incubation is required (4–5 hr.) for the maximum activity relative to protein nitrogen to be attained, since these are more contaminated by inactive protein than either faecal or intestinal preparations. It is interesting to note that trypsin does not appear to act on the protein part of the enzyme, since the protein nitrogen of the incubated enzyme solution remains constant after a certain time, and is not affected by any further incubation, even if more trypsin is added. The decline in enzyme activity appears to be due to inactivation of the enzyme, when incubated in alkaline buffer, rather than to direct trypsin action on the enzyme itself. This inactivation was pointed out by Fischer & Greep (1948) to be much more extensive in the absence of trypsin than when it is present. Thus trypsin, in addition to

digesting contaminating proteins, seems to protect the enzyme to a certain extent against inactivation during incubation in alkaline buffer. Excess of trypsin, however, does not seem to increase the degree of protection of the enzyme; on the contrary, it seems to decrease it, or to cause inhibition of the phosphatase.

SUMMARY

1. Further purification of the alkaline phosphatase, beyond that attainable by fractional precipitation and salting-out procedures, was obtained by incubation with 0.1–0.2 % trypsin at 37° for a period of not more than 4 hr.

2. Addition of more trypsin, or incubation for longer periods, resulted in loss of enzyme activity.

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Reactivation of Alkaline Phosphatases after Dialysis

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(Received 20 October 1948)

Albers, Bayer, Bohnenkamp & Muller (1938) ascribed the inactivation of the kidney alkaline phosphatase through prolonged dialysis to a separation of the phosphatase into an inactive protein apoenzyme and a dialyzable coenzyme or prosthetic group. These workers showed that this coenzyme cannot be magnesium. It appeared to be a labile organic substance, which could be obtained by incubating the holoferment at 100° for a few minutes in a neutral medium. This treatment destroyed the apoenzyme while not affecting the coenzyme. By mixing the latter with an enzyme preparation partially inactivated by dialysis or previous incubation in an acid medium, active phosphatase solutions were obtained. On combining the dialyzable liquid with the inactivated protein solution of dialyzed kidney preparations, with the addition of magnesium, Albers (1939) claimed to have obtained an almost 100 % reactivation. von Euler & Hahn (1947) described a partial reactivation of intestinal phosphatase, inactivated by dialysis at pH 4.5, by the addition of boiled enzyme solution. Cloetens (1941*a, b*, 1942), after repeating Albers's experiments, came to the conclusion that the activating action of a heated enzyme solution (Kochsaft), or the dialysate

liquid, was not due to the presence of a cophosphatase, but more probably to a zinc or cobalt ion, which is present in very small amount and which forms a dissociable complex. After studying the influence of different metallic ions, as well as substances which form weakly dissociable combinations with metals, Cloetens concluded that alkaline phosphatase is composed of a slightly active or completely inactive part, EG_2^1 (i.e. protein + metal-combining groups), and the two metals magnesium and zinc. This postulate was supported by the work of Hove, Elvehjem & Hart (1940), who used zinc salts in combination with amino-acids and with protein split products (from autolyzed mucosal tissue) to activate dialyzed intestinal phosphatase. Thoai, Roche & Roger (1947) reported the inactivation of intestinal phosphatase by prolonged dialysis at 37°, and its complete reactivation by incubation with amino-acids in alkaline medium and determination of the enzyme activity in presence of certain inorganic ions. Comparing these findings with that of Albers, it would appear possible that the dialyzable prosthetic group may be a combination of a metal other than magnesium (possibly zinc) with a compound containing amino-acid groups.

In the present investigation an attempt has been made, using ordinary dialysis as well as electro-dialysis, to obtain a clearer conception of the nature of the dialyzable factors indispensable for the activity of alkaline phosphatase. It has been found that magnesium and a nitrogenous non-protein dialyzable substance, which migrates towards the cathode during electro-dialysis, are responsible for activating the apoenzyme. No evidence for the identity of this dialyzable substance with simple α -amino-acids, or for the presence of zinc, has been obtained. Differences among alkaline phosphatase preparations from different sources have been found, and the results are discussed.

