ON THE PURIFICATION OF THE ALKALINE PHOSPHATASE OF SWINE KIDNEYS

by

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The procedure of ALBERS AND ALBERS[1] is the method of choice for preparation of alkaline phosphatase from swine kidneys. The principles utilized are autolysis of kidneys in 25% alcohol, in the presence of toluene and ethyl acetate, followed by an alcohol fractionation. It has the definite advantage of being generally adequate with other tissues and species[2,3,4] and has even been employed to obtain preparations of acid phosphatase from liver[5] and mushroom[6]. Numerous minor modifications have been introduced, but the essentials of the procedure remain the same. Further purification of preparations obtained with it has also been studied[6,7,8].

Recent attempts in this laboratory to obtain preparations of alkaline phosphatase from swine kidney by the above procedure were completely unsuccessful. The following report describes these results and presents a means of applying the procedure successfully. In addition, an alternate method of preparing the phosphatase was developed, the details of which are presented.

METHODS

Tissues

Kidneys, obtained within two hours after slaughter, were trimmed of excess fat, decapsulated, and frozen. The main portion of the medulla was dissected out and discarded while the tissue was in the semifrozen state. The cortex was washed, frozen, and ground twice while in the frozen state, with the aid of a power-driven meat grinder. The ground tissue was then stored at —18° C.

Protein Nitrogen Determination

The required amount of enzyme solution (or suspension) was placed in a cellophane dialyzing bag (Visking seamless cellulose tubing, 18/32 inches inflated diameter*), tightly knotted, and dialyzed overnight against running tapwater at 5° C. The dialysis bag and contents was then dried, trimmed of excess cellophane, and its nitrogen content determined by the microdistillation Kjeldahl procedure as given by HILLER *et al.*[9]. It was necessary to modify the Kjeldahl procedure as follows: 1. because of the greater amount of organic material 2 ml of concentrated sulfuric acid was used in the digestion, 2. a bit of selenium was added to the digestion as mercury was not efficient in clearing the digests when used alone, and 3. a greater volume of concentrated sodium hydroxide was required in the distillation of the ammonia, owing to the increase in sulfuric acid mentioned above. Blanks were carried out on dialysis bags which contained water. Their nitrogen content was always quite low.

Enzyme Estimation

The procedure of KING *et al.*[10] was used with some alteration. The buffer-substrate mixture had the following composition: 0.005 *M* disodium phenyl phosphate, 0.005 *M* magnesium sulfate,

* The size of tubing used must be standardized as heavier bags with thicker walls retain more enzymatically inactive nitrogen.

References p. 395.

and 0.05 *M* carbonate-bicarbonate buffer (roughly 6:4). The digest consisted of 0.5 ml of enzyme solution (or suspension) added to 10.0 ml of the above buffer-substrate mixture. The digestion period was 15 minutes and was carried out at 25° C. Enzyme action was stopped by adding 4.5 ml of diluted phenol reagent (1:2)[11], and the tubes were kept at 5° C for 1 hour before filtering. With purified enzyme solutions, the protein content was so low that the inhibited digest could be assayed for its phenol content directly, dispensing with the cooling period and filtration. Five ml of filtrate or digest was then mixed with 5 ml of 8% sodium carbonate to develop the color. After standing 1 hour at room temperature, the optical densities were determined in the KLETT-SUMMERSON photoelectric colorimeter (red filter). The colorimeter was standardized with a series of freshly prepared dilutions of the stock standard phenol solution[12] about every two months. This calibration has remained remarkably constant, showing no significant variation in nearly two years.

