

allowed to stand in the refrigerator over night and injected. The results are shown in Table I.

TABLE I.

No. of Rats	Material Injected per Rat	No. Killed	% Killed
	Sheep Super-Immune Serum		
10	10 A.L.D. plus 0.1 cc. serum	7	70
10	10 " " 0.2 " "	5	50
10	10 " " 0.4 " "	0	0
	Human Convalescent Serum		
7	1 A.L.D. plus 0.25 cc. serum	4	57
6	1 " " 0.5 " "	4	66
4	2 " " 1.0 " "	4	100
	Normal Sheep Serum		
10	2 A.L.D. plus 1.0 cc. serum	10	100

*Conclusions.* The results indicate a much higher potency for the sheep serum, one cc. of this serum completely neutralizes 25 average lethal doses of the venom whereas one cc. of the human convalescent serum shows no neutralizing power whatever against 2 average lethal doses of venom.

## 8932 C

## Physical Chemistry of Lipoids. IV. Influence of Narcotics on the Salt-binding Capacity of Lecithin.

MONA SPIEGEL-ADOLF.

*From the Department of Colloid Chemistry, D. J. McCarthy Foundation, Temple University School of Medicine, Philadelphia.*

Direct reactions between lipoids and narcotics were missed in previous studies of viscosimetry<sup>1</sup> and interferometry.<sup>2</sup> In view of the biological importance of lipoids, one must therefore assume that narcotics influence reactions of lipoids with other substances. Nervous excitation is explained by Nernst<sup>3</sup> by changes of ion concentrations occurring at the surface of membranes. Experimental proof of the important rôle of lipoids in artificial polarizable membranes has recently been given.<sup>4</sup> The importance of lipoids in narcosis<sup>5</sup> has been widely assumed. Nevertheless, no data are available that

<sup>1</sup> Handovsky, H., and Wagner, R., *Biochem. Z.*, 1911, **31**, 32.

<sup>2</sup> Spiegel-Adolf, M., *Biochem. J.*, 1932, **26**, 2183.

<sup>3</sup> Nernst, W., *Pflüger's Arch.*, 1908, **122**, 275; **123**, 454.

<sup>4</sup> Spiegel-Adolf, M., *J. Biol. Chem.*, 1936, **114**, xcix.

<sup>5</sup> Henderson, V. E., *Physiol. Rev.*, 1930, **10**, 171.

connect the changes of ion concentration on the cell surface with the lipoids. Former experiments<sup>6</sup> on the salt-binding capacity of lecithin were therefore continued, and the influence of narcotics was examined. The lecithin sols made from egg-lecithin (extra pure Merck, Germany) were either emulsions or were prepared by the method of Keeser<sup>7</sup> (pouring small amounts of boiling alcoholic lecithin solutions into boiling distilled water and boiling the alcohol away). In view of former observations<sup>2</sup> only fresh samples of lecithin sol were used.

Since higher concentrations of lecithin were to be used, conductivity measurements were made instead of the formerly used interferometric determinations. The conductivity (K) was measured in the aqueous lecithin solutions (a), in the salt solutions (b), and in an aqueous solution (c) containing lecithin and salt in the same concentrations as in a and b respectively. The difference (KD) between the calculated values (a + b) and the observed value (c) is used as a measure for the amount of ions bound or adsorbed to lecithin. A similar method has been used by Spiegel-Adolf<sup>8</sup> on globulins. In all experiments it could be shown that the electric conductivity of KCl is decreased by the presence of lecithin. In analogy with the findings in proteins, this fact can be explained by a binding or inactivation of salt-ions by the colloidal lecithin particle.

Quantitatively the salt-binding capacity of lecithin sol depends upon several factors.

TABLE I.\*  
Influence of Ageing.

Time after mixture	5 hr.	24 hr.	48 hr.	72 hr.
KD $\cdot 10^{-3}$	1.22	1.62	1.95	2.12

\* All figures in Tables I-IV refer to 1% lecithin sol. 0.05 N KCl (except Table II) and were measured at 30° C.

