Adsorption of Pancreatic Enzymes[1]

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One of the first experiments on the adsorption of enzymes is described by Vogel (1). In these experiments, an albuminous precipitate was produced by the addition of lead acetate to a solution of pepsin. The precipitate possessed the property of adsorbing pepsin, which could be removed by treatment with hydrogen sulfide and water. The adsorption of pepsin by small particles of calcium phosphate, sulphur, and cholesterol is reported by Burke (2). The separation of the three enzymes of the pancreatic juice has been effected by adsorption methods of Danilewsky (3). In a similar manner, Cohnheim (4) has studied the adsorption of salivary and pancreatic amylase.

Adsorption technique in the separation of enzymes gained considerable impetus through the investigations of Michaelis (5) and Michaelis and Ehrenreich (6), in which adsorbents possessing charges opposite to those of the adsorbates were employed. The adsorption of the enzymes was therefore regarded as a result of the opposite electrical charges of the adsorbate and the adsorbent. Thus it was established "that all substances adsorbable by kaolin must be bases, and all substances adsorbable by alumina must be acids." This conception afforded a criterion for the separation of the enzymes mentioned on the basis of their electrochemical natures (7). Willstätter (8) states that these results apply only to crude enzyme solutions which contain varying amounts of foreign substances. From this investigation and others (9, 10), Willstätter concludes that the nature of the charge of the adsorbent does not necessarily define the nature of the enzyme which is adsorbed, but that the nature of the foreign substance accompanying the enzyme is a controlling factor in the adsorption of specific enzymes by certain adsorbents. Accordingly, an enzyme is considered to consist of an active catalyst and an inert carrier which is colloidal in nature. The carrier is considered to be a substance of high molecular weight, a protein or carbohydrate, and can be separated from the active enzyme and be replaced by another suitable carrier. This so-called carrier theory of the behavior of enzymes with respect to their separation by adsorption methods is substantiated by investigations reported by Fodor (11). According to recent investigations by Dyckerhof and Tawes (12) and Waldschmidt-Leitz and Kofranyl (13), the carrier of pepsin can be replaced by other proteins. Tauber and Kleiner (14), however, point out that the protein carrier of rennin cannot be exchanged for another. Northrup (15) and Sumner (16) show that the decrease in peptic activity is determined by the adsorption of pepsin protein.

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The protein nature of enzymes has been indicated by the work of Tauber and Kleiner (18, 19), in which it was found that rennin was digested with trypsin and pepsin, pepsin with trypsin, trypsin with pepsin, and salivary amylase with trypsin and papain. It was also found by Falk (20) that lipase is digested with trypsin.

The hydrogen-ion activity of the solution of the enzyme substrate affects the adsorption of the enzyme. It is found that the most efficient adsorption, with the least destruction of the enzyme, is at a specific pH value for each enzyme. The factors upon which the pH optimum depends are: the nature of the enzyme preparation, the rate of hydrolysis of the enzyme (21), the nature of the buffer employed to regulate the pH, and the nature of the substrate. The optimum pH for the digestion of olive oil substrate with lipase in the presence of ammonia-ammonium chloride buffer is 9.2, and for the digestion of trybutyrin substrate with lipsase under the same conditions it is 8.3 (22). According to Kunitz and Northrop (23), the optimum for digestion of casein with purified trypsin is 8 to 9, while crude trypsin will digest casein at a pH of about 7.8. The pH for the adsorption of pancreatic amylase is optimum at about 7.2 (24).

In an effort to isolate a pure enzyme, Willstätter and his associates (25) succeeded in preparing very active concentrates of enzymes brought about by preferential adsorption followed by elution to free the adsorbed enzyme from the adsorbent. The maximum adsorption generally occurred at a definite hydrogen-ion activity and the most efficient elution at another. By this technique, these investigators succeeded in splitting some enzyme complexes into their components.

The purpose of this investigation was to study the adsorption of the enzymes, lipase, trypsin, and amylase, from solutions prepared from desiccated pancreatic extract. From these studies it was possible to prescribe an adsorption treatment for this mixture so that any two of the enzymes could be removed by adsorption on certain adsorbents, leaving any one of the enzymes in the "mother liquor" free from the other two. The behavior of the enzyme so isolated was then studied and compared with its behavior before treatment, that is, in the presence of the other two enzymes.

