

## ON LECETHIN.

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### INTRODUCTORY.

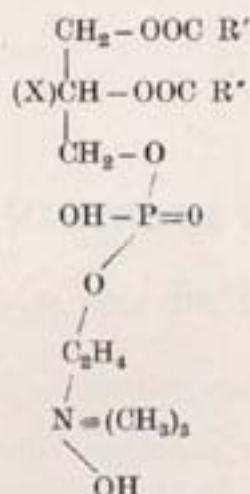
**T**HE organism of the higher vertebrate adjusts itself in many ways to changes of external and internal environment. The brain, the spinal cord and the nervous system in general are involved directly or indirectly in all these adjustments. The chemical components of this great system are predominantly lipoids. It is, therefore, in the careful study of the chemical constituents composing the nervous system, their structure, their decomposition products and, perhaps later, their proper place in the structure of the whole, that an answer may be found to the physico-chemical mechanism underlying nervous processes.

It is not our purpose to survey the history of lipoids, but only to mention briefly those contributions which seem to have a greater bearing on our work. A definite order in the chemistry of lipoids was brought about by extensive and painstaking investigations of Thudichum (1). He not only contributed much to the knowledge of individual lipoids, but proposed a scheme for their classification. His division is as follows:—

1. Monoaminophosphatides (one atom of nitrogen and phosphorus per molecule: lecethin and cephalin).
2. Diaminomonophosphatides (sphingomyelin).
3. Diaminodiphosphatides (assurin).

Perhaps of all lipoids the most widely distributed is lecethin. Gobley (2) was first to obtain and name it. Ulpiani (3), Begell (4), Thierfelder and Stern (5), Maclean (6), (7), (8), (9), (10), Paal and Oehme (11) and Ritter (12) contributed to its isolation, purification and structure. Recently Levene and his co-workers, in a series of investigations (13), (14), (15), (16), (17), (18), (19), (20) have clarified the composition of lecethin and firmly established the nature of the fatty acids bound to its molecule.

These and other investigations have shown that lecethin has probably the formula :—



in which choline, the nitrogen yielding part of the molecule is bound through an ester linkage. The carbon atom (X) is assymmetric (21) being bound to four different atoms or radicles. The symbols R' and R'' stand for different fatty acids attached to the molecule.

The number of fatty acids isolated from different lecethins is quite large. Since our starting material was lecethin derived from egg, we shall restrict ourselves only to the fatty acids of this lecethin. Palmitic  $\text{CH}_3(\text{CH}_2)_{14}\text{COOH}$ , and stearic,  $\text{CH}_3(\text{CH}_2)_{16}\text{COOH}$ , acids have been found. Among the unsaturated acids oleic  $\text{C}_8\text{H}_{17}\text{CH}=\text{CH}(\text{CH}_2)_7\text{COOH}$  is invariably present. Linolic acid, together with the highly unsaturated arachidonic acid, have been also identified. It is interesting to note that the saturated and unsaturated fatty acids are present in about equimolar proportion, indicating that on the average there is one saturated acid to every unsaturated one in the lecethin molecule.

If we now fix our attention on the disposal of the available fatty acids it will be evident that we must have more than one lecethin. The number of available positions is two (R' and R'' in the formula) while there are five fatty acids. As we have noted, one of these positions should be occupied by a saturated fatty acid. It follows that six different lecethins are possible.

On examination of the probable formula of lecethin, it will be noticed that a fatty acid located at R'' will be nearer to the phosphoric acid part of the molecule than in position R'. Since the acid in position R' and R'' are not the same they will affect the chemical properties of the phosphoric acid part of lecethins molecule differently. The reverse is also true—the acids being at different distances will be affected to a different extent by the





In Table I we have calculated the elementary composition of various lecethins which are analytically different. For comparison we have also calculated the analytical figures for lecethin deprived of fatty acids, as well as for lecethins having only one fatty acid. The latter, though not included in the total estimate of the number of possible lecethins, may be, as we shall see, really existant.