1. The results summarized in Table I indicate that the salt-binding capacity of lecithin-sol increases with the time the mixture is allowed to stand. It could be ascertained by special experiments that these changes do not depend upon the ageing of the lecithin sol but upon the time the salt is in contact with the colloid.

2. The amount of salt bound to lecithin sol depends upon the concentration of both salt and lecithin. If the concentration of lecithin sol is kept constant, and the amount of KCl is varied, then the

<sup>6</sup> Spiegel-Adolf, M., *Biochem. J.*, 1935, **29**, 2913.

<sup>7</sup> Keeser, E., *Biochem. Z.*, 1924, **154**, 321.

<sup>8</sup> Spiegel-Adolf, M., *Kolloidchem. Beih.*, 1923, **18**, 275.

graphical presentation suggests the existence of a salt binding maximum in a surplus of salt.

3. The amount of salt bound to lecithin sol depends upon the degree of dispersion of the lecithin sol. Bungenberg, Verberg and Westerkamp<sup>9</sup> have shown recently that even small amounts of contamination prevent the making of a translucent lecithin sol. When a different preparation (Merck, Rahway, 90% pure) was used, the lecithin sol became opaque and milky and the salt-binding capacity of the lecithin sol dropped to about 1/10 of the original value.

In order to elucidate the type of salt fixation to lecithin sol, 2 different series of experiments were undertaken. 1. In aqueous solutions lecithin is negatively charged.<sup>10</sup> Electrophoresis experiments were made using the apparatus of Landsteiner and Pauli.<sup>11</sup> The KCl concentration was varied. Up to 0.1 KCl, no change of the anodic movement of the lecithin sol could be detected. 2. In systematic investigations on globulins<sup>8</sup> it could be shown that globulin calls forth different decreases in conductivity in isonormal solutions of KCl, NaCl and LiCl. This has been explained by the different velocities of the cations and was regarded as a proof that part of the cations had been bound to the globulin. Similar experiments made with lecithin sol gave analogous results. (Table II.)

TABLE II.  
Influence of Cation-Velocity.

Salt	KCl	NaCl	LiCl
KD .10-3	1.62	1.04	0.96

The results of both the electrophoresis and the conductivity measurements seem to indicate that both salt ions are fixed by the lecithin, and that the new compound shows little if any ionisation. Under this assumption, 1% lecithin allowed to stand for 24 hours in contact with 0.05 N KCl binds approximately 18% of the salt.

In another series of experiments, the influence of some narcotics on the salt-binding capacity of lecithin was investigated. In every case all the samples (lecithin-sol, salt solution, lecithin-salt mixture) were treated in the same way in order to exclude possible interferences of changes of the dielectric constants. Some of the results are summarized in Table III.

<sup>9</sup> Bungenberg de Jong, H. G., Verberg, G., and Westerkamp, R. F., *Kolloid Z.*, 1935, **71**, 184.

<sup>10</sup> Thierfelder, H., and Klenk, E., *Die Chemie der Cerebroside und Phosphatide*, Berlin, J. Springer, 1930.

<sup>11</sup> Landsteiner, K., and Pauli, W., *Verh. d. Kongr. f. Inn. Med.*, 1908, **25**, 571.

TABLE III.  
 Influence of Narcotics.

	Ethyl Alcohol	Ether	Chloroform	Chloralhydrate
Lecithin-Salt Narcotic, $KD_n F \cdot 10^{-3}$	1.11	1.10	1.11	1.98
Control without Narcotic, $KD_c \cdot 10^{-3}$	1.95	2.63	2.12	2.18
$KD_n \cdot KD_c \cdot 10^{-4}$	8.4	15.3	10.1	2.0

The figures show that the presence of ethyl alcohol, ether, chloroform, and chloralhydrate decreases the salt binding capacity of lecithin. Since these substances are chemically different, no attempt was made to compare their effectiveness on the salt-binding capacity of lecithin in a quantitative way. For this purpose homologous alcohols were used, since these alcohols have been studied, especially in order to correlate chemical and biological behavior.<sup>5</sup> The results are summarized in Table IV.

