CCXVI. PHYSICAL CHEMISTRY OF LIPOIDS.

V. BEHAVIOUR OF KEPHALIN FROM HUMAN BRAIN TOWARDS ACIDS AND BASES.

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WHILST the reactions between proteins and acids and bases have been extensively studied, no similar systematic investigations have been conducted on lipoids. Yet some phosphatides have features that seem to make them specially fit objects for such investigations. The chemical structures of lecithin and kephalin are nearly completely elucidated, and they form stable colloidal solutions in electrolyte-free water. Finally, kephalin, like proteins, is an ampholyte, the absence of the ampholytic feature from lecithin providing an interesting subject of comparison. In the experiments to be reported here the physico-chemical behaviour of kephalin towards HCl and NaOH was studied.

Some observations on lecithin described by other authors have been confirmed on material derived from the same brains as the kephalin.

The origin, preparation and chemical properties of the kephalin used in these experiments have been the same as reported in a recent paper [Spiegel-Adolf, 1935]. The kephalin was obtained from human brains, cleaned from blood and meninges, dried at 37° and subsequently pulverized. This powder was extracted with ether and then treated with absolute alcohol. This procedure was repeated three times. The preparation thus obtained contained 2.64% of nitrogen and 5.6% of phosphorus. It was entirely soluble in water, ether and chloroform, insoluble in absolute alcohol and acetone. The conductivity of a 1% aqueous solution is $K_{25} = 3.10 \cdot 10^{-4}$ mhos, the $cH = 1.38 \cdot 10^{-6}$. After further purification by means of dialysis, the conductivity dropped to $9.3 \cdot 10^{-5}$ mhos, and the cH rose to $4.71 \cdot 10^{-6}$. Further attempts at purification by electrodialysis, which is so successful in the case of proteins, have not proved to be equally useful in the case of kephalin (and lecithin) solutions. When a positively charged membrane was used on the side of the anode, the final cH of the fluid under electrodialysis did not rise above $1.88 \cdot 10^{-4}$, at which, according to the findings summarized in Tables I and II, kephalin solutions should still be stable. Yet during the electrodialysis the whole of the kephalin (and lecithin) was precipitated on the positively charged membrane. The deposited kephalin could be brought again into aqueous solution which became decidedly more acid than the original solution (cH $9.16 \cdot 10^{-4}$ against $1.38 \cdot 10^{-6}$). As a chemical change in the kephalin could not be excluded, the only purification method used was dialysis. All experiments were made on fresh samples of kephalin, although, unlike lecithin [Spiegel-Adolf, 1932; Fischgold & Chain, 1934], kephalin did not show signs of ageing.

In the first series of experiments various amounts of HCl were added to samples of kephalin. With increasing concentrations of HCl the solutions became more opaque until finally precipitation occurred. At 0.02 *N* HCl in 1% kephalin the solution becomes heterogeneous, though it is still stable. But while, contrary

to Thierfelder & Klenk [1930], watery solutions of kephalin cannot be precipitated by centrifuging (for 25 min. at a rate of 2000–3000 rotations per min.), at HCl concentrations from 0.01 *N* upwards centrifuging becomes effective.

In the different HCl-kephalin mixtures determinations of the conductivity, *cH*, and viscosity were made. The results are summarized in Tables I and II.

Table I.

Final concentration of kephalin = 1%; *faH* = activity coefficient of HCl according to Scatchard; *N* = normality of HCl; *K* = specific conductivity.

Final concentration of HCl <i>N</i>	<i>cH</i>	<i>faH</i>	Bound HCl		
			$n - \frac{c}{faH}$	$K^{30^\circ}_{cor.}$	$K - K_{HCl}$
0	$1.38 \cdot 10^{-6}$	—	—	$3.16 \cdot 10^{-4}$	—
0.0025	$6.44 \cdot 10^{-5}$	—	—	$5.08 \cdot 10^{-4}$	—
0.005	$2.53 \cdot 10^{-4}$	0.937	$4.73 \cdot 10^{-3}$	$7.01 \cdot 10^{-4}$	$5.83 \cdot 10^{-4}$
0.01	$2.28 \cdot 10^{-3}$	0.921	$7.52 \cdot 10^{-3}$	$1.39 \cdot 10^{-3}$	$3.2 \cdot 10^{-4}$
0.015	$5.90 \cdot 10^{-3}$	0.900	$8.45 \cdot 10^{-3}$	$2.50 \cdot 10^{-3}$	$-2.61 \cdot 10^{-4}$
0.02	$1.01 \cdot 10^{-2}$	0.887	$8.60 \cdot 10^{-3}$	$4.50 \cdot 10^{-3}$	$-2.8 \cdot 10^{-4}$

Table II.

Kephalin in all samples = 1%; temperature = 30°; *t*₀ = flowing time of water = 857 sec./5.