#### EXPERIMENTAL.

##### *I. Analysis.*

In the course of the investigation the following analyses were carried out:—

*Total Nitrogen:* The total nitrogen was determined by the method suggested by Pregl (26), using a Parnas and Wagner (27) apparatus for distillation. Copper sulphate was used as the oxidizing agent. For sampling, lecethin was dissolved in methyl alcohol. When cadmium chloride salts were used, carbon tetrachloride was substituted for methyl alcohol. Our analysis usually agreed within two per cent.

*Amino Nitrogen:* The amino nitrogen was determined in the micro apparatus designed by D. D. Van Slyke (28). The solvent used for samples of lecethin was glacial acetic acid, and for the cadmium chloride salt-lecethin, carbon tetrachloride or water. The determination of the amino-nitrogen in cadmium chloride salts is a troublesome procedure. The best method was found to be transference of 1 c.c. of the suspension of the salts in carbon tetrachloride or water to the measuring tube of the apparatus, washing it in with another c.c. of the solvent. A much prolonged period of shaking is necessary to complete the reaction—at least one hour or more. A blank was carried after every determination and shaken for the same period of time. The temperature was about 20°C. The determinations agreed within about 3-4 per cent.

*Carbon and Hydrogen:* These elements were determined by the procedures recommended by Pregl (29). Results are accurate within about one per cent in the case of carbon and two per cent in the case of hydrogen.

*Phosphorus:* The phosphorus was determined according to the method described by Pregl (30). The lecethin was decomposed by the procedure recommended by Lieb and Wintersteiner (31). The analysis agreed within two per cent.

*Determination of the Iodine Number:* The iodine number was determined by the method of Wij. The analysis agreed within about two or three per cent.



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*Determination of Cadmium Chloride:* The cadmium chloride bound to lecethin was determined either by analysis of chlorine by Pregl's method (32) or by the electrolytic method described below.

*Molecular Weight Estimates of the Cadmium Chloride Salts of Lecethin:* K. Rast's method described by Pregl (33) was used. It consists in determining the melting point depression of a known quantity of the camphor in which a known quantity of the unknown has been dissolved. The molecular depression of camphor was taken as 38°. The cadmium chloride salts were not perfectly stable at the temperature employed and had a tendency to give diffuse melting points. The experimental error is therefore considerable and probably amounts to about  $\pm 10$  per cent. The method is, however, valuable in fixing the general size of the molecule.

### II. Preparation.

A commercial preparation of lecethin<sup>1</sup> from eggs was used as a starting material. It was dried by being dissolved in ether and precipitated with acetone. The supernatant liquid was then decanted and the lecethin dried over sulphuric acid in vacuo until constant weight.

*Preparation of Cadmium Chloride Salts of Lecethin:* 230 gms. of lecethin were dissolved in about 2,200 c.c. of 95 per cent ethanol. The mixture was filtered through cheese cloth and to the filtrate 4,800 c.c. of saturated solution of anhydrous cadmium chloride in absolute methyl alcohol were added. During this operation the solution was stirred mechanically. The precipitate of the cadmium chloride salts of lecethin was then left to settle and the supernatant liquid syphoned off and discarded. To the salts, one liter of absolute ethanol was added and the solution stirred again for five minutes. The precipitate was then allowed to settle and the supernatant liquid was syphoned off. The salts were then washed three times with one liter portions of anhydrous ether and dried in vacuo.

*Recrystallization of Lecethin Cadmium Chloride from a Mixture of Ethyl Acetate and Ethyl Alcohol:* About 60 gms. of salts were dissolved in one liter of a solution made up of two parts of ethyl acetate and one part of 80 per cent ethyl alcohol. The mixture was warmed in a water bath to 55°. A small part of the lecethin remained undissolved and was discarded. The solution was placed in a refrigerator at 5° overnight. Crystals of lecethin cadmium chloride separated and these were removed from the mother liquid by decantation. The procedure was repeated once more. The crystals were then washed twice

<sup>1</sup> Throughout this investigation, the word lecethin has been used as referring to certain preparations and not to the pure substance.

with 500 c.c. portions of absolute ethanol, once with anhydrous ether and dried in vacuo over sulphuric acid.

