XC. THE ENZYMIC HYDROLYSIS OF LECITHIN.

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Since the original isolation by Thudichum [1874] of lecithin from brain tissue, the study of this substance in relation to its chemistry and to its physiological significance has been a subject of increasing interest. The work of Willstätter and Lüdecke [1904], of MacLean [1912, 1915] and of Levene and his co-workers [1918, 1921, 1922, 1927] on the nature of its cleavage products on hydrolysis and on the preparation of the phosphatide in a pure state has thrown much light on the chemical nature of lecithin. Within recent years Grün and Limpächer [1927] have succeeded in synthesising, from distearin, phosphoric anhydride and choline, interesting substances, which, if not identical with the naturally occurring phosphatides, are certainly remarkably like them in their physical and chemical properties.

The rôle of phosphatides in metabolism has been a matter of considerable speculation and much experiment. McCollum *et al.* [1912] have shown that the nature of the fatty acid radicals in the lecithin of eggs changes with, and is presumably dependent on, the nature of the fats contained in the diet. Levene and Simms [1921] have shown that, as in the case of the fats themselves, the fatty acid radicals of the lecithin isolated from liver are more unsaturated than those of lecithin from brain or eggs. The view has been repeatedly advanced that the phosphatides play an important part in the metabolism of the fats as a whole. The fact that lecithin is more readily oxidised and hydrolysed than neutral fat and that the lecithin-fat of the liver, where desaturation is thought to take place, is more unsaturated than the fat of other parts of the body, may be taken as evidence supporting this view. Bloor and Knudson [1916, 1917] have observed a definite increase in the phosphatide content of both corpuscles and plasma following the ingestion of a meal containing fat, and Sinclair [1929] has found that the ingestion of cod-liver oil increases the iodine value of the lipoid fatty acids of the mucosa.

The importance of phosphatides as essential fat is well illustrated in the brain. This organ which contains the largest amount of phosphatide loses none of it during starvation, and the inference that this form of fat is essential to the life of the organ would seem justifiable.

The rôle of the body enzymes in relation to lecithin has received considerable attention from various workers. Bokay [1877] investigated the action of the intestinal lipase, and since then many publications dealing with the hydrolysis of lecithin by intestinal and ricinus lipase have appeared. Some of the more recent work in this connection is due to Contardi and Latzer [1927], who studied the hydrolysis of lecithin cadmium chlorides, and by Paal [1929] who found the lipase cleavage of lecithin to give parallel results for the liberation of fatty acid and choline.

Since the study of body phosphatases during recent years has led to such important results in the elucidation of bone formation [Robison, 1923; Robison and Soames, 1924, 1925; Martland and Robison, 1926, 1927] and other physiological processes in health and disease [Kay, 1926, 1928, 1930; Neuberg, 1918, 1926, 1928; Meyerhof, 1928], it seemed possible that a study of the action of an enzyme which would hydrolyse lecithin to liberate phosphate might lead to a better understanding both of the chemical nature of this substance and of its significance in the body processes.

Kay [1926] noted the presence of a feeble "lecithinase" in an extract of kidney, which slowly hydrolysed phosphate from lecithin at 37° and had an optimum p_H of 7·4, about that of the tissues. He regards this as being a different enzyme from kidney phosphatase, the optimum p_H of which is 8·9, and which he has some experimental evidence to show is identical with intestinal and bone phosphatase. The latter Martland and Robison [1926] state to be without action on phosphatides.

The action of a phosphate-liberating enzyme on lecithin is more complex than that of the bone phosphatase on hexose- or glycero-phosphate, since it involves the cleavage of both choline and the glycerol residue. This, however, might be said of the enzymic hydrolysis of any secondary ester of phosphoric acid where two esterified groups must be separated in order to liberate free phosphoric acid; and the optimum p_H for hydrolysis of such esters, 7·7, as found by Asakawa [1929][1], is remarkably near that reported in a former investigation [King and Page, 1930] for the enzyme contained in extracts of intestine and kidney which hydrolysed lecithin most actively at p_H 7·5–7·6. This question of the relation of the enzymes responsible for the hydrolysis of primary and secondary esters of phosphoric acid and of lecithin will be dealt with more fully in a later communication. The investigation detailed in this paper consists of a study of the liberation of phosphoric acid from lecithin under various conditions and of the occurrence and distribution of the enzyme responsible for this hydrolysis in a large number of animal tissues.