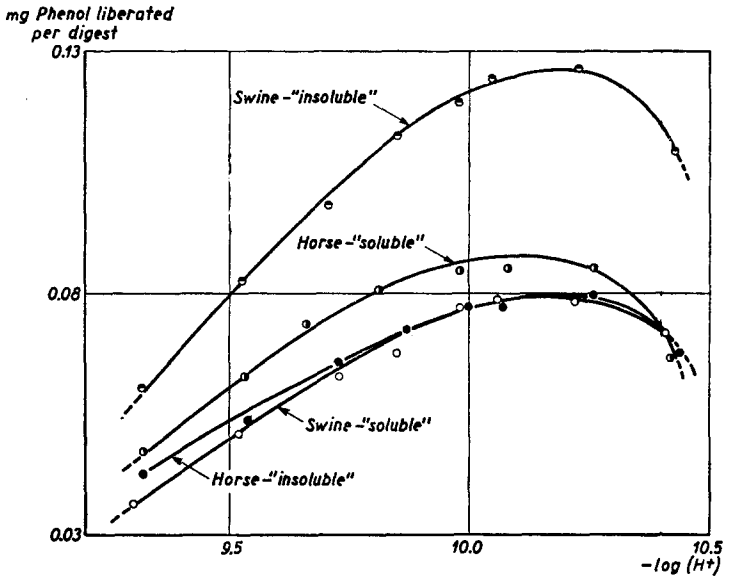

Fig. 1. The rate of hydrolysis of disodium phenylphosphate by the alkaline phosphatases of swine and horse kidney cortex at various pH values. Conditions were those of the standard assay except that the bicarbonate-carbonate ratio of the buffer mixture was varied. "Insoluble" indicates that a homogenate of fresh tissue was the enzyme source, while "soluble" indicates that a purified, filtrable preparation was used. The total activities are relative only.

The pH of the digest was 10.10 ± 0.05, and its buffer capacity was such that, under our conditions, the total pH shift on adding the enzyme solution and carrying out the digestion, was never more than 0.03. All pH determinations were made at 25° C using the Beckman pH meter. Sodium ion corrections for the glass electrode were applied where required.

It is felt that a point of convenience in the use of the carbonate-bicarbonate buffer system as recommended by DELORY AND KING[13] is that the pH shift of the buffer with temperature is such that the mixture which is recommended for use at 37° C[10], also gives optimal action at 25° C (pH 10.1). In support of this, data presented in Fig. 1 give the change in activity with change in pH at 25° C for swine and horse kidney alkaline phosphatases in both the soluble and particulate state*. The pH optimum of the enzyme shifted approximately 0.05 pH units towards the alkaline side when the enzyme was used in the particulate state. If this difference is significant, it might be due to increased alkali stability of the enzyme when attached to cell particulates.

The maximal hydrolysis of substrate allowed was 5%, as up to this point, at least, the rate of reaction was linear for both the enzyme concentration and the digestion time, and hence represented an estimate of initial velocity. A conversion factor for calculating activity at 37° C from activity at 25° C was determined to be 1.60. All units given in this paper are calculated for 25° C and should be multiplied by this factor to obtain units equivalent to KING *et al.*[10]. To determine the activity of the enzyme in the particulate state, samples of the tissue were homogenized in an all glass homogenizer of the POTTER-ELVEHJEM type[14], diluted and assayed in the usual manner.

* The term, particulate state, indicates that no treatment such as autolysis or proteolysis has been used to render the phosphatase soluble.

References p. 395.

RESULTS

The results given in Table I were obtained with sheep and horse kidney cortex, and are typical of the initial portion of the ALBERS procedure. The enzyme was rendered soluble to a considerable extent, and extensive purification resulted from the precipitation of a main part of the contaminating proteins. There is an apparent activity increase resulting from the shaking which has been obtained consistently during the progress of this investigation.

TABLE I

THE PROCEDURE OF H. AND E. ALBERS[1]

Kidney source	Sheep		Horse	
	TPKU*	PU/mg PN*	TPKU*	PU/mg PN*
1. 1 kg ground cortex, 1 liter 50% alcohol and 100 ml 1:1 toluene-ethyl acetate were mixed Suspension	29.9	1.50	19.9	—
2. Agitated for 4 days at room temperature Suspension	42.3	2.28	24.2	1.14
3. Filtered suspension and washed filtercake Combined filtrates	22.9	39.8	16.3	33.3
4. Enzyme precipitated with alcohol Precipitate	16.2	—	9.7	—

* TPKU = total phosphatase units $\cdot 10^{-3}$; PU/mg PN = phosphatase units per mg of protein nitrogen.