Final concentration of HCl <i>N</i>	<i>t</i> ₁ = flowing time in sec./5		After 25 min. of centrifuging
	At once	After 24 hours	
0	1062	1041	Homogeneous
0.0025	1045	1038	"
0.0050	1037	1040	
0.0075	1036	1045	
0.01	Instant	Over 1220	Heterogeneous
0.015	"	Over 3000	"

An examination of the results of the potentiometric *cH* determinations shows that kephalin neutralizes a certain amount of acid, and that this amount increases with increasing HCl concentrations to a maximum value; 1 g. of kephalin combines with $8.6\text{--}8.8 \cdot 10^{-3}$ ml. *N* HCl. According to Jukes [1934] 1 molecule of kephalin in non-aqueous solutions combines with 1 molecule of acid. As Levine & West [1916] found for brain kephalin a molecular weight of 823.7, the normality of a 1% solution ought to be 0.0124. The lower value found in these experiments suggests that not all amino-groups are freely accessible to the acid. This may be explained by the formation of colloidal aggregates in aqueous solutions.

Lecithin does not contain a free amino-acid group, yet with this exception it is very similar in structure to kephalin, so that some experiments with HCl were made with this substance.

The lecithin used in these experiments has been made from the same brains as were used for the preparation of kephalin. After the latter had been precipitated, the alcohol-ether filtrate was concentrated and precipitated by acetone. This manipulation was repeated three times. The resulting preparation gave opaque colloidal solutions in water; it was completely soluble in ether, absolute alcohol and chloroform at 37° and insoluble in acetone. The preparation contained 2% nitrogen and 10.5% phosphorus. It seems therefore that the preparation contains some nitrogen-free phosphatides such as have been described by Chibnall & Channon [1927] in plants. 2% colloidal solutions of lecithin were prepared according to the method of Keiser [1924]. Conductivity and *cH* were measured in the solution, in the various lecithin and HCl mixtures and in the

corresponding HCl solutions. The differences between calculated and measured values were considered as a measure of bound HCl. All measurements were duplicated on egg-lecithin solutions (Merck, egg-lecithin extra pure).

Table III.

Lecithin concentration in all samples = 1 %; I refers to human brain lecithin, II refers to egg lecithin.

Final concentration of HCl <i>N</i>		Conductivity			cH		
		Measured	Calculated	Difference	Measured	Calculated	Difference
0	I	$8.81 \cdot 10^{-5}$	—	—	$2.71 \cdot 10^{-7}$	—	—
	II	$1.93 \cdot 10^{-4}$	—	—	$1.88 \cdot 10^{-4}$	—	—
0.0011	II	$3.31 \cdot 10^{-4}$	$8.05 \cdot 10^{-4}$	$4.74 \cdot 10^{-4}$	$9.92 \cdot 10^{-4}$	$1.31 \cdot 10^{-3}$	$3.16 \cdot 10^{-4}$
0.003	II	—	—	—	$2.15 \cdot 10^{-3}$	$2.66 \cdot 10^{-3}$	$5.1 \cdot 10^{-4}$
0.005	I	$1.81 \cdot 10^{-3}$	$2.39 \cdot 10^{-3}$	$5.8 \cdot 10^{-4}$	$3.53 \cdot 10^{-3}$	$3.97 \cdot 10^{-3}$	$5.7 \cdot 10^{-4}$
	II	$7.63 \cdot 10^{-4}$	$2.51 \cdot 10^{-3}$	$1.75 \cdot 10^{-3}$	$3.67 \cdot 10^{-3}$	$4.16 \cdot 10^{-3}$	$4.9 \cdot 10^{-4}$
0.0075	I	$2.83 \cdot 10^{-3}$	$3.53 \cdot 10^{-3}$	$7.0 \cdot 10^{-4}$	$5.90 \cdot 10^{-3}$	$6.40 \cdot 10^{-3}$	$5.0 \cdot 10^{-4}$
	II	$2.44 \cdot 10^{-3}$	$3.71 \cdot 10^{-3}$	$1.27 \cdot 10^{-3}$	$5.90 \cdot 10^{-3}$	$6.33 \cdot 10^{-3}$	$4.3 \cdot 10^{-4}$
0.01	I	$3.72 \cdot 10^{-3}$	$4.80 \cdot 10^{-3}$	$1.08 \cdot 10^{-3}$	$7.79 \cdot 10^{-3}$	$8.43 \cdot 10^{-3}$	$6.4 \cdot 10^{-4}$
	II	$3.90 \cdot 10^{-3}$	$4.86 \cdot 10^{-3}$	$9.6 \cdot 10^{-4}$	$8.27 \cdot 10^{-3}$	$8.55 \cdot 10^{-3}$	$2.8 \cdot 10^{-4}$
0.015	I	$5.56 \cdot 10^{-3}$	$6.88 \cdot 10^{-3}$	$1.32 \cdot 10^{-3}$	$1.16 \cdot 10^{-2}$	$1.25 \cdot 10^{-2}$	$9.0 \cdot 10^{-4}$
	II	$6.17 \cdot 10^{-3}$	$7.12 \cdot 10^{-3}$	$9.5 \cdot 10^{-4}$	—	—	—