*An Electrolytic Method for the Determination of Cadmium in the Cadmium Chloride Salt of Lecethin.*

Any gravimetric method for the analysis of the amount of cadmium chloride found in lecethin requires a considerable amount of labor. We therefore have attempted to substitute for this method the usual electrolytic deposition of cadmium and have found that with a few minor changes it works well.

The apparatus consisted of a 150 c.c. beaker into which two platinum electrodes were inserted, the one an ordinary platinum wire, the other a piece of platinum gauze, approximately  $1\frac{1}{2} \times \frac{3}{4}$  inches. The former served as the anode, the latter as the cathode. The electrodes were connected to a source of direct current having the pressure of about four volts and of such a density that it produced a steady flow of gas bubbles from both electrodes.

After several trials, the following procedure was adopted. About 0.5 grms. of finely powdered cadmium chloride salts of lecethin were transferred to the beaker and stirred with 50 c.c. of water. A few drops of phenolphthallin were added and the solution made distinctly alkaline with sodium hydroxide. Then 10 c.c. of potassium cyanide (freshly prepared 100 grms. in 100 c.c. of  $H_2O$ ) were added and the solution carefully stirred. A few drops of caprylic alcohol were then added to prevent foaming during electrolysis.

Prior to electrolysis the cathode was washed with water, then with absolute alcohol and ether and dried. Its weight was recorded. This procedure was repeated after the deposition of cadmium. Table II gives the details of a typical determination. 0.4992 grms. of salts were used in this analysis and the cathode, prior to electrolysis, weighed 1.5020 grms. Using the last figure of this table, the percentage of cadmium in the salt was

$$\%Cd = \frac{0.0630}{0.4992} \times 100 = 12.62.$$

TABLE II.

*Electrolysis of cadmium chloride salt of lecethin.*

Time for electrolysis	Weight of the electrode	Weight of cadmium
hrs.	g.	g.
(1)	(2)	(3)
2.45	1.5624	0.0604
3.30	1.5635	0.0615
4.45	1.5643	0.0623
5.30	1.5651	0.0631
6.30	1.5650	0.0630



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In routine analysis the solution was, during the first hours of electrolysis, occasionally stirred with a glass rod and then left overnight to remove the last traces of cadmium. The length of time for electrolysis may easily be found by reweighing the electrode during the course of electrolysis until a constant weight is obtained.

There is little doubt in our minds that the determination of cadmium in lecethin salt depends upon a partial decomposition of this salt in water, and a subsequent ionization of the liberated cadmium chloride. The electrolysis of the salts is also possible from a 50 mol. per cent solution of ethanol in water. Small amounts of water are thus sufficient to produce a decomposition of the salt. It follows that in preparation of the salts, water must be avoided, unless care is taken to wash out, with some solvent, the free lecethin thus liberated.

It was interesting to see whether electrolysis could be affected from solvent in which the cadmium chloride salts of lecethin are noticeably soluble. Benzene dissolved the salts extremely well. Carbon tetrachloride and chloroform no doubt dissolved a considerable amount.

A number of experiments indicated that in solvents in which salts form apparently true solutions (benzene for instance) no electrolysis takes place. The better the solution from the point of view of absence of colloidal formation, the worse is its electrolytic decomposition.

This leads us to believe that the cadmium chloride salts of lecethin, not unlike other salts of lipoids, are completely unionized in solvents in which they are particularly soluble and that the colloidal nature of watery solutions, in this case, is primarily due to a decomposition of the salts and the liberation of free lecethin.

The result of an electrolysis of cadmium chloride salts of lecethin is free lecethin. The electrolysis is therefore a method of obtaining free lecethin from its salt. It is, however, doubtful that such a lecethin will retain its original degree of unsaturation, since it necessarily will come in contact with gas generated by electrolysis. This, however, may be avoided by surrounding the electrodes with membranes permeable to crystalloids, but impermeable to lecethin. If this can be achieved the method may become of considerable use, since the liberation of lecethin electrolytically does not involve any loss of material, while in the usual procedure (through the decomposition of the salt with alkali in some organic solvent), wastes a considerable amount of the material.