When applying the ALBERS procedure to swine kidney cortex, the results given in Table II-A were obtained. Obviously, little or no soluble enzyme was produced by the autolysis. Numerous modifications of the method were tried with similar results. Redistilling solvents, varying the relative and absolute amounts of toluene and ethyl acetate, or the amount of alcohol used, made little difference. Successful preparations with sheep kidney (Table I) and horse kidney (Table I and II-B) made it appear that the difficulty might be traced to the source of tissue.

Among possible causes for the difference, are the lack of a necessary autolytic enzyme in the swine tissue, or the presence of an interfering substance of some type. It was thought that autolysis of a mixture of horse and swine kidney would be interesting. If the first postulate were true, the missing factor might be supplied with the added horse kidney. The results (Table II-C) of this experiment can only be indicative, but they do suggest that the horse kidney phosphatase was purified in the usual manner, in the presence of swine kidney enzyme which remained insoluble. It would seem that a deficiency in autolytic enzymes was not the difficulty. As for an inhibitor, if present, it must have been circumscribed in its action, as the solubilization of the horse kidney enzyme appeared to be unaffected.

A characteristic of swine tissue is the presence of a high content of lipid. Inasmuch as excessive fat might interfere with the isolation, a defatted, dehydrated kidney preparation (Viobin Co.) was used in the procedure (Table III) and adequate solubilization was obtained. Since the activity of this material was low, indicating the possibility of rigorous treatment in preparation, some swine kidney powder was prepared

References p. 395.

TABLE II

MIXED TISSUE ALBERS PREPARATION

	A	B	C
Swine kidney (g)	100		50
Horse kidney (g)		100	50
50% alcohol (ml)	100	100	100
1:1 PhMe–EtOAc (ml)	10	10	10
	Relative Enzyme Activity		
Suspension — initial	373	257	326
Suspension — 3 days' shaking*	355	297	378
Filtrate — 3 days' shaking	7	313	173

* At room temperature.

TABLE III

USE OF TREATED SWINE KIDNEY PREPARATIONS IN THE ALBERS PROCEDURE

Kidney	"Viobin"*	"Extracted"**	
	PU/ml	PU/ml	PU/mg PN
1. 20 g powder, 80 ml water, 100 ml 50% alcohol and 10 ml 1:1 toluene-ethyl acetate were mixed Suspension	4.02	10.9	1.18
2. Agitated 3–4 days at room temperature Suspension	5.14	15.2	1.66
3. Filtered Filtrate	4.21	12.3	60.4

* Dehydrated, defatted kidney powder obtained from the Viobin Corp., Monticello, Ill., U.S.A.
** Ground kidney cortex powder obtained by treatment with alcohol, ether and acetone at room temperature or lower.

by extraction with organic solvents under mild conditions and tested in the preparation. Again, a thoroughly satisfactory preparation resulted, as is seen in Table III. It would seem from this that the presence of fatty material in excessive amounts was the factor interfering in the isolation procedure, however, other effects of the organic solvents on kidney components have not been ruled out.

An alternate procedure was sought, in part to avoid the above difficulty, and more particularly, to afford an alternate means of preparing the enzyme, a consideration of importance in further work on alkaline phosphatase contemplated in this laboratory. At first, it was thought that the procedure of PERLMANN AND FERRY[15] could be applied to advantage, to obtain purified preparations of both the acid and the alkaline phosphatases. Attempts in this direction only confirmed the conclusions of SARLES[16] that this procedure was not well adapted for purification purposes. PERLMANN AND FERRY used a *clear* extract of beef kidney, obtained by centrifugation of a saline extract, as the starting material in their separation. It is known that alkaline phosphatase is firmly

References p. 395.

bound to cellular structures[17,18] and obtaining the enzyme in the soluble form is one of the greatest initial barriers to any scheme for the purification of the enzyme[19]. If the beef kidney extract had been centrifuged to clearness, it should have lost practically all of its alkaline phosphatase activity to the sediment, unless the phosphatase of beef kidneys is unlike that of other species. It appears possible that PERLMAN AND FERRY were dealing with renal alkaline phosphatase that was still attached to the cell particulates.