The results of Table III show that both samples of lecithin bind HCl, but that according to their chemical difference (as indicated by their different cH) the binding capacity reaches its maximum value at a different HCl concentration. The fact that lecithin in aqueous solutions binds HCl is rather unexpected, since according to Jukes [1934] lecithin is an internally neutralized compound and has no free amino-group. But systematic investigations of the salt-binding capacity of lecithin solutions [Spiegel-Adolf, 1936] have given evidence of the sorptive power of lecithin. Furthermore, former experiments on globulins [Spiegel-Adolf, 1930] have shown that acid binding can be supplemented by acid sorption.

The behaviour of kephalin and HCl is very similar to the behaviour observed with proteins and acid, except that computation, based on the cH and conductivity data, shows that the ionization of the kephalin-HCl complex appears to decrease gradually.

These results seem to suggest the existence of a compound of kephalin and HCl in solutions. However, Thierfelder & Klenk [1930] reviewing the results of the literature on HCl precipitates of kephalin came to a different conclusion. In order to elucidate this question, special experiments were undertaken.

1. A solution containing 0.25 % kephalin and 0.025 *N* HCl shows partial precipitation. A chloride determination in the filtrate, after destruction of kephalin with HNO_3 , shows that it contains only 90 % of the original amount of chlorine. This suggests that about 0.0025 *N* HCl has been precipitated with a part of the kephalin.

2. The conductivity was measured in a 0.25 % kephalin solution, in a 0.025 *N* HCl solution, and in a solution containing 0.025 *N* HCl and 0.25 % kephalin. The sum of the conductivities of pure kephalin and pure HCl was larger than the conductivity observed in the solution containing both substances. This difference in conductivities was 10 times larger than the conductivity of the pure kephalin solution. Calculated in terms of HCl-normality, the apparent loss in conductivity corresponded to an amount of 0.0025 *N*.

These results seem to suggest that HCl combines with kephalin not only in solutions but becomes part of the precipitate when precipitation occurs. By repeated centrifuging and replacement of the watery acid layer by pure water, most of the acid can be eliminated. This is due probably to hydrolysis of the lipid-acid compound. The acid kephalin flocculate, when redissolved in water, settled at an acid concentration which primarily did not interfere with stability in centrifuging, but neutralization with alkali restored the original behaviour. These findings about the formation of kephalin-HCl compounds are perhaps not entirely devoid of biological interest, thus e.g. Peters & Man [1934] claim that part of the chloride of the serum is combined with lipid.

The results of the viscosity determinations made with an Ostwald viscosimeter at a constant temperature of 30° show with increasing concentration of HCl a steady drop in viscosity, which only begins to rise again at a stage preceding precipitation. The initial drop becomes less distinct if the mixtures stand for 24 hours. These observations on the changes of kephalin viscosities upon addition of acids are very similar to those reported by Handovsky & Wagner [1911] on lecithin under comparable conditions. But the behaviour of kephalin is in this respect markedly different from the behaviour observed in proteins, for with the latter increasing HCl concentrations produce at first a rise in viscosity and ionization.

Analogous determinations were made on mixtures of kephalin and NaOH. The results are summarized in Tables IV and V.

Table IV.

Final kephalin concentration = 1 %; f_{aOH} = activity coefficient of NaOH;
 N = normality of the NaOH.

Final concentration of NaOH N	$K^{30^\circ}_{cor.}$	cOH	f_{aOH}	$N - \frac{cOH}{f_{aOH}}$
0	$3.12 \cdot 10^{-4}$	$3.88 \cdot 10^{-7}$	—	—
0.0025	$4.24 \cdot 10^{-4}$	$2.92 \cdot 10^{-7}$	—	—
0.005	$5.84 \cdot 10^{-4}$	$7.56 \cdot 10^{-7}$	0.928	0.004999
0.01	$7.49 \cdot 10^{-4}$	$1.81 \cdot 10^{-6}$	0.901	0.0099
0.015	$10.21 \cdot 10^{-4}$	$1.42 \cdot 10^{-5}$	0.882	0.0149
0.02	$13.00 \cdot 10^{-4}$	$7.50 \cdot 10^{-5}$	0.860	0.0199
0.05	$57.00 \cdot 10^{-4}$	$1.17 \cdot 10^{-2}$	0.819	0.0356

Table V.

The final concentration of kephalin is 1 %; temperature = 30°;
 t_0 = flowing time of water = 857 sec./5.

