CLXXX. THE EFFECT OF INTRAVENOUS PHOSPHATIDE ON THE SUGAR EXCRETION OF THE PHLORIDZINISED DOG.

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PARENTERAL injection of fats has never been a successful procedure. Failure of absorption is at least partially due to the physical properties of these substances. Fatty acids and their glycerol esters are notoriously insoluble in water, consequently, subcutaneous injection is followed by only very partial absorption and, usually, by the production of a foreign body tumour. Intravenous injection is seldom well borne because of emboli and further because it can readily be shown that many of the fat particles must be phagocytosed by the reticulo-endothelial system to remove them from the blood. They appear to act usually more as foreign bodies than as metabolites.

Conversion of fatty acid into the soluble sodium or organic base (mono, di, triethanolamine) salt suggests itself as a possible mode for the organism to deal with these insoluble substances. From the work of Page and Allen [1930], however, it appears improbable that the bulk of lipin could be transported or utilised in this manner. Actually the animal organism appears to make use of the solubility conferred by esterification of fatty acid with glycerol-phosphoric acid-base, the combination being known as phosphatide.

Evidence gathered by Page and Pasternak [1932] indicates that of all lipins studied, phosphatide was most easily introduced into the body. They found that after the intravenous injection of phosphatide (mainly kephalin) in amounts not exceeding 1·2 g./kg. into the rabbit, that the phosphatide could be recovered only in relatively small amounts from the blood or tissues. The liver was the organ most enriched by the injection. Subcutaneously or intraperitoneally injected phosphatide is rapidly absorbed if given in the proper emulsion form. Whether the material is actually burned remains an open question.

We were much interested in a recent report by Jost [1931], that phosphatide, especially kephalin from heart, seemed to be converted into sugar by the perfused dog liver. The sugar production of the liver of a dog starved and phloridzinised for four days in two experiments averaged 215 mg./1000 cc.

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blood after the injection of 50 cc. Ringer solution. In five experiments in which 5–8 g. of kephalin were perfused, the sugar rose after the phosphatide injection to an average of 448 mg. The perfusion volume of blood was 1600 cc.; therefore, subtracting the amount of sugar produced by the control, 215 mg., from 448 mg. there remains 233 mg. per litre or 372 mg. for 1600 cc. When as much as 8 g. of kephalin were employed for the perfusion it is not clear, however, why the glycerol or some contaminant could not account for this extra sugar. Jost may be perfectly correct in his interpretation but at the moment it is not convincing to us. The phloridzinised dog seems an excellent means of testing the hypothesis of the conversion of phosphatide into sugar, therefore we have undertaken these experiments.

Methods.

Phosphatide. The mixed phosphatide was prepared from human brain according to a method already described in detail [Rudy and Page, 1930; Page and Bülow, 1931]. Cerebrosides were eliminated but no effort was made to separate lecithin from kephalin. The latter substance predominates in such a mixture. 10 % water emulsions were easily made from the partially dried material. Iodine number and neutralisation values were not markedly affected by this procedure[1].

Phloridzinised dogs. The dogs were kept in metabolism cages and the urine was collected every 24 hours under toluene. For the first 48 hours the animals were starved and then allowed 100 g. of cooked liver daily. Estimations on the urine were not made until the fifth day which is given as the first day in the protocols. 1 g. of phloridzin (B.D.H.) was suspended in 7 cc. of warm sterile olive oil, well ground in a mortar and daily injected subcutaneously. Female dogs were used from which the labia had been removed. We are very grateful to Dr E. B. Verney for these animals. At the end of the 24-hour periods the residual urine in the bladder was removed by catheterisation.

Injections of phosphatide or glucose were made into a cannulated femoral vein. The introduction of the cannula was performed under the influence of the local anaesthetic percain. Injection of 100 cc. of fluid usually required 1·5 hours.

Chemical procedures. Nitrogen was determined by the Kjeldahl method on 5 cc. of urine. Glucose was estimated by the Benedict method on urine diluted 25 times with water. All reagents were standardised.

Results.

The results obtained with three of the seven animals treated are quoted and seem to us quite definite.

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	Day	Urine volume cc.	Glucose g.	Nitrogen g.	D/N	Remarks
Dog 1	1	615	34·10	6·60	5·17	
	2	380	25·95	6·36	4·08	
	3	350	20·85	5·40	3·86	
	4	460	25·85	7·93	3·26	
	5	385	21·85	6·58	3·32	
	6	456	23·80	6·60	3·60	
	7	719	25·20	7·10	3·55	
	8	849	23·80	6·65	3·58	
	9	755	23·10	6·22	3·71	100 cc. 10 % glucose injected on 9th day
	10	850	30·25	7·08	4·27	100 cc. 10 % phosphatide injected on 10th day
	11	715	21·90	6·04	3·63	Dog killed on 11th day *Post mortem* revealed large abscess spreading over whole of back
Dog 2	1	340	38·80	8·25	4·70	
	2	492	49·50	10·59	4·68	
	3	543	53·80	11·74	4·58	
	4	327	36·30	6·79	5·35	
	5	386	36·80	11·20	3·29	
	6	253	25·30	6·97	3·63	
	7	262	26·80	7·42	3·61	
	8	293	26·60	7·28	3·66	100 cc. 10 % glucose injected intravenously on 8th day
	9	643	34·80	7·40	4·70	
	10	454	27·10	7·10	3·82	70 cc. of 10 % phosphatide injected intravenously on 10th day
	11	550	29·55	7·85	3·76	
	12	295	29·80	8·64	3·45	Dog killed on 12th day *Post mortem* revealed abscess on back
Dog 3	1	459	33·20	6·74	4·93	
	2	489	40·60	13·70	2·96	
	3	545	31·00	11·35	2·73	
	4	453	28·00	7·50	3·73	
	5	386	27·50	7·00	3·93	
	6	480	28·00	7·46	3·75	
	7	498	28·00	8·00	3·50	
	8	355	29·80	8·20	3·64	
	9	515	29·40	8·40	3·50	100 cc. 10 % glucose injected intravenously on 9th day
	10	565	38·20	9·30	4·10	
	11	958	31·90	8·86	3·60	
	12	582	27·10	8·03	3·37	
	13	535	27·90	7·83	3·56	100 cc. 10 % phosphatide injected intravenously on 13th day
	14	364	28·60	7·90	3·62	
	15	485	28·70	7·80	3·68	Dog killed on 15th day

Discussion.

It will be noted that the D/N ratios are considerably higher than those which have been reported in the past few years. This question has formed the subject of a separate communication and will not be discussed further than to state that these high ratios are associated quite specifically with the feeding of liver. Dr Stanley Benedict has been good enough to verify these results.

Glucose injected as a control for the recovery of added sugar was excreted adequately, and a corresponding rise in D/N ratio was observed. The ratios were also quite constant so that we believed that should extra sugar be formed

from a phosphatide injection in the phloridzinised animal we should be able to detect it. It seems clear from the above results that injection of phosphatide does not increase the extra sugar. There is only the slightest suggestion that any rise in D/N ratio occurs and this, if it is a real effect, might well be due to the conversion of glycerol into sugar.

SUMMARY.

Injection of aqueous brain phosphatide emulsion into phloridzinised dogs does not appear to alter the D/N ratio of the urine appreciably. As far as the phloridzinised animals are concerned, under the conditions of our experiment, there does not seem to be any conversion of fatty acid into sugar.

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