Bile salts have been reported as having the property of solubilizing enzymes. Thus, cytochrome oxidase[20] and leucocyte alkaline phosphatase[21] have been reported to be solubilized in this manner. The possibility that this property could be of aid in enzyme extraction for purposes of purification was tested. When swine kidney was homogenized in the presence of bile salt, the phosphatase did not sediment at 15,000 R.P.M. as it did in controls without added bile salt. It thus appeared that solubilization had been obtained. However, filtration of such bile salt treated homogenates through filter paper with the addition of filter-aid gave a filtrate with only slight activity. It appears that in this case bile salts did not yield a true solution of enzyme, but rather increased the stability of the dispersion.

The procedure finally adopted involved the use of proteolysis to give a true solution of the enzyme (Table IV). The essential steps of the procedure are:

1. *Pancreatin solubilization.* The use of pancreatic enzymes in bringing alkaline phosphatase into solution, while simultaneously digesting contaminating proteins, was introduced by EHRENSVARD[22], though suggested by the work of HORI[23], and has been applied in various forms since then by numerous other investigators[24,25,26,27]. The alkaline phosphatase content of the pancreatin used in this investigation was so low that there was no possibility of significant contamination of the kidney enzyme. The conditions of the digestion were studied by varying the amount of pancreatin, digestion time, and digestion p_H. Those adopted here represent the best compromise. Highly active pancreatin results in greater digestion and causes excessive enzyme destruction, in addition to rendering the initial filtration quite difficult. Reduction in the amount of pancreatin and shortening the digestion period may be of aid in such cases. Efforts to improve this stage of the preparation are in progress, as it will be noted from Table IV that complete solution of the enzyme has not been obtained. The filtration of the pancreatin digest should be carried out on the largest Buchner funnels available (32 cm diameter or greater) taking care not to overload the funnel.

2. *Ammonium sulfate fractionation.* Ammonium sulfate was used at this stage of the purification to avoid handling the large volumes of liquid entailed by organic solvent fractionation. The final enzyme-containing filter cake is sucked as free of mother liquor as possible to avoid excessive contamination of the subsequent alcohol fractionation step with ammonium sulfate. However, this cake should not be dried, for extraction then becomes difficult, presumably due to the presence of contaminating lipids.

3. *Alcohol fractionation.* Considerable purification is obtained with alcohol by virtue of the fact that fractional precipitation, and denaturation of contaminating proteins by alcohol at room temperature, occur simultaneously. All alcohol additions were made with rapid stirring while adding the alcohol slowly through a capillary. Sodium bicarbonate is required in the extract used in this fractionation in order to prevent *reversible* inactivation of the enzyme, presumably due to excessive acidity. In general, during the course of this investigation, it was found necessary to maintain p_H values of the enzyme solutions obtained on the alkaline side of p_H 7 to prevent *reversible* loss of activity.

References p. 395.