Final concentration of NaOH N	0	0.005	0.01	0.015	0.02
At once	1010	1270	1369	1290	1218
After 1 hour	—	1059	1027	1003	969
After 2 hours	—	1018	984	979	952
After 24 hours	1010	963	944	937	922

Since the reaction of NaOH with kephalin may lead to some chemical changes of the kephalin, attempts were made to ascertain whether the highest NaOH concentrations used produce a splitting of kephalin. As a breakdown of the kephalin molecule by alkali ought to liberate fatty acids, conductivity methods could be used in order to trace an increase in conducting material.

Samples containing 1 % kephalin and 0.02 N or 0.05 N NaOH were exactly neutralized after 24 hours. The conductivities of these solutions were nearly identical with the sum of the conductivities of controls containing either pure

kephalin or pure alkali. A slight decrease in the conductivities of the mixtures was explained by the inactivating effect of kephalin on the salt. A splitting of kephalin in the alkali concentrations used in Tables IV and V can therefore be disregarded. The optical changes in kephalin on addition of alkali proved to be reversible on neutralization.

An attempt was made to determine the binding capacity of kephalin for NaOH. According to the figures in Table IV the NaOH-binding capacity exceeds by far the combining power of kephalin for HCl. Apparently in excess of alkali a maximum value could also be reached.

Viscosity measurements of kephalin at varying NaOH concentrations reveal a great similarity in behaviour to proteins, especially globulins [Spiegel-Adolf, 1930]. Here too, with increasing amounts of NaOH, the viscosity increases and drops again on further addition of alkali. The maximum viscosity is reached at a much lower concentration of alkali than the maximum binding capacity. Yet all these changes occur in the first hours after mixing kephalin and alkali. After several hours of standing, an increase of alkali has uniformly produced a corresponding decrease of viscosity.

The fact that kephalin, though combining with acid like a protein, shows no increase in viscosity like lecithin may be of some interest to the biologist. The non-swelling of nervous substance in acids [Bauer, 1911; Spiegel, 1921] has been attributed by Hooker & Fischer [1912] to reactions of lecithin prevailing over the reactions of proteins. This explanation gains new support from the results on kephalin reported here. Thus according to Singer [1926] brain contains more kephalin than lecithin. Recent studies of Spiegel & Spiegel-Adolf [1936] have shown that in dead and in living brains the polarization indices—a convenient measure of the swelling and the permeability of tissues—show a marked decrease on addition of alkali, whilst they are unchanged or only minimally lowered in the presence of acids.

SUMMARY.

1. Purified kephalin from human brains was studied in regard to its reactions with HCl and NaOH.
2. Measurements of the conductivity and of the ϵ H show that kephalin combines with HCl. In the presence of an excess of HCl 1 g. kephalin neutralizes 8.6–8.8.10⁻³ ml. *N* HCl. Further increase of HCl precipitates a compound of kephalin and HCl.
3. The viscosity of kephalin is decreased by additions of HCl and begins only to rise again at a stage preceding flocculation. At this stage only can kephalin be precipitated by centrifuging.
4. Kephalin neutralizes NaOH, the binding capacity increasing with the concentration of alkali.
5. In fresh mixtures of kephalin and NaOH of increasing concentrations, the viscosity passes through a maximum. After 24 hours, the samples show only a steady decline in viscosity with increasing NaOH concentrations.
6. Some biological aspects of these findings are discussed.

REFERENCES.

- Bauer (1911). *Arb. Neurol. Inst. Univ. Wien*, **19**, 87.
Chibnall & Channon (1927). *Biochem. J.* **21**, 233.
Fischgold & Chain (1934). *Biochem. J.* **28**, 2044.
Handovsky & Wagner (1911). *Biochem. Z.* **31**, 32.
Hooker & Fischer (1912). *Kolloidzshr.* **10**, 283.
Jukes (1934). *J. biol. Chem.* **107**, 783.
Keeser (1924). *Biochem. Z.* **154**, 321.
Levine & West (1916). *J. biol. Chem.* **24**, 41.
Peters & Man (1934). *J. biol. Chem.* **107**, 23.
Singer (1926). *Biochem. Z.* **179**, 432.
Spiegel (1921). *Pflüg. Arch. ges. Physiol.* **192**, 225.
Spiegel-Adolf (1930). *Die Globuline.* (Dresden-Leipzig: Steinkopff.)
—— (1932). *Biochem. J.* **26**, 2183.
—— (1935). *J. Amer. chem. Soc.* **57**, 431.
—— (1936). (In the press.)
Spiegel & Spiegel-Adolf (1936). *Amer. J. Psychiat.* **92**, 1145.
Thierfelder & Klenk (1930). *Die Chemie der Cerebroside und Phosphatide.* (Berlin: Springer.)