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### *The Solubility of Cadmium Chloride Salts of Lecethin in Various Solvents and the Composition of the Final Products.*

The original lecethin, the cadmium chloride salt prepared from it and the same salt recrystallized from an ethyl acetate and ethanol mixture were subjected to analysis. The results of these are found in Table III.

TABLE III.  
*Analysis of lecethin preparations.*

Preparation	Per cent by weight				
	Amino-N	N	P	CdCl <sub>2</sub>	Iodine No.
(1)	(2)	(3)	(4)	(5)	(6)
Original dried	0.48	2.00	3.81		66.5
CdCl <sub>2</sub> -salt	0.345	1.91	2.95	20.7	47.1
CdCl <sub>2</sub> -salt recrystallized from ethyl acetate-ethanol	0.338	1.89	2.97	22.40	43.4

Neither of these preparations fulfil the requirements of pure lecethin, since all of them contain amino nitrogen. Neither nitrogen nor phosphorus are present in the quantities expected in lecethin.

It has been of interest to investigate what would be the effect of washing such preparations with various solvents or mixture of solvents. If the impurities have different solubilities, we will end with products differing in elementary composition from the original substance. This has been already amply proved in the case of cephalin salts which are more soluble in ether than the lecethin salts.

The experiments were carried in the following way. 0.50, 1.00 or 2.00 grams portions of cadmium chloride salt of lecethin recrystallized from ethyl acetone-ethanol were placed in small flasks which were then filled with 75 c.c. of various solvents. To the flasks were added five or six small glass balls and the flasks subjected to vigorous shaking in an air thermostat at  $25 \pm 0.5^\circ\text{C}$ . for twenty or more hours. Under these conditions the salt is broken up into a very fine state of subdivision, the glass balls acting quite efficiently as a ball mill. Such a suspension passes readily through ordinary filter paper. The separation of the solid phase from the liquid one by filtration was found to be impractical.

It is, however, possible to affect the separation by letting the flasks stand for a certain length of time. After an elapse of 48 hours or more (in a



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water thermostat kept at  $25 \pm 0.1^\circ$ ) the settling was complete except where ether was used as a solvent. In this case, a noticeable opalescence persisted almost indefinitely.

After the precipitate has settled down 50 c.c. of supernatant liquid were carefully syphoned off, aliquot parts transferred to weighed beakers and the beakers placed in a vacuum desiccator at room temperature until dry. Then they were reweighed and the weight of the solids determined.

The flasks were refilled with 50 c.c. portions of fresh solvent and the procedure of equilibration, settling and analysis repeated. Thus, every time 50 c.c. out of 75 c.c. were removed and replaced with fresh solvent.

The amount of solid phase at any saturation may be calculated from the following considerations:—

Let  $P_n$  be the amount in mgs. of solid phase at saturation  $n$  and the solubility (mgs. dissolved in 75 c.c.)  $S_n$ . At the next saturation, we similarly have  $P_{n+1}$  and  $S_{n+1}$ .  $P_{n+1}$  would be equal to  $P_n - S_{n+1}$  if all of the 75 c.c. of solvent were removed at  $n$  saturation, but since we left in the flask 25 c.c. at this saturation, we must subtract from  $S_{n+1} - \frac{2}{3}S_n$  and we have:

$$P_{n+1} = P_n - (S_{n+1} - \frac{2}{3}S_n)$$

or 
$$P_{n+1} = P_n - S_{n+1} + 0.333S_n.$$

In Table IV is found a summary of these experiments. In column (5) will be found the values of solid left undissolved, calculated by the equation just derived. Column (6) gives the percentage of the salt dissolved and was calculated as follows: From the initial amount of salt [Column (3) No. 1, 2, and 3] the amount left in the flasks at the final saturation [Column (3) No. 4, 5, and 6 for ether] was subtracted, giving thus the amount dissolved, in all flasks for all saturations. The per cent. of the salt dissolved, with reference to the initial amount, was then calculated.

The cadmium chloride salts left undissolved were dried and subjected to the analysis reported in Table V. We are indebted for some of the data reported in this table to Dr. G. Weiler and Dr. A. Schöeller, Berlin, Germany. The values for oxygen were, as usual, calculated by difference.