TABLE IV

AN ALTERNATE PROCEDURE FOR PREPARING ALKALINE PHOSPHATASE FROM SWINE KIDNEYS

	PU/ml	TPKU	PU/mg PN
1. 2 kg ground swine kidney cortex, 2 liters water, 100 ml toluene and 40 g pancreatin were mixed. To p_H 8.0 with 20% Na_2CO_3 Suspension	10.9	45.8	1.33
2. Digested for one week readjusting p_H when required. The digest temperature was 25° C Suspension	9.46	40.7	3.1
3. Added 200 g Hyflo- and 200 g Standard Super cel. Filtered with a large Buchner. Washed cake with 1 liter water Filtrate	6.83	24.1	7.5
4. Added 25 g $(NH_4)_2SO_4$ per 100 ml filtrate; dissolved with slow stirring over night at 5° C. Added 50 g Hyflo- and 50 g Standard Super cel; filtered on a 32 cm Buchner. Washed cake with 200 ml of water containing 50 g $(NH_4)_2SO_4$ Combined filtrates	6.72	26.2	11.4
5. Added 25 g $(NH_4)_2SO_4$ per 100 ml of filtrate and dissolved as above. Added 30 g Hyflo- and 30 g Standard Super cel and filtered on a 32 cm Buchner; washed cake with 200 ml water containing 100 g $(NH_4)_2SO_4$. Sucked cake quite free of mother liquor. Discarded filtrate. Stirred cake with 400 ml 0.05 *M* $NaHCO_3$ for 30 minutes. Filtered with a Buchner. Washed cake with 2 consecutive washes of 200 ml H_2O each Combined filtrates	28.1	22.5	13.8
6. Added 40 ml of 95% ethyl alcohol per 60 ml of filtrate through a capillary with good stirring (room temperature). Let stand over night. Added 30 g Hyflo- and 30 g Standard Super cel and filtered on a 17.5 cm Buchner. Washed cake with 200 ml alcohol (4:6 95% alcohol-water) Combined filtrates	15.9	21.3	44.2
7. Added 80 ml 95% alcohol to every 100 ml of above filtrate as above, but at 5° C. Added 10 g Hyflo-and 10 g Standard Super cel; filtered on a 17.5 cm Buchner. Stirred cake with 200 ml 0.05 *M* $NaHCO_4$ 30 minutes at 5° C, filtered with suction, and washed cake twice with 100 ml water per wash Combined filtrates	52.2	20.1	118
8. Placed filtrates in a large cellophane dialyzing bag and dialyzed against two changes of 8–10 liter approx. 0.01 *M* $NaHCO_4$ for 48 hours (at 5° C) with toluene in the dialyzing bag Solution	40.4	19.2	122
9. Added 2 vol cold acetone in the manner of the alcohol additions (5° C). Filtered with suction (with 5 g aid if desired) and washed cake with cold acetone and then ether. Dryed *in vacuo* Cake	—	18.8	130

4. *Dialysis.* This procedure removed ammonium sulfate carried along from the first fractionation. In addition, a considerable amount of other low molecular weight nitrogen-containing impurities were removed. Quantitative enzyme yields were obtained even after dialysis for 4 days, provided the dialysis water was maintained near neutrality by the addition of sodium bicarbonate.

Attempts at further purification of this dialyzed solution by fractionation at 5° C with saturated ammonium sulfate, alcohol or acetone, afforded only slight increase in activity. The potency of the best preparation obtained in these attempts was 169 PU/mgPN, consequently, it appeared that further purification of this solution by conventional means was not promising. This could be due to the low protein content of

References p. 395.

these solutions, as NORTHROP has emphasized the need for high protein concentration in purification work of this type[28]. Hence, the enzyme was precipitated by 66% acetone and, when desired, was preserved for further use as the dry preparation. The concentration of acetone used is higher than that which gave precipitation of ALBERS preparations[8], possibly owing to the high protein dilution, or altered precipitation properties resulting from differences in the method of preparation. Solutions containing around 2% protein or more can readily be obtained by extracting these dry preparations with dilute sodium bicarbonate. In practice, it has been found of advantage to prepare the concentrated bicarbonate extract, immediately after the preparation of the semidry powder. This solution when stored at —18° C contains all of the enzyme and is remarkably stable. The enzyme yield was found to be somewhat poorer if the powder was stored.

DISCUSSION

No attempts to crystallize the preparations obtained in this investigation have been made as it is felt that further purification is still necessary. Carbohydrate is still present, as indicated by the MOLISCH test, and this is always indicative of potential crystallization difficulties. In general, attempts at crystallization of the alkaline phosphatases as derived from kidneys, feces and intestinal mucosa have been singularly unsuccessful. A reported preparation of crystalline alkaline phosphatase[7] has recently been shown to consist of crystalline magnesium salts mixed with enzymatically active, but amorphous protein[8]. ALBERS also reported obtaining such inorganic crystalline salts in his concentrated preparations[29].