Experimental.

That there is an enzyme present in autolysing tissues which is responsible for the liberation of free phosphate from compounds other than those usually

[1] Asakawa investigated the action of kidney phosphatase on a large number of primary and secondary esters of phosphoric acid, including diphenyl, dicresyl, diglyceryl and diethyl phosphate.

referred to as "acid-soluble" may be inferred from the fact that the "free phosphate" in a trichloroacetic acid extract of a tissue made after a few days' autolysis is greater than the "total phosphate" present in such an extract of the fresh tissue. This is shown by Table I where are given the amounts, at different times, of free and total phosphate present in the trichloroacetic acid extracts of aliquot portions of composite samples of rabbit liver and kidney, which were ground with sand and water and allowed to autolyse in the presence of chloroform. It is probable that this extra phosphate from a non-acid-soluble source is largely derived from lecithin, and it seemed worth while to investigate the enzyme responsible for this hydrolysis.

Table I.

	Kidney		Liver	
	Free phosphate	Total phosphate	Free phosphate	Total phosphate
Days	mg. P in 1 cc. of trichloroacetic acid extract		mg. P in 1 cc. of trichloroacetic acid extract	
0	0·113	0·128	0·211	0·265
1	0·115	—	0·242	—
4	0·131	—	—	0·269
5	0·134	0·134	0·271	—
7	0·141	—	0·289	—
9	0·148	—	0·298	0·306
11	0·149	0·150	0·304	—
13	0·153	—	0·318	—
15	0·155	—	—	—

MacLean [1927] has pointed out that probably in none of the researches on the action of enzymes on lecithin has pure lecithin been used. The obvious impurity of commercial lecithin, must, of course, complicate any results where this material is used as a substrate. One brand of egg-lecithin, which was tried, was only 40 % precipitable by cadmium chloride, and this precipitate, when freed from cadmium and dissolved in ether, gave only a very small precipitate when poured into acetone.

With purified lecithins from different sources, however, there seems to be very little difference in the amount of hydrolysis by the phosphate-splitting enzyme. Table II contains the results of the hydrolysis of three different lecithins by different amounts of enzyme solutions prepared by extracting ground-up beef kidney and small intestine of rabbit with 20 times their weight of distilled water saturated with chloroform. The lecithins used in these experiments were prepared from eggs, from brain, and from Kahlbaum's lecithin, by the method of Levene and Rolf [1927]. Emulsions of the lecithins were made by running 1 volume of 2·5 % alcoholic solution of the lecithin into 10 volumes of hot borate buffer (Palitzsch) at p_H 7·5, with vigorous shaking. This gives a permanent emulsion of lecithin which contains 0·5 mg. of phosphorus in 5 cc. and practically no free phosphate. For the enzymic hydrolysis a series of test-tubes containing 5 cc. of lecithin emulsion and various amounts of the tissue extract were incubated in the presence of a drop of chloroform and toluene for 48 hours at 37·5°. Controls consisted of

5 cc. of borate buffer *plus* the same amounts of extract. Previous work [King and Page, 1930] had shown that there is practically no increase in the free phosphate of a lecithin emulsion itself when incubated at this temperature, so controls of lecithin emulsion *plus* water were omitted. At the end of the 48 hours the hydrolysis was stopped by the addition of 25 % trichloroacetic acid, and the inorganic phosphorus of the filtrate estimated by the Martland and Robison [1924] modification of the Briggs colorimetric procedure. The experiments were done in duplicate and the figures are the average of duplicate determinations.

