaction between bromine and lecithin sol, but fail to explain the effect of hypnotics.

2. The effect of lecithin addition to proteins depends on the salt content of the latter. In an electrolyte-free medium, flocculation occurs; if there is an excess of salt, no changes can be detected by help of the interferometer. Between those two extremes there are salt concentrations at which lecithin-sol additions cause a decrease of the refractive index of the mixture.

3. For lecithin sols of a given age, different concentrations of neutral salts are required to avoid flocculation by the same amount of serum-albumin or

pseudoglobulin. In mixtures containing both kinds of proteins with just sufficient salt to prevent flocculation by serum-albumin, the serum-albumin-neutral salt compound seems to inhibit to some degree the flocculating power of pseudoglobulin, the final effect depending on the albumin/globulin ratio.

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