Aside from insufficient purity, a possible explanation for the lack of success in crystallization of the phosphatases might be that the enzyme isolated is not an entity, but a mixture of several closely related but not entirely similar phosphate ester hydrolyzing enzymes, *i.e.*, a family of enzymes[30].

It appears that there may exist fundamental differences between the usual order of crystalline enzymes and the alkaline phosphatases. Possibly the nature of the enzyme under consideration is such that it could not exist in the crystalline state. In the main, most of the crystalline enzymes appear to belong to the class sometimes termed "lyo-enzymes" *i.e.*, freely soluble and easily extractable from the cells which contain them. Alkaline phosphatase, on the other hand, is difficult to separate from cellular residues. The enzyme is liberated from cellular residues by autolysis, or proteolysis, hence, the linkage between phosphatase and cell-structure might be of a peptide nature. It is possible that this bond could be ruptured in a random manner, *i.e.*, at differing peptide linkages, and thus result in molecular heterogeneity, which, if great enough, might inhibit crystallization, if not render it impossible. Molecular weight is another property in which there exists the possibility of differences between the usual crystalline enzymes and alkaline phosphatase. ALBERS[29] reported a molecular weight of 6 to 10 thousand for renal alkaline phosphatase, and regarded these figures as upper limits. This is a lower value than that usually obtained for the crystalline enzymes. Variable permeability of the glomerular membrane for serum, fecal and renal phosphatase preparations is of possible interest in this regard[31]. Reports of the passage of the enzyme through collodion membranes may be found in the literature[29].

It will be recalled that the shaking involved in the ALBERS procedure for the preparation of alkaline phosphatase resulted in increased activity in this investigation. ALBERS

References p. 395.

did not report such an increase possibly because he did not determine initial tissue enzyme activity. The increased activity has not been previously cited to the best of our knowledge, and has not been further studied here. However, it may have significance in the determination of alkaline phosphatase activities in homogenates. The possibility of increased activity after solubilization might render the use of the whole homogenate less secure from a quantitative standpoint. Such determinations have been advanced by MOOG[32], STAFFORD, MCSHAN AND MEYER[33], DRABKIN[34] and others.

ALBERS[29] did, however, report an increase of up to 4 fold in apparent enzyme activity on precipitation of the phosphatase from the crude autolysate followed by resolution of the precipitate. He postulated the presence of an inhibitor in the autolysate which was separated from the enzyme during the precipitation process. During many trials, we did not observe similar results (Table I), possibly because the method of estimating activity differs from that in ALBERS studies.

Lack of success when the ALBERS procedure was used with swine kidney tissue, if due to the cause postulated, is probably dependent upon the manner of feeding and possibly the strain of animal, and hence would not necessarily be universal in appearance. However, the possibility of the appearance of such an interference in almost any tissue or species should be kept in mind. For example, numerous assays for alkaline phosphatase in the tissues of animals under various experimental conditions have been carried out using filtered autolysates. If the experimental conditions were such that excessive fatty deposition occurred in the tissue under investigation, there would always exist the possibility of obtaining low assay values due to incomplete autolytic liberation of the phosphatase.

SUMMARY

Recent attempts in this laboratory to obtain purified alkaline phosphatase from swine kidney cortex, using the procedure of ALBERS AND ALBERS were singularly unsuccessful, although the procedure readily gave purified preparations from sheep and horse kidneys. Varying the conditions of the autolysis were without effect on this result. Autolysis of a 1:1 mixture of swine and horse kidney cortex gave results indicating that the enzyme from horse kidney was solubilized and purified at the same time that there was no effect on the enzyme of swine kidney. Further investigation showed that organic solvent extraction removed or destroyed the interference present in fresh swine kidneys. From this it seems likely that an excess of fatty material in the swine kidneys was interfering with the isolation, although other organic solvent effects have not been ruled out.