Table II. *Hydrolysis of different lecithins by varying amounts of tissue extract.*

	cc. of extract	Free P extract and buffered lecithin mg.	Free P extract and buffer mg.	Increase in free P mg.
(1)	Rabbit intestinal extract and egg-lecithin:			
	1	0·208	0·184	0·024
	3	0·698	0·545	0·153
	5	1·137	0·937	0·200
	10	2·280	1·840	0·440
	Rabbit intestinal extract and purified Kahlbaum's lecithin:			
	1	0·240	0·199	0·041
	3	0·714	0·560	0·154
	5	1·154	0·948	0·206
	10	2·191	1·837	0·354
	Rabbit intestinal extract and brain-lecithin:			
	1	—	0·161	—
	3	0·647	0·546	0·101
	5	1·108	0·923	0·185
	10	2·243	1·833	0·410
(2)	Beef kidney extract and egg-lecithin:			
	1	0·176	0·162	0·014
	3	0·589	0·462	0·127
	5	1·001	0·791	0·210
	10	1·951	1·561	0·390
	Beef kidney extract and purified Kahlbaum's lecithin:			
	1	—	0·162	—
	3	0·592	0·462	0·130
	5	0·981	0·791	0·190
	10	1·869	1·561	0·308

It has been shown by Kay [1926] that different buffer solutions have an effect on the amount of hydrolysis of glycerophosphate by kidney phosphatase. The hydrolysis in presence of borate buffer is always considerably less than that obtained when glycine buffer is used. No such effect as this was noticed in the enzymic hydrolysis of lecithin. The amount of hydrolysis in the presence of borate buffer at p_H 7·5 was practically the same with intestinal and kidney extracts as in the absence of borate, the lecithin emulsion being made up in distilled water and the mixture of lecithin and extract adjusted to p_H 7·5 by the careful addition of acid and alkali. The veronal buffer of Michaelis [1930] is very useful for the range covered by phosphate-hydrolysing enzymes, but appears to have no advantage over the borate mixture of Palitzsch in the

hydrolysis of lecithin, as the extent of hydrolysis appears to be very nearly the same in the presence of either buffer. Thus 2 cc. of rabbit intestinal extract liberated 0·115 mg. of phosphorus from 5 cc. of lecithin emulsion in the presence of veronal buffer and 0·105 mg. in the presence of borate; and 2 cc. of rabbit kidney extract liberated 0·111 mg. phosphorus with the veronal, and 0·103 mg. with the borate buffer during 48 hours' hydrolysis.

The hydrolysis of lecithin in borate buffer at varying times and at different hydrogen ion concentrations is illustrated in Fig. 1. Mixtures of 8 cc. of beef

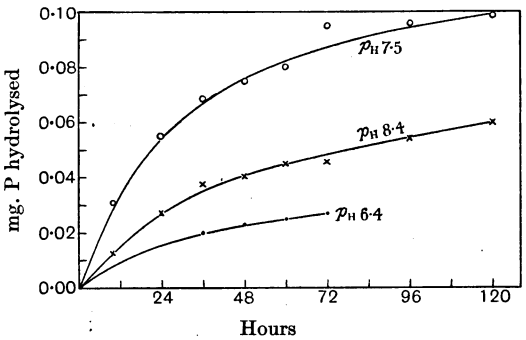

Fig. 1. Hydrolysis of lecithin at different p_H.

kidney extract, 40 cc. of 0·25 % lecithin emulsion (in water) and 40 cc. of borate buffer (of the desired p_H) were kept in the thermostat over a period of several days. 6 cc. portions were removed every 12 hours into 1 cc. of 25 % trichloroacetic acid and the inorganic phosphorus estimated in the filtrate. At the optimal p_H there was a rapid increase in the free phosphate during the first 60 hours, and thereafter a decrease in the rate of hydrolysis until equilibrium seemed to be approached between the fourth and fifth days.

As about 75 % of the hydrolysis had occurred during the first 48 hours, this was adopted as the standard period of time for comparative hydrolysis of lecithin by different tissue extracts. The method used for estimating the amount of the lecithin-hydrolysing enzyme in any tissue is as follows. The sample, freed from adhering tissue, is weighed, ground with sand and transferred with 20 times its weight of water saturated with chloroform to an Erlenmeyer flask. It is allowed to stand at room temperature, with occasional shaking, for 48 hours, when the mixture is filtered through cotton wool, adjusted to p_H 7·5 and preserved with a little toluene in the refrigerator. Longer periods of extraction, frequent grinding of the mixture or repeated extraction with smaller quantities of water do not appear to increase appreciably the amount of enzyme extracted. In the case of kidneys and similar tissues, the whole organ is taken, or it is minced up finely with scissors and an aliquot portion used for grinding and extraction. Intestines are freed from fat and extraneous tissue, split open lengthwise and gently washed out with a little water before mincing. Bones are freed as far as possible from muscle,