Proper adjustment of this pH, temperature control, and the use of solutions of bile salts in the digestion mixture were employed to furnish a more nearly natural environment.

Experimental

Materials.—The source of enzymes was desiccated pancreatic extract which contained the three enzymes, lipase, trypsin, and amylase. Pancreatic extracts were the Triple Strength Pancreatin furnished through the courtesy of the Eli Lilly and Company of Indianapolis and Armour and Company of Chicago. Both of these materials gave comparable results.

The adsorbents and their sources or methods of preparation were as follows:

Aluminum oxide on asbestos support was prepared by adding 250 ml. of 3 molar ammonium hydroxide to 250 ml. of aluminum sulfate in the presence of 10 g. of asbestos fiber. The hydrous aluminum oxide was thus precipitated on the asbestos fiber. This mass was dried at 100°C. and activated at 200°C. for 24 hours.

Hydrous aluminum oxide gel was prepared in the same manner as the above, but in the absence of the asbestos fiber. The gel was dried and activated at 250°C.

Lloyd's Reagent was obtained from E. H. Sargent and Company of Chicago, Illinois.

Animal charcoal was purchased from Diagger and Company, Chicago, Illinois.

Silica gel was grade 150-F-1000-3060, obtained from the Davison Chemical Corporation, Silica Gel Division, Baltimore, Maryland.

Cholesterol was obtained from the Pfanstiehl Chemical Company, Waukegan, Illinois.

Carborundum was supplied by the Carbon and Carbide Corporation, Niagara Falls, New York.

Permutite was purchased from the Chicago Apparatus Company, Chicago, Illinois.

Bone charcoal was purchased from the Diagger and Company, Chicago, Illinois.

Magnesium silicate was made by diluting sodium silicate with distilled water to a specific gravity of 1.06 and adding a molar solution of magnesium chloride to the silicate as long as the precipitation occurred. The precipitate was washed free of chlorides, dried in an oven at 100°C., and activated at 200°C. for 24 hours.

Graphite, the fine flaky grade, was purchased from the Chicago Apparatus Company, Chicago, Illinois.

Clay (bauxite) was prepared by activating bauxite at 200°C. for 24 hours. The bauxite was obtained from the Chicago Apparatus Company, Chicago, Illinois.

Kaolin (colloidal) was obtained from Merck and Company, Rahway, New Jersey.

Palladium black was bought from E. H. Sargent and Company, Chicago, Illinois.

The *egg albumen* used was an imported product from China.

Coconut charcoal was obtained from Eimer and Amend Company, New York.

Ferric oxide was obtained from the Chicago Apparatus Company, Chicago, Illinois.

Activated alumina was grade A., 14-20 mesh from the Alcoa Ore Company, East St. Louis, Illinois.

Blood charcoal was bought from Diagger and Company, Chicago, Illinois.

Pumice stone was prepared by treating with concentrated hydrochloric acid, washing with water, and activating for 4 hours at 250°C.

Hydrous aluminum oxide gel in alcohol was prepared in the same manner as the hydrous aluminum oxide gel mentioned above. Alcohol was added at the time of adsorption.

Procedure.—The technique for the adsorption of the enzymes was as follows: Ten grams of the triple strength pancreatin was suspended in 500 ml. of distilled water, to which had been added sodium carbonate and hydrochloric acid to produce a pH of 7.4. The mixture was allowed to stand for 12 hours and then centrifuged for 20 minutes at 2,000 r.p.m. To 100 ml. of the solution after centrifugation was added 10 g. of an adsorbent and the mixture was incubated at 37°C. for 24 hours. The mixture was then centrifuged for 20 minutes at 2,000 r.p.m., and the clear solution was used for determining the enzyme or enzymes that were removed by the adsorbent. A negative test for an enzyme in this clear solution was taken as the indication that it had been removed by the adsorbent.

Tests for lipase were made with olive oil as the substrate in the following manner: To 2 ml. of olive oil was added 15 ml. of the solution after treatment with the adsorbent and the mixture adjusted to a pH of 8 and incubated at 37°C. for 24 hours. The presence of lipolytic activity was indicated by the production of fatty acids as measured by 0.1 N. potassium hydroxide solution required to adjust the pH back to 8.