From inspection of Table IV, it is apparent that our preparation behaved differently in different solvents. The solubility is least in ether. The values recorded in the table are probably too high, due to the difficulty in separating the last trace of the precipitate. Replacing carbon tetrachloride in a mixture with ethanol with benzene increases the solubility. The factor is from 1.22 to 1.25.

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TABLE IV.

*The solubility of cadmium chloride salt of lecithin in various solvents.*

No.	No. of saturations	Initial amount of CdCl <sub>2</sub> -lecithin g.	Solubility: mg. CdCl <sub>2</sub> -L dissolved in 75 c.c.	Amount of solid phase mg.	Per cent dissolved
(1)	(2)	(3)	(4)	(5)	(6)
75 c.c. (C <sub>2</sub> H <sub>5</sub> ) <sub>2</sub> O					
1	1	0.50	14.0	486	(1+2+3) - (4+5+6)
2	1	1.00	25.5	974.5	..
3	1	2.00	47.0	1953	11.8
4	9	0.50	8.0	409	..
5	9	1.00	20.0	874	..
6	9	2.00	24.0	1806	..
55 c.c. (CH <sub>3</sub> ) <sub>2</sub> CO + 20 c.c. CHCl <sub>3</sub>					
1	1	0.50	63	437	(1+2+3) - (4+5+6)
2	1	1.00	92	908	..
3	1	2.00	126	1874	34.6
4	11	0.50	24	217	..
5	11	1.00	32	611	..
6	11	2.00	46	1464	..
50 c.c. C <sub>2</sub> H <sub>5</sub> OH + 25 c.c. CCl <sub>4</sub>					
1	1	0.50	141	359	(1+2+3) - (4+7+8)
2	1	1.00	173	827	..
3	1	2.00	228	1772	..
4	5	0.50	76	127	58.5
5	5	1.00	111	520	..
6	5	2.00	146	1408	..
7	9	1.00	70.5	283	..
8	9	2.00	115	1402	..
50 c.c. C <sub>2</sub> H <sub>5</sub> OH + 25 c.c. C <sub>6</sub> H <sub>6</sub> (after 2d sat. changed to 66.67 c.c. C <sub>2</sub> H <sub>5</sub> OH + 8.33 c.c. C <sub>6</sub> H <sub>6</sub> )					
1	1	0.25	143	107	(1+2+3) - (4+7+9)
2	1	0.50	172	328	..
3	1	1.00	216	784	..
4	3	0.25	69	7.5	..
5	3	0.50	101	167	85.4
6	3	1.00	127	580	..
7	6	0.50	67.5	24.5	..
8	6	1.00	91	398	..
9	9	1.00	85	224	..



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It is doubtful if the dielectric constant of the solvent governs the solubility of the salts in this instance. The dielectric constants (20°) of these two solvents, as determined by King and Patrick (34) is about the same: 17.0 for  $C_2H_5OH-CCl_4$  and 16.6 for  $C_2H_5OH-C_6H_6$ . We must therefore look for an explanation elsewhere, since, furthermore, a lowering of the dielectric in this case results in an increased solubility, while in ether, which has a dielectric constant of about 4, the solubility is less.

TABLE V.

