DISC ELECTROPHORESIS OF TURKEY SERUM ALKALINE PHOSPHATASE ISOENZYMES USING POLYACRYLAMIDE GEL

Thesis for the Degree of M. S.
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THESIS

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ABSTRACT

DISC ELECTROPHORESIS OF TURKEY SERUM
ALKALINE PHOSPHATASE ISOENZYMES
USING POLYACRYLAMIDE GEL

By

John Richard Beck

In this study the isoenzymes of alkaline phosphatase in turkey serum were separated by disc electrophoresis using polyacrylamide gel as the supporting medium. Comparisons made by previous workers between polyacrylamide gel and several other supporting mediums used in electrophoresis have generally indicated that polyacrylamide gel will yield the greatest resolution of alkaline phosphatase isoenzymes.

The author was unable to adapt polyacrylamide gel disc electrophoresis procedures that had been described by previous workers as successful in separating the isoenzymes of avian alkaline phosphatase for use in this laboratory.

After a period of laboratory experimentation, a rapid and repeatable procedure that yielded excellent resolution of alkaline phosphatase isoenzymes was devised. This procedure involved a step-by-step determination of the following: a suitable gel system, buffer systems, staining procedure and sample preparation.

Test subjects consisted of 103 turkeys maintained for the "vibrator" condition at Michigan State University, average age at first sample was 15 months ± 2 months. The females were housed in individual bird cages and the males kept on a litter floor. A second flock of 46 female turkeys was also tested, these birds were of the Nicholas Broad Breasted White egg-laying strain. These birds were housed in two-bird cages and they were 42 weeks of age at time of sampling. Approximately one-half of the Nicholas strain and all of the "vibrator" birds received a standard turkey breeder ration. The remainder of the Nicholas strain received the same ration except it contained 10 percent dried poultry anaphage. The protein content of both rations was similar.

Blood samples were obtained from the "vibrator" flock on two occasions: one week after the birds were subjected to a forced molt, when egg production was zero, and at a later date when the hens were in peak egg production. The Nicholas strain was sampled a single time when these birds were also in peak egg production. Egg production records were kept in order to identify laying intensity. Semen was collected on three occasions and individual semen production was calculated as the average of the two most similar yields.

Thirteen distinct alkaline phosphatase bands were isolated from the serum of the turkeys used in this study.

The thirteen different bands were observed to occur in thirteen different patterns, each pattern being classified as a separate zymogram. Zymogram types II, V, VI and IX appeared only in the "vibrator" birds and zymogram types VIII and XI appeared only in the Nicholas strain. Two zymogram types, VI and X were observed in females only and zymogram type XIII was observed to be limited to the males.

Zymogram type III was observed in the force-molted non-laying "vibrator" turkeys only and types V, VI and X appeared only in the "vibrator" turkeys after they had been stimulated into peak egg production. Zymogram type XII was observed in birds classified as high intensity layers in both the force-molted and the peak egg production periods. In the Nicholas strain, zymogram types I and XI were normally associated with the birds classified as high intensity layers.

Isoenzyme banding patterns did not seem to be influenced by either the "vibrator" condition or the difference in the diet fed to the Nicholas strain.

The various levels of semen production did not seem to be associated with any particular zymogram type. However, when grouping together the two highest semen producing groups with the two highest groups of laying intensity, zymogram types IV and V were commonly observed. Under the assumption that egg-laying intensity would

represent reproductive ability in the females and semen production would represent reproductive ability in the males it may be possible to predict the potential reproductive ability in individuals displaying zymogram types IV and V as opposed to the remaining zymogram types.

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By

John Richard Beck

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INTRODUCTION

Electrophoresis can be loosely defined as the use of a minute electrical current to separate protein samples that are applied to some sort of supportative medium. In the past a wide variety of enzymes and other proteins have been separated using such things as: cellulose acetate strips, paper, agar gel, starch gel and finally, polyacrylamide gel as the supporting medium. The advantage of using polyacrylamide gel lies in its greater resolution capacity for all kinds of proteins. Unlike the other forms of supporting medium, polyacrylamide gel separates on the basis of both molecular size and electrical charge.

Interaction of the polyacrylamide gel and protein species is nonexistent. The pore size of the gel can be varied by changing the ratio of acrylamide to bisacrylamide and this allows the gel to act as a very effective molecular sieve. The bisacrylamide functions as a cross linking agent in the formation of synthetic polymers from acrylamide. The pore size can be varied to accommodate proteins of almost any molecular weight.