The presence of amylase in the enzyme solutions after treatment with the adsorbents was detected with the use of starch as the substrate. Five ml. of the solution was added to 10 ml. of a 1% starch solution, and the mixture was adjusted to a pH of 7.2 and incubated at 37°C. for 24 hours. Amylase activity was indicated by tests with iodine, fuchsin, and Fehling's solution which detected the presence or absence of hydrolytic products of the starch.

A solution of casein in normal sodium acetate solution was used as the substrate in the detection of trypsin. Two ml. of the enzyme solution, after treatment with the adsorbents, was added to 10 ml. of the casein solution and the mixture adjusted to a pH of 8 and incubated at 37°C. for 6 hours. The absence of trypsin in the solution was indicated by the production of a white precipitate upon the addition of a few drops of acetic acid.

In order to improve the tests for the three enzymes in the solutions, a few drops of bile salt were added to the digestion mixtures. The bile salt produced a more natural environment for the enzymes and, as a result, increased their respective activities. This proved especially valuable in the lipase tests.

Data and Results.—The data in Table I show the adsorption of the enzymes by the various adsorbents studied. The enzyme adsorbed is indicated by the minus sign (—), which indicates that the enzyme was not present in the solution remaining after treatment with the adsorbent. A positive sign (+) signifies the presence of hydrolytic products in the substrate and indicates that the enzyme was not removed by treatment with the adsorbent.

In general, it is noticed that the aluminum-containing adsorbents manifest a selective adsorbtive capacity for lipase and trypsin. Likewise, the silica containing adsorbents and egg albumen, blood charcoal, and bone charcoal seem to adsorb amylase preferentially.

In Table II the adsorbents are listed according to their abilities to adsorb each of the three enzymes studied. From the table it may be seen that lipase and trypsin are adsorbed by the aluminum- and the silica-bearing compounds more readily than by any of the other adsorbents. Amylase is best adsorbed by the charcoals and egg albumen.

By virtue of the selective adsorption of the enzymes in pancreatin by the adsorbents studied, it is possible to choose certain adsorbents and devise a technique by means of which a complete separation of the three enzymes may be effected. By this means, the mixture of the three enzymes may be treated in such a manner that the solution remaining after treatment will contain only one of the active enzymes.

TABLE I.—Removal of Enzymes from Pancreatin with Various Adsorbents

Adsorbent	Enzymes		
	Lipase	Trypsin	Amylase
$Al(OH)_3$ gel in alcohol	—	—	+
$Al(OH)_3$ gel	—	—	+
Al_2O_3 on asbestos	—	—	+
Activated alumina	—	—	+
Bauxite	—	—	+
Kaolin	—	—	+
Silica gel	—	+	+
Lloyd's Reagent	—	—	+
Magnesium silicate	+	—	—
Permutite	+	—	+
Soapstone	—	+	—
Carborundum	+	—	+
Pumice	+	—	+
Palladium black	—	+	+
Animal charcoal	—	+	+
Blood charcoal	—	+	—
Bone charcoal	—	+	—
Cocoanut charcoal	—	+	+
Graphite	—	+	+
Cholesterol	—	+	+
Egg albumin	+	—	[1]

+, presence of the enzyme after the mixture has been treated with the adsorbent.

—, absence of the enzyme after the mixture has been treated with the adsorbent.

TABLE II.—The Adsorbents Which Adsorb Each Enzyme

Enzyme Adsorbed	Adsorbent
Lipase*	$Al(OH)_3$ gel in alcohol
	$Al(OH)_3$ gel
	Al_2O_3 on asbestos
	Activated alumina
	Bauxite
	Kaolin
	Silica gel
	Lloyd's Reagent
	Soapstone
	Palladium black
	Animal charcoal
	Blood charcoal
	Bone charcoal
	Cocoanut charcoal
	Graphite
	Cholesterol
Trypsin	$Al(OH)_3$ gel in alcohol
	$Al(OH)_3$ gel
	Al_2O_3 on asbestos
	Activated alumina
	Bauxite
	Kaolin
	Lloyd's Reagent
	Magnesium silicate
	Permutite
	Carborundum
	Pumice
Amylase	Magnesium silicate
	Soapstone
	Blood charcoal
	Bone charcoal
	Egg albumin

*No assay showing the quantity of lipase present in the triple strength pancreatin was available. The experiments of this investigation indicated a low content of lipase which probably accounts for the fact that so many adsorbents were found to leave the mixture free from lipase.