split open and freed from marrow before weighing. (Owing to the low "lecithinase" activity of bone, only 10 times the weight of water is used to make the extract instead of 20 times as in the case of other tissues.) To test the enzymic potency of an extract, two test-tubes containing 5 cc. of Palitzsch's borate buffer solution at p_H 7·5, 5 cc. of the 0·25 % lecithin emulsion, and 2 cc. of the tissue extract are incubated at 37·5° in the presence of a drop of chloroform and toluene. Controls consisting of buffer and extract and buffer and lecithin are prepared and incubated in the same way. The amount of free phosphate in the buffer and lecithin tubes is usually very small and may be neglected. At the end of the 48 hours the hydrolysis is stopped by the addition of 3 cc. of 20 % trichloroacetic acid, and the inorganic phosphorus is estimated in the filtrate. The amount of phosphate liberated is taken as a measure of the enzymic activity of the extract and hence of the tissue.

The hydrolysis of lecithin to yield free phosphate probably occurs in two stages, the first of which should yield either a glyceridephosphate or choline phosphate, while the second would involve the cleavage of either or both of these compounds to give free phosphate. Since the glyceridephosphate would probably, and choline phosphate almost certainly, be more soluble than lecithin in trichloroacetic acid solution, it was considered worth while to estimate the total as well as the inorganic phosphorus in the trichloroacetic acid filtrates. In general, the increase in total acid-soluble phosphorus of the tubes containing lecithin and extract over that of the controls ran parallel with, and was slightly greater than, the increase in the inorganic phosphorus.

While phosphatase has an optimum p_H of 8·9 for its action on glycerophosphoric acid, quite different from that of lecithinase, it is conceivable that in some way it may be concerned in the liberation of inorganic phosphorus from lecithin. It was therefore interesting to determine whether the phosphatase activity of the extracts ran parallel with the phosphate-hydrolysing enzyme for lecithin. Such did not appear to be the case. The extracts of bone, which generally showed a high phosphatase activity, were usually without any appreciable action on lecithin. The intestinal and kidney extracts, on the other hand, had a high enzymic activity for both glycerophosphate and for lecithin, but there was considerable variation in the values obtained and no relation could be seen between the phosphatase and lecithinase activities. The method used for determining the phosphatase activity of the extracts consisted in placing 5 cc. of 0·3 % sodium β-glycerophosphate, 5 cc. of glycine buffer and 0·5 cc. of extract (all of p_H 8·9) in each of four test-tubes. 2 cc. of 25 % trichloroacetic acid were added immediately to two of the tubes and the other two were kept at 37·5° in the presence of toluene for exactly 2 hours, and 2 cc. of trichloroacetic acid then added. The increase in free phosphate was taken as the measure of phosphatase activity.

Table III contains the results of a considerable number of determinations of the lecithinase and phosphatase activity of mammalian and avian tissues.