EXPERIMENTAL

King & Dolan's (1934) electro-dialyzing apparatus has been used throughout. It consists of a glass cell of suitable capacity containing two units of electrode chambers immersed in the enzyme solution. Each unit consists of a parchment dialyzing thimble containing the electrode surrounded by distilled water. Because of the shortage of platinum this has been used only for the cathode, while a carbon electrode has been found quite satisfactory as an anode. The two electrode chambers were either connected together, as when water is circulated through them, or left unconnected, according to the requirements of the experiment. A mechanical stirrer was used, since the bubbling of N_2 was found to cause some inactivation due to frothing. A mercury-vapour-lamp transformer was used as the source of direct current. In some cases a 6 V. battery was used. An ammeter was included in the circuit. The current passing through the enzyme solution was as low as 1.5 ma. and was never allowed to exceed 10 ma.

Enzyme solution and determination of activity. The solutions being dialyzed always contained the enzyme in its highest available state of purity, prepared and the activity determined according to the methods already described (Abul-Fadl, King, Roche & Thoai, 1949; Abul-Fadl & King, 1949).

Nitrogen determination. The micro-Kjeldahl method was used, and was carried out on 0.2 ml. of the clear enzyme solution on which the activity had been previously determined. For protein N the proteins were precipitated with molybdic acid. Digestion was carried out with 2 ml. 50% (v/v) H_2SO_4 containing 1% SeO_2 , a small electric heater being used.

Micro-detection of certain metals (cf. British Drug Houses Ltd. *Book of Organic Reagents for Analytical Use including Spot Tests*). Magnesium was tested for by the *p*-nitro-benzeneazo- α -naphthol test which is sensitive to 2 μg . Mg; calcium by the picrolonic acid test which is sensitive to 0.01 μg . Ca; and zinc by the resorcinol test, sensitive to 2 μg . Zn.

Procedure for dialysis. The enzyme solution was subjected to prolonged electro-dialysis against running distilled water, continuously stirring and using an electric current of 1–3 ma. The enzyme activity was determined at regular intervals, with and without the addition of Mg^{++} to the buffer-substrate mixture. As the activity started to decrease, the two electrode chambers were disconnected from each other, and distilled water was very slowly passed through each chamber separately, and collected in separate containers; or if necessary the water circulation was stopped. The current was gradually increased.

The effect of the anode and cathode liquids, as well as certain other substances, was then tried on the enzyme solution which had been partially inactivated by electro-dialysis.

RESULTS

Kidney phosphatase. Table 1 shows the changes in enzyme activity in absence and in presence of magnesium, and in total nitrogen and protein nitrogen, taking place during electro-dialysis of kidney alkaline

Table 1. *Effect of electro-dialysis on the activity of kidney alkaline phosphatase*

(Slow current of running distilled water in the electrode chambers; electric current started with 1 ma. and gradually increased to 10 ma.)

Duration of electro-dialysis (hr.)	Enzyme activity (units/ml.)		mg. N/ml.	
	Without Mg	With 0.01 M-Mg	Total	Protein
0	56	84	4.6	1.30
2	56	84	4.5	1.30
4	56	84	4.3	1.30
6	56	84	4.2	1.30
8	53	84	4.1	1.30
10	50	84	4.0	1.30
12	46	84	3.9	1.30
14	40	82	3.8	1.28
16	34	80	3.7	1.27
18	28	53	3.6	1.25
20	20	36	—	—
26	6	9	3.4	1.23
30	3	4	—	—
34	2	2	3.2	1.20
36	0.8	0.8	—	—
40	0.3	0.3	3.0	1.20

phosphatase solution. A progressive loss of activity occurs, and it appears that at least two important factors are lost during the course of the dialysis. The first of these is magnesium, the addition of which restores the original activity of the enzyme solution during the first 16 hr. of electro-dialysis, although the time depends on the current strength. After that a sudden drop in activity takes place followed by a comparatively rapid inactivation of the enzyme. This suggests the splitting off of another factor which cannot be identical with magnesium since the latter, even in increased amounts, fails to restore the initial enzyme activity. It is noticed that the more inactivation takes place the more this factor is lost, and the less the response of the enzyme towards magnesium until finally the latter has almost no effect on the enzyme activity.

The total nitrogen falls gradually with electro-dialysis. The protein nitrogen remains constant for a time, and then decreases gradually, the solution becoming turbid especially around the cathode chamber. There is a tendency for the protein nitrogen to remain constant again near the end of the dialysis.