*The composition of cadmium chloride salts of lecethin.*

Source: Table IV column (5). No. of table IV and sol- vent: (1)	Element or compound (2)	Per cent by weight in the $CdCl_2$ salt (3)	Per cent by weight in lecethin (4)	G.-atom per 100g. (5)	P N (6)	Weight of lecethin con- taining one gram-atom. 100/(5) (7)
4+5+6 $(C_2H_5)_2O$	P	2.90	3.70	0.119	0.81	840
	N	1.62	2.06	0.147		680
	$CdCl_2$	21.50	..	0.1173	..	850
	C	48.95	62.35	..	..	..
	H	8.20	10.45	..	..	..
	O	16.83	21.44	..	..	..
	Amino-N Mol. wt.	0.265 ..	.. ..	.. ..	.. ..	.. ..
4+5+6 $(CH_3)_2CO +$ $CHCl_3$	P	2.95	3.93	0.127	0.82	790
	N	1.70	2.17	0.155		650
	$CdCl_2$	24.95	..	0.1362	..	735
	C	48.05	64.10	..	..	..
	H	8.15	10.90	..	..	..
	O	14.20	18.90	..	..	..
	Amino-N Mol. wt.	0.35 ..	.. ..	.. ..	.. ..	.. ..
4+7+8 $C_2H_5OH +$ $CCl_4$	P	3.34	4.72	0.152	1.03	660
	N	1.46	2.06	0.147		680
	$CdCl_2$	29.25	..	0.1595	..	630
	C	44.50	62.90	..	..	..
	H	7.52	10.65	..	..	..
	O	13.93	19.67	..	..	..
	Amino-N Mol. wt.	Trace ..	.. ..	.. ..	.. ..	.. ..
4+7+9 $C_2H_5OH +$ $C_6H_6$	P	3.06	4.19	0.135	0.94	740
	N	1.44	2.01	0.144		700
	$CdCl_2$	27.00	..	0.1473	..	680
	C	46.70	64.00	..	..	..
	H	7.50	10.27	..	..	..
	O	14.30	19.53	..	..	..
	Amino-N Mol. wt.	None ..	.. ..	.. ..	.. ..	.. ..

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An inspection of Tables IV and V leads us to the following conclusions:—

The monoaminophosphatides are less soluble in all of the solvents investigated than the impurities associated with them. The  $\frac{P}{N}$  ratio of our starting material, calculated from Table III for free lecethin is 0.71. All the ratios recorded in Table V are higher than this.

One of the most troublesome impurities of lecethin is cephalin which is very similar in composition to lecethin, but its nitrogen is in the form of amino nitrogen. Therefore, the amount of amino nitrogen serves as an index of the freedom of lecethin from that impurity. The per cent in our starting material, calculated with respect to free lecethin, is 0.435 per cent. The per cent for ether washed lecethin is 0.34. For lecethin extracted with  $(\text{CH}_3)_2\text{CO} - \text{CHCl}_3$  mixture the amount is 0.47. The other two samples contained either a negligible amount or none at all. These findings show that in acetone-chloroform mixtures, lecethin is probably more soluble than cephalin, since the amount of amino nitrogen in the sample increased. This solvent cannot be used for purification of lecethin. The relative solubility of these two substances, according to our determinations, confirms the fact that cephalin is more soluble in ether than lecethin. All these considerations, it is understood, apply only to the cadmium chloride salts of these substances.

The next point of interest seems to be the evaluation of the relative efficiency of the solvents used. A complete solution of the problem cannot be given without an elementary analysis at every saturation, but the following approximate calculation may serve as a useful index. The amino nitrogen content cannot serve as a basis of comparison since in two of the four samples we found practically none, but the  $\frac{P}{N}$  ratio may serve well this purpose. The efficiency of a given solvent in this case is directly proportional to the increase in the  $\frac{P}{N}$  ratio and is inversely proportional to the amount of material dissolved which is necessary to effect it. In other words:—

$$(\text{C}_2\text{H}_5)_2\text{O} : \frac{0.81-0.71}{11.8} = 0.0085$$

$$(\text{CH}_3)_2\text{CO} + \text{CHCl}_3 : \frac{0.82-0.71}{34.6} = 0.0032$$

$$\text{C}_2\text{H}_5\text{OH} + \text{CCl}_4 : \frac{1.03-0.71}{58.5} = 0.0055$$

$$\text{C}_2\text{H}_5\text{OH} + \text{C}_6\text{H}_6 : \frac{0.94-0.71}{85.4} = 0.0027.$$



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Using these criteria, we must conclude that ether is the most efficient solvent. Next to ether the best of the solvents is  $C_2H_5OH + CCl_4$ . This comparison does not include the separation of lecethin from cephalin. The ratio of phosphorus over nitrogen does not distinguish between the two, but it serves only as an index of the relative quantities of monophosphatides in the material investigated.