Table III. *Hydrolysis of lecithin and of glycerophosphate by tissue extracts.*

Free P hydrolysed from lecithin mg.	Total P hydrolysed from lecithin (acid-soluble) mg.	Free P hydrolysed from glycero-phosphate mg.
Pig kidney		
0·030	—	0·223
0·020	—	—
Beef kidney		
0·060	—	—
0·031	—	0·179
0·084	—	—
Beef suprarenal		
0·001	—	—
0·012	—	—
Dog kidney		
0·024	—	0·151
0·049	—	0·174
Dog intestine		
0·024	—	0·223
Human baby (acute intestinal diarrhoea)		
Large intestine		
0·006	0·006	0·056
Small intestine		
0·012	0·012	0·069
Human baby (stillborn)		
Small intestine		
0·012	—	0·176
Kidneys		
0·013	—	0·003
Rabbit liver		
0·011	—	0·058
0·022	0·059	0·104
0·006	0·006	0·048
Rabbit suprarenals		
0·008	0·008	0·006
0·008	0·032	0·072
0·011	0·020	0·012
0·003	0·004	0·018
Rabbit pancreas		
0·005	0·049	0·001
0·009	0·059	0·008
0·006	0·006	0·003
Rabbit spleen		
0·015	0·018	0·097
0·021	0·022	—
0·034	0·042	—
0·029	0·039	0·027
Rabbit testes		
0·008	0·011	0·316
0·023	0·024	0·336
Rabbit ovaries		
0·006	0·009	0·101
0·010	0·021	0·059
Rabbit brain		
0·006	0·012	0·021
0·011	0·043	0·022
0·023	0·048	0·033
0·008	0·010	0·020
Rabbit lung		
0·009	0·006	0·065
0·012	0·036	0·105
0·005	0·015	0·074
Rabbit aorta		
0·000	0·010	0·000
0·000	0·009	0·000
Rabbit skeletal muscle		
0·003	0·013	0·005
0·001	0·029	—
0·000	0·007	0·011
0·002	0·005	0·002
Rabbit heart		
0·003	0·024	0·008
0·002	0·002	0·009
0·002	0·013	0·010
0·005	0·006	0·013
Rabbit large intestine		
0·009	0·010	0·014
0·017	0·150	0·024
0·005	0·011	0·019
0·006	0·005	0·015
Rabbit small intestine		
0·024	—	0·075
0·068	—	0·104
0·057	0·088	0·268
0·010	0·010	0·064
0·020	0·064	0·074
0·046	0·227	0·236
0·027	—	0·379
0·020	—	0·040
0·030	0·086	0·219
0·117	—	0·255
0·137	—	0·114
Rabbit kidney		
0·006	—	0·251
0·042	0·063	0·214
0·029	0·036	0·314
0·009	0·030	0·172
0·035	—	0·252
0·024	—	0·112
0·049	0·062	0·224
0·098	—	0·227
0·014	0·060	0·179
Rabbit bone		
0·021	—	0·134
0·010	—	0·231
0·024	0·105	0·307
0·007	0·007	0·286
0·001	0·013	0·309
0·012	—	0·174
0·024	—	0·145
0·009	—	0·112
0·003 (old)	0·022	0·052
0·029 (young)	0·073	0·536
0·076 (rickets)	0·252	0·267
Chicken liver		
0·031	0·038	0·117
0·021	0·032	0·105
0·021	0·016	0·088
0·005	0·016	0 028

Table III (*contd.*).

Free P hydrolysed from lecithin mg.	Total P hydrolysed from lecithin (acid-soluble) mg.	Free P hydrolysed from glycero-phosphate mg.
Chicken gizzard		
0·004	0·007	0·004
0·001	0·017	0·004
0·000	—	0·007
0·008	0·017	0·009
Chicken pancreas		
0·006	0·019	0·012
0·002	0·031	0·014
0·041	0·083	0·012
0·022	0·021	0·005
Chicken spleen		
0·010	0·054	0·018
0·020	0·069	0·012
0·001	0·020	0·009
Chicken proventriculus		
0·013	0·031	0·015
0·017	0·038	0·029
0·002	0·037	0·010
Chicken testes		
0·040	0·061	0·096
0·012	0·024	0·047
0·008	0·010	0·008
0·013	0·017	0·055
0·007	0·016	0·039
0·011	0·060	0·051
0·016	0·059	0·027
Chicken brain		
0·010	0·018	0·036
0·008	0·027	0·041
0·006	0·011	0·024
0·015	0·015	0·016
Chicken lung		
0·006	0·017	0·023
0·001	0·007	0·011
0·002	0·002	0·011
0·011	0·017	0·054
Chicken blood vessels		
0·014	0·020	0·037
0·001	0·004	0·005
0·009	0·014	0·039
Chicken skeletal muscle		
0·005	0·007	0·008
0·002	0·017	0·004
—	0·008	0·002
0·007	0·007	0·005
Chicken cardiac muscle		
0·001	0·002	0·018
0·004	0·017	0·008
0·008	0·016	0·013
Chicken large intestine		
0·031	0·065	0·062
0·005	0·031	0·021
0·012	0·068	0·017
0·021	0·033	0·014
Chicken small intestine		
0·023	0·039	0·027
0·015	0·091	0·030
0·019	0·189	0·035
0·046	0·056	0·051
0·012	0·125	0·066
0·008	0·082	0·032
0·006	0·110	0·034
0·006	0·076	0·018
0·007	0·036	0·050
Chicken kidney		
0·022	—	0·230
0·017	0·022	0·059
0·031	0·088	0·138
0·038	0·178	0·183
0·053	0·054	0·344
0·016	0·043	0·277
0·012	0·023	0·227
0·032	—	0·256
0·036	0·079	0·260
0·020	0·058	0·254
0·026	0·032	0·196
Chicken bone		
0·020	—	0·331
0·004	—	0·046
0·000	0·005	0·035
0·003	0·035	0·185
0·007	0·010	0·157
0·011	0·024	0·358
0·021	0·024	0·387
0·016	—	0·178
0·028	—	0·076
0·001	0·044	0·085
0·000	0·037	0·110
0·003	0·008	0·132

Inactivation of the enzyme.