Table 2 shows the strong activating power of the clear cathode liquid after filtration from the inactive precipitate. The cathode liquid alone has no activity, but it has considerable activating power when mixed with the apoenzyme, and this is much more pronounced in the presence of magnesium. The three experiments were carried out on the same solution

Table 2. *Effect of the liquids in the electrode chambers on kidney alkaline phosphatase partially inactivated by electro dialysis*

(These experiments were carried out on a single enzyme solution which was dialyzed in three stages. At the end of each stage the enzyme solution was mixed with the liquids from the electrode chambers collected during the period of dialysis. The water was circulated through the dialyzing thimbles as slowly as possible.)

	Activity (units/ml.)	
	Without Mg	With 0.01 M-Mg
Stage 1 of dialysis:		
Dialyzed enzyme solution (30 hr.) (+ water, 1 : 1)	28	53
After 2 hr. further dialysis (+ water, 1 : 1)	20	36
Enzyme solution + anode liquid (1 : 1)	20	36
Enzyme solution + cathode liquid (1 : 1)	38	62
Enzyme solution + anode + cathode liquids (1 : 0.5 : 0.5)	38	61
Cathode liquid alone	0	0
Anode liquid alone	0	0
Cathode + anode liquids	0	0
Stage 2 of dialysis:		
Dialyzed enzyme solution (total 35 hr.) (+ water, 1 : 1)	8	11
After 1 hr. further dialysis (+ water, 1 : 1)	7	9
Enzyme solution + anode liquid (1 : 1)	7	9
Enzyme solution + cathode liquid (1 : 1)	9	15
Stage 3 of dialysis:		
Enzyme solution after prolonged dialysis (+ water, 1 : 1)	5	5.5
Further dialysis (+ water, 1 : 1)	0	0
Enzyme solution + anode liquid (1 : 1)	0	0
Enzyme solution + cathode liquid (1 : 1)	1.2	5.3

which had been electro dialyzed in three stages. The activation is very marked during the initial stage of electro dialysis, but after prolonged electro dialysis the cathode liquid does not fully restore the initial activity of the enzyme. It seems probable that the apoenzyme is affected by the prolonged dialysis.

The anode liquid, inactive in itself, has neither activating nor inhibiting action when mixed with the enzyme solution being dialyzed.

Table 3 shows the effect of magnesium, zinc, amino-acids and cathode liquid added to two enzyme solutions of different initial activities which had been partially inactivated by electro dialysis, singly and

in different combinations. Zinc in 0.0002–0.0005 M-concentrations and in presence of 0.01 M-magnesium shows a slight activating property, but inhibits in higher concentrations. Its activating effect, however, is very small compared with that of the cathode liquid. A combination of cathode liquid and

Table 3. *Effect of magnesium and zinc salts and amino-acids compared with that of cathode liquid on the kidney alkaline phosphatase partially inactivated by prolonged electro dialysis*

(Two enzyme solutions A and B of different initial activity were used.)

	A (units/ml.)		B (units/ml.)	
	No Mg	0.01 M-Mg	No Mg	0.01 M-Mg
Original solutions	40	60	13	20
After prolonged dialysis	3.0	3.4	2.7	3.0
Effect of zinc salt:				
0.01 M	0.0	0.5	0.0	0.7
0.001 M	1.6	5.6	0.7	9.6
0.0005 M	2.0	10.5	1.2	11.5
0.0002 M	2.5	10.5	1.5	11.5
0.0001 M	2.7	5.8	2.0	3.5
Incubation with amino-acids:				
Glycine (0.001 M)	0	0	0	0
Glycine (0.0001 M)	1.5	1.0	0.7	0.5
Alanine (0.001 M)	0	0	0	0
Alanine (0.0001 M)	2.0	1.5	1.5	1.2
Enzyme solution	28	55	7	18.5
mixed with cathode liquid (1 : 1)				
Enzyme solution	6	22.5	2.5	9.4
mixed with cathode liquid, and 0.0002 M-zinc added				

0.0002 M-zinc has less activating power than the cathode liquid alone. The amino-acids (glycine and alanine) in different concentrations, even after previous incubation with the inactive enzyme, do not exhibit any activating property; on the contrary, they inhibit, especially in presence of magnesium.