A further insight into the nature of the products obtained may be gathered from an inspection of column (7) of Table V. There can be very little doubt that the substance isolated from the  $C_2H_5OH - CCl_4$  mixture is a pure monophosphatide, free from amino nitrogen, excepting a trace of it, and therefore practically free from cephalin. These conclusions are formed from the good agreement of the weight of lecethin per one gram atom of elements or compounds analyzed. The molecular weight estimate agrees very well with the analytical figures.

The product obtained from  $C_2H_5OH - C_6H_6$  is of considerable purity, the analyses, considering our experimental error, agree satisfactorily.

A comparison of elementary compositions of lecethin found in Table I with the one obtained (Table V) reveals that none of the two lecethin preparations agree with the elementary composition required by a single lecethin. This is not surprising in the light of the review of the whole problem in the introductory part of this paper. The curious part of this comparison is that none of the lecethins having two fatty acids attached to the molecule will satisfy the requirements of the analyses obtained for the sample from  $C_2H_5OH - CCl_4$ . The good agreement of the molecular weight estimate precludes the presence of any impurities of smaller molecular weight. Even if this is not admitted, these impurities must contain P and N in the same proportion as the lecethin, and in addition, bind  $CdCl_2$  to the same extent, which makes their existence highly improbable. The more plausible explanation to our mind is that the preparation isolated contains not only lecethins containing two fatty acids, but one or more lecethins containing only one fatty acid. As far as we are aware, this is the first indication of the possibility of the existence of such a compound.

The preparation derived from  $C_2H_5OH - C_6H_6$  consist probably of lecethins containing two fatty acids with a small admixture of lecethins containing one fatty acid.

Beside the consideration derived from these analyses, there is an independent way of judging the purity of a chemical substance. For a single

chemical individual the phase rule predicts that in a system containing a liquid phase and a solid phase, the temperature, composition of the liquid phase, etc., being kept constant, the solubility can have but a single value which should be independent of the amount of solid phase.

Consulting Table IV and comparing the solubilities in  $C_2H_5OH-CCl_4$  at final saturation (Nos. 4, 7, and 8), and similarly the solubilities in  $C_2H_5OH-C_6H_6$  (Nos. 4, 7, and 9), we come to the following conclusion: neither of these substances could be single chemical individuals since their solubility depends upon the amount of solid phase. It is curious to note that there is a far better agreement between the solubilities in  $C_2H_5OH-C_6H_6$ , than in  $C_2H_5OH-CCl_4$ . The explanation seems to lie in the considerable chemical differences of the lecithins composing the first preparation, while the second, containing chiefly lecithins with two fatty acids, is more chemically homogeneous.

No doubt in the years to come, the chemistry of lipoids will deal with single species of molecules. To our mind, the application of the Phase Rule to such preparations is highly desirable. In any preparation, it should be considered one of the final criteria for a single chemical individual.

#### SUMMARY.

1. On the basis of the present knowledge of lecethin, the total number of possible lecethins derived from egg was estimated and found to be not less than eighteen.

2. An electrolytic method for the determination of cadmium in the cadmium chloride salt of lecethin is described. The electrolysis of these salts was tested in various solvents and certain conclusions concerning the nature of such solutions drawn.

3. The solubility of the cadmium chloride salts of lecethin obtained from a preparation of lecethin from eggs was studied in several solvents. After extensive washing of the salt with these solvents, the residue was subjected to complete elementary analysis as well as molecular weight estimates. Upon a simultaneous consideration of analytical data and solubility measurements, the following conclusions were drawn.

(a) Ether is the most efficient solvent for separating impurities from monoaminophosphatides.



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(b) It is possible to obtain lecethin free from cephalin and having a  $\frac{P}{N}$  ratio equal to one, by extracting the cadmium chloride preparation with ethanol-benzene and ethanol-carbon tetrachloride mixtures.

(c) The product obtained from ethanol-carbon tetrachloride indicated that it was composed not only of lecethins having two fatty acids, but also of a large proportion of lecethin having but one fatty acid.

(d) The phase rule test was applied to the products obtained. The importance of this general law to the chemistry of lipoids is emphasized.

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