When an active tissue extract is kept at body temperature at different hydrogen ion concentrations, a marked destruction of the enzyme occurs at reactions which are not near neutrality. Fig. 2 illustrates the results which were obtained on the inactivation of an extract of rabbit kidney. 10 cc. quantities of the extract were adjusted to different p_H by the careful addition of a few drops of $N/10$ acid or alkali. After being left in the thermostat at 38° for 24 hours, the samples were adjusted to p_H 7·6 by adding more acid or alkali. Water was then added to all of them to bring the volume to 12 cc.

The lecithinase and phosphatase activities were determined on 2 cc. and 0·5 cc. portions, respectively, and the results compared with the activity of the original extract. Between p_H 7·0 and 8·0 there appeared to be very little loss of activity towards either lecithin or glycerophosphate. Above p_H 8, and below p_H 6, however, there was a marked diminution in the activity of the extracts.

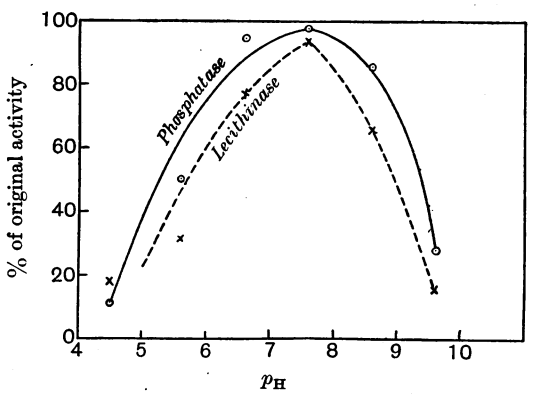

Fig. 2. Inactivation of the enzyme.

Hydrolysis of lecithin at different temperatures.

The optimum temperature for the hydrolysis of lecithin appears to be that of the body. Incubation of hydrolysing mixtures at lower or higher temperatures than 38° results in a smaller increase in the free and total acid-

Table IV. *Hydrolysis of lecithin at different temperatures.*

	Days	Free P mg.	Increase in free P mg.	Total acid-soluble P mg.	Increase in total acid-soluble P mg.
(1) Temperature 11°	0	0·220	—	0·244	—
	1	0·221	0·001	0·245	0·001
	2	0·223	0·003	0·246	0·012
	3	0·224	0·004	0·251	0·007
	4	0·227	0·007	0·254	0·010
	5	0·231	0·011	0·266	0·022
(2) Temperature 23°	0	0·220	—	0·244	—
	1	0·224	0·004	0·253	0·009
	2	0·235	0·015	0·275	0·031
	3	0·245	0·025	0·278	0·034
	4	0·254	0·034	0·285	0·041
	5	0·261	0·041	0·302	0·058
(3) Temperature 38°	0	0·220	—	0·244	—
	1	0·232	0·012	0·262	0·018
	2	0·252	0·032	0·272	0·028
	3	0·261	0·041	0·298	0·054
	4	0·276	0·056	0·296	0·052
	5	0·288	0·068	0·314	0·070
(4) Temperature 57°	0	0·220	—	0·244	—
	1	0·221	0·001	0·261	0·017
	2	0·226	0·006	0·261	0·017
	3	0·231	0·011	0·282	0·038
	4	0·240	0·020	0·278	0·034
	5	0·244	0·024	—	—

soluble phosphorus. Two test-tubes, each containing 20 cc. of buffer (p_H 7·5), 20 cc. of lecithin emulsion and 10 cc. of kidney extract, were kept in thermostats at different temperatures for a period of 5 days, 8 cc. being withdrawn each day into 2 cc. of 25 % trichloroacetic acid for the determination of free and total phosphorus (Table IV).