 TABLE IV.  
 Influence of Homologous Alcohols.

	KD	Dielectric constants Aqua = 80	Partition-coeff. 5) Oil: water	Sorption <sup>13</sup> Picric acid to charcoal	Narcotic effect 5)
Methyl Alcohol	$95 \times 10^{-5}$	32	50	98	.57
Ethyl "	78 "	26	1:30	91	.29
N-Propyl "	73 "	22	1:8	75	.11
I-Butyl "	10 "	19	6:1	64	.045
N-Butyl "	2 "	—	$\infty$ :12	—	.038
I-Amyl "	0 "	16	$\infty$ :12	—	.023

<sup>12</sup> Winterstein, H., *Die Narkose*, 1926, Springer, Berlin.

<sup>13</sup> Thomas, A. W., *Colloid Chemistry*, 1934, McGraw-Hill, New York and London.

The results show that the capacity of alcohols to decrease the salt-binding capacity of lecithin becomes more marked as the length of the chain increases. In presence of amyl alcohol the salt binding capacity has practically disappeared. At the same time the opacity of the lecithin sol increases, as well as its sensitiveness to salt precipitation. A similar behavior has been observed by Freundlich and Rona,<sup>14</sup> when colloidal ferric hydroxyde was treated with narcotics.

Several possible explanations are to be considered: 1. the dielectric constants of the homologous alcohols decrease with increasing chain length. It has been mentioned above that the experimental method used excluded an influence of changes in the dielectric constants upon the salt conductivity. Nevertheless, in presence of a

<sup>14</sup> Freundlich, H., and Rona, P., *Biochem. Z.*, 1917, **181**, 87.

lipoid, such changes could account for a loss of conductivity, as long as the narcotic is more soluble in water. But this explanation fails with the higher alcohols in which the lipoid solubility prevails. 2. It seems more probable that the different alcohols differ in effect on the sorption capacity of colloidal lecithin. Freundlich and Rona<sup>14</sup> have explained the sensitizing action of narcotics upon ferric sol through sorption of the narcotics to the surface of the colloid. Heymann and Boye<sup>15</sup> working on the same series of alcohols have shown a very similar phenomenon to the one observed on lecithin. In their experiments, the sorption capacity of charcoal for various acids decreased with increasing chain length and molecular polarization of the added alcohols. 3. The relation between salt-binding capacity and degree of dispersion in the lecithin sols have been mentioned above. Therefore, in the case of lecithin, the influence of those alcohols upon the dispersion of the sols should in itself lower the salt-binding capacity of lecithin. Chloralhydrate which has less effect upon the salt-binding capacity of lecithin sol has been found to increase the dispersion of lecithin sol.<sup>2</sup> If it is warranted to assume that egg-lecithin behaves physicochemically like human lecithin, then the diminished ability of the lipoids in the cellular surface films to fix salt ions should diminish the reactivity of the cell upon stimuli. Changes in ion concentrations must reach a higher threshold in order to act upon the lipoids of the cell surface or of its interior. In this connection, it seems of interest that there is a certain parallel between the power of homologous alcohols to lower the salt-binding capacity of lecithin and their narcotic effect.

### 8933 P

#### Localization of Pain Following Faradic Stimulation of the Common Bile Duct.

ROBERT ZOLLINGER AND CARL W. WALTER. (Introduced by E. C. Cutler.)

*From the Surgical Clinic of the Peter Bent Brigham Hospital, Boston, Mass.*

Previous studies<sup>1</sup> have demonstrated the inability to reproduce pain referred to the back by mechanical distention of the gallbladder or common duct in conscious patients. Pain, characteristic of the

---

<sup>15</sup> Heymann, E., and Boye, E., *Z. f. phys. Chem.*, 1930, **150A**, 219.

<sup>1</sup> Zollinger, Robert, *Proc. Soc. Exp. Biol. and Med.*, 1933, **30**, 1260.