Table III indicates the conditions required to carry out this concentration of a single enzyme. In some instances the first treatment with a given adsorbent will remove one of the enzymes and only part of the second, which makes it necessary to treat the solution a second time with the same adsorbent to remove completely the second enzyme and produce a solution containing only one enzyme.

TABLE III.—Adsorption Technique which, when Applied to Pancreatin, Will Remove All but One of the Enzymes, Lipase, Trypsin, or Amylase

ADSORPTION METHOD LEAVING LIPASE IN SOLUTION (pH = 6-7.6)

Adsorbent	Enzyme removed	Enzyme remaining
1. Mg-silicate	trypsin, some amylase	some amylase, lipase
2. Mg-silicate	amylase	lipase
1. Permutite	trypsin	amylase, lipase
2. Mg-silicate	amylase	lipase

ADSORPTION METHOD LEAVING TRYPSIN IN SOLUTION (pH = 6-7.6)

Adsorbent	Enzyme removed	Enzyme remaining
1. Soapstone	lipase, some amylase	some amylase, trypsin
2. Soapstone	amylase	trypsin
1. Soapstone	lipase, some amylase	some amylase, trypsin
2. Egg albumin	amylase	trypsin
1. Cocoanut charcoal	lipase	amylase, trypsin
2. Egg albumin	amylase	trypsin
1. Cocoanut charcoal	lipase	amylase, trypsin
2. Bone charcoal	amylase	trypsin
1. Blood charcoal	lipase, some amylase	some amylase, trypsin
2. Blood charcoal	amylase	trypsin

ADSORPTION METHOD LEAVING AMYLASE IN SOLUTION (pH = 6-7.6)

Adsorbent	Enzyme removed	Enzyme remaining
1. Al_2O_3 on asbestos	lipase, some trypsin	some trypsin, amylase
2. Al_2O_3 on asbestos	trypsin	amylase
1. Bauxite	lipase, some trypsin	some trypsin, amylase
2. Bauxite	trypsin	amylase
1. $Al(OH)_3$ gel	lipase, some trypsin	some trypsin, amylase
2. Permutite	trypsin	amylase

TABLE IV.—Activity of Enzymes of the Pancreatic Mixture Compared with Their Activities after Isolation by means of Adsorption from the Other Enzymes

enzyme	in presence of	in absence of	substrate	enzyme solution (ml)	enzyme potency
Lipase	trypsin amylase		2 ml. olive oil	15	8.5 ml. 0.1 *N* KOH to neutralize fatty acid after 2 hours digestion
Lipase		trypsin amylase	2 ml. olive oil	15	14.2 ml. 0.1 *N* KOH to neutralize fatty acid after digestion for 2 hours
Trypsin	lipase amylase		10 ml. case in solution	2	complete digestion in 5 minutes
Trypsin		lipase amylase	10 ml. case in solution	2 2	complete digestion in 1 minute
Amylase	lipase trypsin		10 ml. starch paste	5	complete digestion in 4 minutes
Amylase		lipase trypsin	10 ml. starch paste	5	complete digestion in 0.5 minute

This difficulty may be eliminated by using double the weight of the adsorbent per unit volume of the enzyme solution.

In all instances it was found that the activity of the single enzyme remaining after the treatment of the mixture of the three with the suitable adsorption technique was greater than when it was present in the original mixture of the three enzymes. The data in Table IV indicate the activity of each of the enzymes, lipase, trypsin, and amylase, in the solutions treated with the suitable adsorbents as compared with the activity of the enzymes in the presence of the other two.

Summary

1. The following materials are reported as new adsorbents for certain enzymes: soapstone for lipase and amylase; bauxite for lipase and trypsin; graphite for lipase; carborundum for trypsin; and magnesium silicate for trypsin and amylase.

2. From studies involving adsorption of the enzymes, lipase, amylase, and trypsin, which occur in desiccated pancreatin, it was possible to develop adsorption technique by means of which any two of the enzymes may be removed from the third.

3. The potency of the enzyme thus isolated was found to be much greater than when the enzyme was in the presence of the other two.

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