Table 4 shows that the activating substance present in the cathode liquid is organic in nature as the ash is not active. It is stable to boiling for 10 min. but is destroyed by a slight excess of ammonia.

A cathode liquid with high coenzyme activity contained 3.9 mg. total solids/ml. and yielded 0.60 mg. of ash, mainly alkaline earths. 'Spot' tests for calcium and for zinc in the ash were negative, while a slight positive reaction for magnesium was obtained. The total nitrogen, 0.66 mg./ml., was equivalent to 20 % of the organic matter. This is consistent with the possibility of an amino-acid or peptide structure being present. Protein was apparently absent since there was no detectable precipitate with molybdic acid.

Table 4. *Effect of boiling, ashing and other treatments on the activating property of the cathode liquid on the kidney phosphatase*

	Activity (units/ml.)	
	No Mg	0.01 M-Mg
Kidney phosphatase after 8 hr. electro-dialysis	12.6	19
Enzyme solution + cathode liquid	20	60
Enzyme solution + boiled cathode liquid	20	60
Enzyme solution + redissolved ash*	12.0	18.5
Enzyme solution + cathode liquid treated with 0.05 N-ammonia solution	12.6	19.5

* Cathode liquid evaporated to dryness, residue ignited, and ash dissolved in HCl, evaporated on water bath to dryness, then dissolved in water, neutralized and diluted to original volume.

Intestinal, faecal and liver phosphatase. Table 5 illustrates the effect of electrodialysis on these alkaline phosphatases. The enzyme activity/ml. remains almost constant for the first 20 hr.; then a

Table 5. *Effect of electrodialysis on intestinal, faecal and liver phosphatases, and the effect of the electrode chamber liquids on the enzyme activity*

	Activity (units/ml.)	
	No Mg	0.01 M-Mg
Intestinal phosphatase (+ water, 1 : 1)	140	200
Electrodialysis against running distilled water, 1.5 increased to 10 ma.:		
After 2 hr.	140	200
" 6 "	140	200
" 10 "	130	200
" 16 "	125	200
" 20 "	115	180
" 24 "	90	130
" 28 "	40	55
" 30 "	28	38
Enzyme solution + anode liquid (1 : 1)	18	27
Enzymesolution + cathodeliquid(1 : 1)	36	45
Dialysis for further 4 hr.:		
Enzyme solution (+ water 1 : 1)	0.4	0.4
" (+ anode 1 : 1)	0	0
" (+ cathode 1 : 1)	1.2	1.4
" (+ anode + cathode)	0	0
Faecal phosphatase solution (+ water 1 : 1)	180	240
After 30 hr. electrodialysis against running distilled water, 1.5-10 ma.	65	95
Enzyme solution + anode liquid (1 : 1)	34	70
Enzyme solution + cathode liquid (1 : 1)	75	120
Liver phosphatase solution (+ water 1 : 1)	96	120
After 20 hr. electrodialysis against running distilled water, 5 ma.	35	50
Enzyme solution + anode liquid (1 : 1)	15	22
Enzyme solution + cathode liquid (1 : 1)	45	65

sudden drop takes place. The cathode liquid showed a slight activating effect, but not as much as that noticed in the case of the kidney phosphatase. In some cases the cathode liquid was without any detectable activating effect, and the dialyzed enzyme lost its activity without any evidence of separation of a coferment, contrary to what was always found with the kidney phosphatase.

On the other hand, the anode liquids showed a tendency to inhibit the intestinal, faecal and liver phosphatases. The pH of these liquids ranged from 2.3 to 4.7, and the inhibitory effect was more pronounced with the more acid solutions. The kidney enzyme was not sensitive to the same solution.

Effects of magnesium on phosphatases. Fig. 1 shows the effects of different magnesium concentrations on the activity of crude and purified intestinal and kidney phosphatase preparations. The enhancing

Fig. 1. Effect of Mg on crude and purified alkaline phosphatases. Hydrolysis of 0.005 M-sodium phenyl phosphate in carbonate-bicarbonate buffer, pH 10; $q \text{ Mg} = -\log [\text{Mg}^{++}]$.

action of magnesium on kidney phosphatase is much more pronounced than that on the intestinal enzyme. With crude enzyme preparations there is an optimum concentration of magnesium beyond which the enzyme is inhibited. With purified enzyme

preparations, on the other hand, excess of magnesium has no such inhibitory effect.