A procedure was developed for the isolation of alkaline phosphatase from fresh swine kidney cortex which avoided the difficulty mentioned above. Principles employed were pancreatin solubilization, ammonium sulfate fractionation, alcohol fractionation and selective denaturation, followed by dialysis. Specific activity of the material obtained is comparable with that of other kidney alkaline phosphatase preparations reported in the literature.

RÉSUMÉ

Les tentatives récentes dans ce laboratoire pour obtenir de la phosphatase alcaline purifiée de l'écorce rénale de porc, par le procédé d'ALBERS ET ALBERS, étaient singulièrement privées de succès, quoique le procédé ait donné effectivement des préparations purifiées de rein de mouton et de cheval. Les variations des conditions de l'autolyse ne modifiaient en rien ce résultat. L'autolyse d'un mélange à 1:1 de l'écorce rénale de porc et de cheval donna des résultats indiquant que l'enzyme de rein de cheval était solubilisé et purifié en même temps que l'enzyme de rein de porc ne subissait aucu effet. Des recherches plus poussées montrèrent que l'extraction par un solvent organique éliminait, ou détruisait l'interférence présente dans les reins frais de porc. Il paraît donc qu'un excès de matière grasse dans les reins de porc entravait l'isolation, quoique d'autres effets du solvant organique ne soient pas exclus.

Nous avons développé un procédé pour l'isolation de phosphatases alcaline de l'écorce rénale fraîche de porc, procédé qui évitait les inconvénients mentionés ci-dessus. Les principes utilisés

References p. 395.

étaient la solubilisation par la pancréatine, le fractionnement au sulfate d'ammonium, le fractionnement à l'alcool et la dénaturation sélective, suivies de dialyse. L'activité spécifique de la matière obtenue est comparable à celle d'autres préparations de phosphatase alcaline rénale rapportées dans la littérature.

ZUSAMMENFASSUNG

Versuche welche vor kurzem in diesem Laboratorium unternommen worden waren, um mit Hilfe des Verfahrens von ALBERS UND ALBERS gereinigte alkalische Phosphatase aus Schweinenierenrinde zu erhalten, blieben merkwürdig erfolglos, obwohl das Verfahren leicht aus Schaf- und Pferdenieren gereinigte Präparate lieferte. Veränderung der Autolyse-Bedingungen hatte keinen Einfluss auf dieses Ergebnis. Die Resultate der Autolyse einer 1:1-Mischung von Schweine- und Pferdenierenrinde weisen darauf hin, dass das Pferdenieren-Enzym in Lösung gebracht und gereinigt wurde während auf das Schweinenieren-Enzym keine Wirkung ausgeübt wurde. Nähere Untersuchungen zeigten, dass die in den frischen Schweinenieren vorkommende Störung durch Extraktion mit organischen Lösungsmitteln entfernt oder vernichtet wurde. Es scheint also, dass ein Überschuss an Fettstoffen in den Schweinenieren die Isolierung störte, obwohl andere Wirkungen der organischen Lösungsmittel nicht ausgeschlossen sind.

Es wurde ein Verfahren zur Isolierung von alkalischer Phosphatase aus frischer Schweinenierenrinde entwickelt, welches die obenerwähnten Schwierigkeiten umgeht. Die Methoden welche hierbei Anwendung fanden waren: löslich machen mit Hilfe von Pankreatin, Fraktionierung mit Alkohol und selektive Denaturierung, gefolgt durch Dialyse. Die spezifische Aktivität des erhaltenen Materials ist vergleichbar mit derjenigen von anderen, in der Literatur erwähnten Präparaten von alkalischer Nierenphosphatase.