The effect of hydrolysis products on the rate of hydrolysis.

If the velocity constants for a unimolecular reaction,

$$k = \frac{1}{t} \log \frac{a}{a-x},$$

are calculated from the data of hydrolysis experiments, it is seen that there is a very marked difference in the behaviour of the hydrolysing mixtures according to the enzyme preparation used. For some kidney preparations the values for k are fairly constant over a period of several days. For others, and especially for intestinal preparations, the reaction velocities show a marked and steady decline and soon reach values which are much smaller than those for the original rates of hydrolysis. The data for four experiments which were carried out to test this point are contained in Table V.

Table V. *Rate of hydrolysis of lecithin.*

Time hrs.	Free P hydrolysed mg.	k	Time hrs.	Free P hydrolysed mg.	k	Total acid-soluble P hydrolysed mg.	k
Rabbit kidney extracts:							
24	0·021	0·000778	8	0·017	0·00186	0·037	0·00418
48	0·034	0·000648	24	0·058	0·00223	0·093	0·00372
72	0·062	0·000803	32	0·073	0·00214	0·109	0·00333
96	0·083	0·000824	48	0·103	0·00209	0·143	0·00304
120	0·091	0·000731	59	0·122	0·00206	0·170	0·00305
144	0·106	0·000722	72	0·127	0·00176	0·173	0·00255
168	0·120	0·000714	94	0·136	0·00147	0·170	0·00192
192	0·120	0·000623	Total lecithin-P 0·500 mg.				
216	0·127	0·000592					
240	0·134	0·000567					
Total lecithin-P 0·500 mg.							
Rabbit intestinal extracts:							
24	0·159	0·00693	24	0·012	0·000566	0·031	0·001461
48	0·171	0·00379	48	0·013	0·000302	0·032	0·000759
72	0·191	0·00290	72	0·012	0·000189	0·042	0·000673
96	0·199	0·00229	96	0·015	0·000176	0·051	0·000622
120	0·228	0·00220	120	0·023	0·000214	—	—
144	0·231	0·00187	144	0·024	0·000189	0·069	0·000572
168	0·232	0·00161	168	0·029	0·000196	0·077	0·000553
192	0·233	0·00142	192	0·031	0·000184	0·077	0·000484
216	0·239	0·00130	216	0·033	0·000174	—	—
240	0·244	0·00121	Total lecithin-P 0·400 mg.				
Total lecithin-P 0·500 mg.							
Enzyme solutions dialysed 48 hours against running water.			Enzyme solutions electrodialysed against tap-water till nearly free of inorganic phosphate.				

It would seem that this decline in the values of k is more probably due to the slow destruction of the enzyme in the hydrolysing mixture than to the

accumulation of the products of hydrolysis. The increases of inorganic phosphate in the experiments cited are not sufficiently different in amount to account, on the basis of inhibition due to accumulation of a product of hydrolysis, for the large variations in the values for the velocity constants.

Several attempts were made to ascertain the effect on the rate of hydrolysis of added inorganic phosphate, but such small differences as were observed in the amounts hydrolysed were irregular and could not be attributed to the extra phosphate present.

Since the cleavage of inorganic phosphorus from lecithin also involves the removal of choline, it was of interest to determine what influence, if any, choline had on the amount of hydrolysis as estimated by the liberation of phosphate. Table VI contains the results of an experiment in which choline, in varying amounts, was added to digesting mixtures of lecithin and rabbit kidney extract. The addition of the choline caused no diminution in the amount of phosphorus liberated, and the variations in the values for k between the solutions of different choline content are no greater than the variations in the values for the individual solutions.