Effect of amino-acids. Table 6 shows the effect of amino-acids on kidney and faecal enzymes. Both non-dialyzed kidney and faecal enzymes are activated by some amino-acids. The dialyzed kidney enzyme shows only feeble activation with cystine and cysteine, while glycine does not activate. The

Table 6. *Effect of amino-acids on faecal and kidney alkaline phosphatase before and after dialysis*

(Ordinary dialysis in cellophan bags at room temperature against distilled water.)

	Activity (units/ml.)	
	No Mg	0.01 M-Mg
Non-dialyzed kidney phosphatase*	17.3	26.3
Glycine (0.002 M)	27.8	30.5
Cysteine (0.0005 M)	27.0	29.3
Histidine (0.001 M)	27.0	32.5
Cystine (0.001 M)	27.0	31.0
Dialyzed kidney phosphatase* (36 hr.)	9.0	10.5
Glycine (0.0002 M)	8.5	9.0
Cystine (0.0005 M)	10.6	12.4
Cysteine (0.0005 M)	10.0	11.5
Non-dialyzed faecal phosphatase*	82.5	112.0
Glycine (0.0002 M)	96.0	125.0
Dialyzed faecal phosphatase* (36 hr.)	14.0	15.2
Glycine (0.0002 M)	35.7	46.5
Alanine (0.0005 M)	36.0	45.8
Cysteine (0.0005 M)	14.6	16.5
Cystine (0.0005 M)	14.6	16.5
Histidine (0.0005 M)	45.7	62.5
Histidine (0.001 M)	45.7	62.0
Histidine (0.002 M)	45.0	62.0
Tyrosine (0.001 M)	20.3	25.6
Tryptophan (0.001 M)	17.8	22.0

* Different preparations.

dialyzed faecal enzyme, on the other hand, shows a marked activation with amino-acids, that with histidine being especially noteworthy. This does not seem to be lessened with increasing concentrations of histidine up to 0.002 M. Cystine and cysteine, on the other hand, have no effect and tryptophan only a slight activating effect.

Effect of formaldehyde. Table 7 shows the effect of formaldehyde on the phosphatases obtained from different tissue autolysates. By inclusion of 0.1 ml. of 20 % neutral formaldehyde solution in the buffer-substrate-enzyme mixture (without previous incubation of the enzyme with the formaldehyde solution) a 95–100 % inhibition was attained with all tissue alkaline phosphatases, except those of intestinal mucosa and faeces, where the inhibition was 70 %.

Attempts were made to reactivate a kidney alkaline phosphatase that had lost most of its activity through formaldehyde treatment. Directly after adding the formaldehyde solution to the enzyme

preparation a slight excess (over the theoretical to combine with the formaldehyde) of ammonia solution was added. The activity was then determined,

Table 7. *Effect of formaldehyde on the alkaline phosphatase of tissue extracts*

	Activity (units/100 ml.)		Inhibition (%)
	Without formal- dehyde	Formal- dehyde (0.5%)	
Ox kidney	138.5	5	96
Human kidney	77.3	1.5	97
Adrenal glands	165	0	100
Dog bile diluted 1 : 200	28.5	0	100
Bone (young rabbit)	162	1.5	99
Dog faecal	75	24	68
Human intestinal	165	60	64
Liver	97	0	100
Pancreas	9	0	100
Spleen	120	0	100
Thyroid	47	0	100

and the effects of different amino-acids (glycine, alanine, histidine, arginine, tyrosine and tryptophan) as well as of the cathode liquid were tried. It was not possible to restore the activity by means of any of these substances.

DISCUSSION

These experiments support the hypothesis of Albers *et al.* (1938) and of Albers (1939) that phosphatase consists of an apo- and a co-enzyme. This is well illustrated with kidney phosphatase by a progressive loss of enzyme activity during electrodialysis, due to splitting off a factor (or factors) essential for enzyme activity which migrates towards the cathode. Thus the liquid in the cathode chamber shows strong reactivating properties when added to the holoferment which has been partially inactivated by electrodialysis. The liquid in the anode chamber, on the other hand, has no action on the enzyme activity. The anode and cathode liquids possess no enzymic activity either alone or combined.