REFERENCES

1 H. ALBERS AND E. ALBERS, *Z. physiol. Chem.*, 232 (1935) 189.
2 D. ALBERS, *Z. physiol. Chem.*, 265 (1940) 129.
3 H. VON EULER AND A. FONO, *Arkiv Kemi, Mineral. Geol.*, 25A (1948) No. 23.
4 B. EK, H. V. EULER, AND L. HAHN, *Arkiv Kemi, Mineral. Geol.*, 25B (1948) No. 3.
5 A. RUFFO, *Boll. soc. ital. biol. sper.*, 18 (1943) 171.
6 S. BOUCHILLOUX, Y. DEERIEN, J. ROCHE, AND M. ROGER, *Bull. soc. chim. biol.*, 30 (1948) 417.
7 N.-V. THOAI, J. ROCHE, AND L. SARTORI, *Compt. rend. soc. biol.*, 138 (1944) 47.
8 M. A. M. ABUL-FADL, E. J. KING, J. ROCHE, AND N.-V. THOAI, *Biochem. J.*, 44 (1949) 428.
9 A. HILLER, J. PLAZIN, AND D. D. VAN SLYKE, *J. Biol. Chem.*, 176 (1948) 1401.
10 E. J. KING, G. A. D. HASLEWOOD, G. E. DELORY, AND D. BEALL, *Lancet*, I (1942) 207.
11 O. FOLIN AND Y. CIOCALTEU, *J. Biol. Chem.*, 73 (1927) 627.
12 E. J. KING AND A. R. ARMSTRONG, *Can. Med. Assoc. J.*, 31 (1934) 376.
13 G. E. DELORY AND E. J. KING, *Biochem. J.*, 39 (1945) 245.
14 V. R. POTTER AND C. A. ELVEHJEM, *J. Biol. Chem.*, 114 (1936) 495.
15 G. E. PERLMANN AND R. M. FERRY, *J. Biol. Chem.*, 142 (1942) 513.
16 H. SARLES, *Compt. rend. soc. biol.*, 141 (1947) 1071.
17 E. A. KABAT, *Science*, 93 (1941) 43.
18 F. MOOG AND H. B. STEINBACH, *J. Cellular Comp. Physiol.*, 28 (1946) 209.
19 H. ERDTMAN, *Z. physiol. Chem.*, 172 (1927) 182.
20 W. W. WAINIO, S. J. COOPERSTEIN, S. KOLLEN, AND B. EICHEL, *J. Biol. Chem.*, 173 (1948) 145.
21 D. M. CRAM AND R. J. ROSSITER, *Can. J. Research*, E, 27 (1949) 290.
22 G. EHRENSVARD, *Z. physiol. Chem.*, 217 (1933) 274.
23 W. HORI, *J. Biochem.*, 16 (1932) 433.
24 G. SCHMIDT AND S. J. THANNHAUSER, *J. Biol. Chem.*, 149 (1943) 369.
25 C. J. FISCHER AND R. O. GREEP, *Arch. Biochem.*, 16 (1948) 199.
26 M. A. M. ABUL-FADL AND E. J. KING, *Biochem. J.*, 44 (1949) 431.
27 M. A. M. ABUL-FADL AND E. J. KING, *Biochem. J.*, 44 (1949) 434.
28 J. H. NORTHROP, M. KUNITZ, AND R. M. HERRIOTT, *Crystalline Enzymes*, Columbia University Press, New York 1948.
29 H. ALBERS AND E. ALBERS, *Z. physiol. Chem.*, 232 (1935) 165.
30 P. P. COHEN AND R. W. MCGILVERY, *Ann. Rev. Biochem.*, 19 (1950) 43.
31 Y. P. CHEN, S. FREEMAN, AND A. C. IVY, *J. Biol. Chem.*, 132 (1940) 445.
32 F. MOOG, *J. Cellular Comp. Physiol.*, 28 (1946) 197.
33 R. O. STAFFORD, W. H. MCSHAN, AND R. K. MEYER, *Endocrinology*, 41 (1947) 45.
34 D. L. DRABKIN AND J. B. MARSH, *J. Biol. Chem.*, 168 (1947) 777; 171 (1947) 455.

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