Another important advantage of polyacrylamide gel over other mediums is its greater ability to separate proteins

on the basis of their electrical charge. Unlike the other supporting mediums a uniform voltage gradient is attained in which all protein fractions of a sample will receive an equal amount of electrical attraction from the electrodes at all levels in the gel. Ions in the buffer function as leading, trailing and counter ions. At the onset of current the leading ion precedes the protein migrating anodally (downward). The trailing ion, moving at a slower rate anodally overtakes the protein. At the same time the counter ion migrates cathodally (upward). The combined movement of these three ions creates a uniform linear voltage gradient.

Alkaline phosphatase is a group of non-specific enzymes capable of hydrolizing a large number of primary phosphate esters. In vivo, these enzymes are associated with calcification and bone formation. The obvious role of alkaline phosphatase during egg formation has led to a variety of investigations. Through the use of electrophoretic techniques the different forms of alkaline phosphatase (akp), termed isoenzymes, have been separated and studied. Some of the earlier papers attempted to define the inheritance of akp isoenzymes in several species of fowl, however, the electrophoretic medium used was starch gel in most cases. In a comparison of agar gel, starch gel and polyacrylamide gel it was observed that the latter was capable of the greatest resolution of akp isoenzymes in chicken serum (Tamaki and Tanabe, 1970).

The onset of egg production in Japanese quail has been observed to alter the migration rate of one isoenzyme in polyacrylamide gel and the similar attainment of sexual maturity in the males caused the disappearance of one isoenzyme band. These results suggest the influence of factors other than genetics upon the composition and electrophoretic expression of akp isoenzymes. In human medicine it has long been ascertained that diseases involving bone and liver have profound effects on the isoenzymes as well as total enzyme activity in serum.

In human medicine electrophoretic assay procedures have been clinically used in the diagnosis of a variety of diseases. A similar procedure applicable to fowl might possess commercial value in the selection for higher egg production if the relation of egg formation and akp isoenzymes was made more explicit.

REVIEW OF LITERATURE

Alkaline phosphatase (orthophosphoric monoester phosphohydrolase, E.C. 3.1.3.1) is a group of non-specific enzymes capable of hydrolyzing a large number of primary phosphate esters regardless of the nature of the organic radical (Posen, 1967). The term "isoenzyme" has been used by Harris (1969) to describe different forms of an enzyme exhibiting identical or very similar enzyme activities.

In vivo, these enzymes are associated with calcification and bone formation (Pearse, 1968). It has been proposed by Bide (1970) that the increased levels of alkaline phosphatase (akp) in chicken plasma are the result of an increased food consumption stimulated by increased metabolic demand rather than being directly related to bone formation and calcification.

In an attempt to attain a background for developing a rapid and repeatable procedure for the separation of turkey serum akp isoenzymes on polyacrylamide gel many papers and books were reviewed, these papers and books are listed in the section of this thesis entitled "General References".

Considerable expertise can be gained by reviewing these publications.

In studying the literature it was found that a prodigious amount of laboratory investigation has been accomplished with preliminary studies of either serum or plasma akp and the separation of its isoenzymes. A number of things have been shown to influence the activity of akp in chicken serum and plasma. Lust and Squibb (1967), Motzok (1950) and Sanger et al. (1966) have described the effects of certain pathological conditions on total serum akp activity. Reports by Wilcox (1963), Bide (1970) and Bide and Dorward (1970) have indicated the influence of starvation upon akp activity in chicken serum. An increased plasma akp in high producing hens as compared to that of low producing hens was observed by Gutowska et al. (1943), Tanabe and Wilcox (1960) and Wilcox et al. (1962).

Svozil and Pavel (1971) reported that akp activity reached its peak in chickens just prior to egg production, sharply diminished at onset of lay and then gradually increased. Their data did not indicate that the akp activity recorded in layers could be correlated with these birds' akp activity in their pre-egg laying measurement. A correlation between enzyme activity and laying intensity was reached only at the fourth month of egg laying. Rao et al. (1969) also observed maximum enzyme activity in

chickens preceding egg production. In addition, pre-lay hens were reported to possess greater akp activity than the same hens following one year of egg production. Following selection for high akp activity, Wilcox et al. (1962) reported a heritability of .36 and estimated genetic correlation at .50 between akp level in chicken plasma and egg production. A phenotypic correlation of .24 was reported by Wilcox et al. (1963) between egg production and serum akp activity in White Leghorn hens. Auchinachie and Emslie (1934) and Stutts et al. (1957) were unable to correlate plasma akp with egg production in chickens and Common (1936) observed no correlation between plasma akp and intensity of lay. Using starch gel electrophoresis, Engh and Wilcox (1971) reported that "fast" band akp frequencies in fifteen strains of chickens selected for high egg production ranged from .29 to .96 and thereby failed to indicate any correlations between the "fast" isoenzyme and high egg production.