The activating effect of the cathode liquid is mainly due to two factors: (A) magnesium (which is possibly replaceable by other metals), which can be easily split off and is responsible for the whole activating property of the cathode liquid during the early stages of electrodialysis, especially with weak currents; (B) an organic factor (possibly nitrogenous), which is not easily detachable from the apo-enzyme, but splits off quickly with strong currents, or suddenly after prolonged ordinary dialysis. This factor withstands prolonged boiling, but is readily destroyed when treated with ammonia, excess alkali or acid. It is not identical with any of the amino-acids, since the latter do not exhibit a similar effect.

Magnesium seems to be essential for the activation of the holoferment. When the latter is carefully freed from magnesium and then added to the partially inactivated enzyme, it will produce no effect; but when magnesium is added as well the phosphomonoesterase activity of the mixture is much more than could be produced by adding magnesium alone. On the other hand, magnesium alone has no effect on the completely inactive enzyme, but in presence of the organic factors the activity is restored, although in no case to the initial level. Complete inactivation of the enzyme is probably accompanied by denaturation of the apoenzyme.

Under the conditions of these experiments this organic factor is essential for the reactivation of the dialyzed holoferment from ox kidney. It does not require any previous incubation, nor has it any inhibitory effect when present in excess. In contrast, it has no such action on the non-dialyzed kidney enzyme, nor on the electro-dialyzed inactive enzyme prepared from liver or intestine. A possible explanation is that in the case of the liver and intestinal enzymes this factor is so strongly bound to the apoenzyme that the latter is inactivated by prolonged dialysis before any splitting of the coenzyme takes place.

These facts seem to indicate that the kidney phosphatase consists of three main components which are indispensable factors for the enzymic activity: a specific protein portion; a specific dialyzable organic group or groups; and magnesium. Magnesium produces its effect only in the presence of the dialyzable organic group or groups.

The two-metal theory involving zinc seems to be highly improbable since no evidence that zinc is indispensable has been obtained.

The behaviour of faecal, intestinal and liver phosphatases during dialysis has been found to differ from that of the kidney enzyme under the same experimental conditions.

The following are the results of a great number of experiments which have been carried out in this connexion:

(1) By ordinary dialysis in cellophane bags against distilled water, or feebly alkaline water at room temperature, it was observed that the kidney enzyme progressively decreased in activity. After 24 hr. dialysis the activity was greatly decreased, but it could be restored to the original level through the addition of optimum concentrations of magnesium. On further prolonged dialysis the activity still declined and after 48 hr. a marked loss of phosphatase activity took place. The enzyme was still activated by magnesium, but this could no longer restore the activity to its original value. The faecal, intestinal and liver enzymes, on the other hand, were markedly stable under the same conditions. Although a limited decrease in activity took place

magnesium could still restore the original activity. Beyond this no further decline in activity took place, and the enzymes remained stable during 72 hr. dialysis in one experiment. With more prolonged dialysis activity was maintained for about 8–10 days at room temperature, following which it rapidly declined. This phenomenon can be explained on the basis of the previously mentioned postulate that the coenzyme, while very strongly bound to the apoenzyme in the case of faecal, intestinal and liver phosphatases, can be comparatively easily split in the case of the kidney enzyme.

(2) On electro-dialysis the kidney enzyme was comparatively more stable than the other phosphatases. This could be partially accounted for, in many cases, by the marked sensitivity of these enzymes to anions. The anode liquid inhibited these enzymes, while it was quite harmless to the kidney enzyme. If the water circulating in the anode chamber was stopped during electro-dialysis, the intestinal phosphatase was inhibited very quickly (even if dialysis was carried out at 0°), while the kidney enzyme, on the other hand, was not affected.