Table VI. *Effect of choline on the hydrolysis of lecithin.*

	No choline				0·005 *M* choline			
Time hrs.	Free P hydrolysed mg.	k	Total acid-soluble P hydrolysed mg.	k	Free P hydrolysed mg.	k	Total acid-soluble P hydrolysed mg.	k
14	0·020	0·001286	0·031	0·001995	0·020	0·001286	0·030	0·001939
28	0·028	0·000899	0·042	0·001331	0·030	0·000964	0·041	0·001336
38	0·036	0·000863	0·056	0·001362	0·040	0·000960	0·056	0·001369
49	0·055	0·001035	0·054	0·001021	0·055	0·001035	0·057	0·001081
62	0·060	0·000901	0·061	0·000917	0·061	0·000918	0·068	0·001031
75	0·066	0·000822	0·072	0·000906	0·071	0·000892	0·075	0·000948
86	0·067	0·000730	0·084	0·000933	0·074	0·000814	0·082	0·000907
96	0·078	0·000771	0·074	0·000729	0·081	0·000802	0·083	0·000824
110	0·095	0·000836	0·085	0·000737	—	—	—	—
	0·01 *M* choline				0·04 *M* choline			
14	0·023	0·001499	0·022	0·001421	—	—	0·022	0·001421
28	0·033	0·001071	0·039	0·001268	0·028	0·000899	0·030	0·000968
38	0·041	0·000984	0·054	0·001314	0·040	0·000960	0·048	0·001157
49	0·054	0·001017	0·052	0·000980	0·052	0·000980	—	—
62	0·064	0·000967	0·064	0·000967	0·060	0·000914	—	—
76	0·072	0·000904	—	—	0·067	0·000840	—	—
86	0·076	0·000837	0·078	0·000861	0·072	0·000790	—	—
96	—	—	—	—	0·075	0·000739	0·077	0·000761
110	0·094	0·000827	—	—	—	—	—	—

Total lecithin-P 0·500 mg.

Enzyme solution electrodialysed against tap-water.

The presence of glycerol in the hydrolysing mixtures had no effect in concentrations up to 0·4 *M*, the highest investigated. An experiment similar to the foregoing was carried out with lecithin, kidney extract and varying amounts of added glycerol. A sample equivalent to 0·500 mg. of lecithin-P

was removed from each flask at various periods and the free and total P estimated in the trichloroacetic acid filtrates (Table VII).

Table VII. *Effect of glycerol on the hydrolysis of lecithin.*

	No glycerol		0·01 *M* glycerol		0·1 *M* glycerol		0·4 *M* glycerol	
Time hrs.	Free P hydrolysed mg.	Total acid-soluble P hydrolysed mg.	Free P hydrolysed mg.	Total acid-soluble P hydrolysed mg.	Free P hydrolysed mg.	Total acid-soluble P hydrolysed mg.	Free P hydrolysed mg.	Total acid soluble P hydrolysed mg.
13	0·007	0·019	0·007	0·011	0·003	0·003	0·009	0·010
24	0·019	0·020	0·020	0·027	0·015	0·013	0·014	0·014
35	0·017	0·031	0·018	0·026	0·016	0·016	0·013	—
48	0·030	0·033	0·032	0·024	0·027	—	0·027	0·031
61	0·030	0·050	0·033	0·048	0·027	0·032	0·031	0·039
80	0·042	0·054	0·042	0·054	0·037	—	0·037	0·040
95	—	0·059	0·046	0·058	0·043	0·051	0·041	0·042
105	0·044	0·059	0·046	0·056	—	—	0·041	0·044
120	0·057	0·060	0·057	0·063	0·045	—	0·059	—

SUMMARY.

1. An enzyme capable of hydrolysing lecithin to liberate inorganic phosphate is present in various animal tissues.

2. The optimal hydrogen ion concentration for the action of this enzyme is at about p_H 7·5, while that of phosphatase is at p_H 8·9. For this reason, and because the distribution of "lecithinase" does not correspond with that of phosphatase, they are thought not to be the same enzyme.

3. The relative "lecithinase" activity of different tissues is as follows: (in decreasing order)—kidney, small intestine, spleen, liver, testes, pancreas, large intestine, brain, ovaries, bone, suprarenals, lung, blood vessels, cardiac muscle, skeletal muscle.

The enzyme is fairly stable at a neutral reaction but is rapidly destroyed when kept in an acid or alkaline medium at 38°.

4. Lecithin appears to be hydrolysed most rapidly by the enzyme at body temperature.

5. The addition of inorganic phosphate and choline (products of hydrolysis) and of glycerol to hydrolysing mixtures of lecithin and the enzyme does not appear to affect appreciably either the rate or amount of hydrolysis.

The technical work and colorimetric analyses in connection with this investigation were done by Miss Margaret Shaw.

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