Although several factors have been known to influence the degree of activation of phosphatase by magnesium (e.g. concentration of magnesium, nature of substrate and its concentration, degree of enzyme purity, concentration and age, cf. Fischer & Greep, 1948), yet there have been strong indications that certain phosphatases are more activated by magnesium than others. Armstrong (1935) and Schmidt & Thannhauser (1943) described a 25% increase in the activity of faecal and intestinal phosphatases with optimum magnesium concentration. Drill, Annegers & Ivy (1944) also reported slight enhancement by magnesium of the elevated serum phosphatase during hepatic damage. Our findings concerning faecal, intestinal and liver phosphatases are in agreement with those of these authors. The kidney enzyme, on the other hand, has always been found to be much more activated by magnesium than either faecal, intestinal or liver phosphatases. This can again be explained on the basis of variation of affinity between the apoenzyme and its coenzyme and magnesium, in different phosphatases. When this affinity is very strong, the enzyme, as it naturally occurs, will be almost saturated with its requirements of magnesium, and hence the effect of any added amount will be only slight. On the other hand, when this affinity is weak, the enzyme will lose its magnesium readily during its extraction from the tissues and by mere ageing or by dilution, and thus the effect of added magnesium will be pronounced (cf. Fischer & Greep, 1948).

Amino-acids are shown here to activate both kidney and intestinal non-dialyzed enzymes. After prolonged dialysis of these enzymes, however, the effect of amino-acids on kidney enzyme was almost

negligible, while their strong activating influence was still persistent with the intestinal enzyme. This suggests that amino-acids exert their effect through certain groups in the coenzyme, which are easily split from the kidney enzyme while being relatively stable in the intestinal enzyme during dialysis.

The activation of kidney phosphatase by cysteine has been similarly reported by Williams & Watson (1940), who found that cysteine in low concentrations activates the alkaline phosphatase of bone, while with higher concentrations inhibition occurs.

On the other hand, Waldschmidt-Leitz, Schaffner & Bauer (1933), Bodansky (1936*a, b*) and Thannhauser, Reichel & Grattan (1937) reported that thiol compounds such as cysteine decrease the phosphatase activity.

With the dialyzed faecal phosphatase preparation in Table 6 both cysteine and cystine failed to reactivate the enzyme, while other amino-acids did so, especially histidine whose effect was marked up to a concentration of 0.002*M*. Bodansky (1948) has recently reported the inhibitory effect of L-histidine on the activity of intestinal, bone and kidney phosphatases using higher concentrations (0.0064*M*) than used here. He also pointed out that the basic amino-acids, L-histidine and L-lysine exert a greater inhibitory effect on rat-bone and kidney phosphatases than on intestinal phosphatase, whereas the reverse holds for the dicarboxylic L-glutamic acid.

The effect of formaldehyde on the different alkaline phosphatases provides a further distinction between kidney phosphatase and the intestinal and faecal enzymes. This phenomenon was previously noticed by Gould (1944). Bodansky (1937) also showed a difference between the intestinal phosphatase and other tissue phosphatases in connexion with their behaviour towards bile salts. The intestinal enzyme is less sensitive towards this inhibitor than the other phosphatases. It seems that the amino groups of the

apoenzyme are essential for the phosphatase activity. Attempts to reactivate the formaldehyde-inhibited enzyme by addition of ammonium salts, amino-acids or cathode liquid were unsuccessful.

Owing to the fact that the kidney alkaline phosphatase has not yet been obtained in a satisfactory degree of purity, an investigation of the nature of the dialyzable coenzyme is not yet possible.

SUMMARY

1. The mechanism of enzyme inhibition during electrodialysis of faecal, intestinal, kidney and liver alkaline phosphatases has been studied.

2. The migration of a substance with strong reactivating properties towards the cathode during dialysis of the different phosphatases was most clearly demonstrable with the kidney enzyme. The properties of this substance have been investigated and compared with certain other substances.

3. No evidence in favour of the two-metal theory involving zinc could be obtained.

4. The presence of three main components seems to be indispensable for the normal alkaline phosphomonoesterase activity: a specific protein apoenzyme; an organic dialyzable coenzyme; and magnesium.

5. The activation of the enzyme by magnesium, and possibly certain other metals and amino-acids, takes place only in the presence of the dialyzable coenzyme.

6. The differences among the alkaline phosphatases of different origins are probably due to different affinities between the apoenzyme and its coenzyme, and possibly to slight variations in the nature of the apoenzyme in different phosphatases.

7. The apoenzyme appears to contain amino groups, which are essential for the phosphatase activity.

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