Electrophoresis of Japanese quail serum on polyacrylamide gel by Savage et al. (1970b) detected an increased mobility of the slowest moving band upon onset of lay. In his study the attainment of sexual maturity caused the disappearance of one isoenzyme band.

A number of different methods of separating the isoenzymes of akp have been utilized with varying success.

Deactivation of isoenzymes by: heat (Moss et al., 1972; Soto, 1972; It-Koon Tan et al., 1972), urea (Bide, 1970; Horne et al., 1969) and L-phenylalanine (Green and Antiss, 1971; Warnock, 1966; Johnson et al., 1972) have been useful in determining tissue origin.

The stability of akp appears to be influenced by the nature of the serum in which it is dissolved. Moss et al. (1972) have observed that the pH, urea concentration and protein concentration of the serum are minor factors influencing enzyme stability.

With the use of neuraminidase-treated serum, Beckman et al. (1966); Law (1967); Robinson and Pierce (1964) and Yong (1967) demonstrated that the electrophoretic mobility of the various isoenzymes is in a large part due to their sialic acid content. Dymling (1966) concluded that akp isoenzymes could not be separated on the basis of sialic acid content or molecular weight after filtration on Sephadex G-200 and treatment with neuraminidase.

The removal of sialic acid residues by neuraminidase is reported by Warnes (1972) to decrease the mobility of human liver akp isoenzyme but has no effect on enzyme activity.

The isoenzymes of alkaline phosphatase have been separated by use of Sephadex G-200 chromatography (Dunne et al., 1967; Smith et al., 1968) DEAE cellulose (anion

exchange) (Dunne et al., 1968) or electrophoresis using a variety of supporting mediums.

Electrophoretic separation of akp has been conducted on paper (Baker and Pellegrino, 1954), cellulose acetate (Kitchener et al., 1965; Korner, 1962; Bergerman and Blethen, 1972; Soto, 1972; Romel et al., 1968; Horne et al., 1969), agar gel (Demetriou and Beattie, 1971; Maeda et al., 1972; Rawston, 1971; Dymling, 1966; Haije and De Jong, 1963; Kramer, 1968; Suzuki et al., 1969; Tamaki and Tanabe, 1970; Winkelman et al., 1972), starch gel (Arfors et al., 1963; Chiandussi et al., 1962; Gahne, 1963; Green and Antiss, 1961; Wilcox 1966; Hodson et al., 1962; Keiding, 1959; Langman et al., 1966; Robson and Harris, 1965; Smithies, 1959; Taswell and Jeffers, 1963). Electrophoretic separation has also been conducted on polyacrylamide gel (Barakat et al., 1971; Brown and Manley, 1970; Epstein et al., 1967; Green et al., 1972; Johnson et al., 1972; Kaplan and Rogers, 1969; Kazuga, 1972; Savage et al., 1970b; Savage, 1972; Smith et al., 1968; Sussman and Gottlieb, 1969; Tamaki and Tanabe, 1970; Walker and Pollard, 1971).

The term "zymogram" was proposed by Hunter and Markert (1957) to refer to cellulose strips in which the location of enzymes was demonstrated by histochemical methods. This term has subsequently been used in the literature to describe any electrophoretic supporting medium that has

been specifically stained for alkaline phosphatase activity.

The fact that serum akp is subject to genetical influence has been reported in humans (Boyer, 1961; Robson and Harris, 1965; Beckman et al., 1966), sheep (Rasmussen, 1965), cattle (Gahne, 1963), Drosophilia melanogaster (Beckman and Johnson, 1964), bacteria (Garen, 1960), Japanese quail (Maeda et al., 1972; Savage et al., 1970a; Savage, 1972), pigeons (Brown and Manley, 1970), chickens (Law and Munro, 1965; Tamaki and Tanabe, 1970; Wilcox, 1963; Wilcox, 1966; Stutts et al., 1957) and in turkeys (Stevens and Garza, 1968).

The great bulk of investigations using electrophoretic methods has involved humans. The primary interest has been directed to the relation of human akp isoenzymes to pathological conditions involving liver and bone disorders. The following supporting mediums have been used in an effort to find a reliable, clinically useful, diagnostic tool for human medicine:

starch gel: Skillen et al., 1972; Rosenberg, 1958; Newton, 1967; Keiding, 1959; Hodson et al., 1962.

agar gel: Haije and De Jong, 1963; Yong, 1967.

cellulose acetate: Bergerman and Blethen, 1972.

polyacrylamide gel: Barakat et al., 1971; Connell, 1970; Epstein et al., 1967; Kaplan and Rogers, 1969; Kazuga, 1972; Smith et al., 1968.

Comparison of the different supporting mediums has suggested that polyacrylamide gels yield the greatest resolution of akp isoenzymes. Japanese quail serum has been separated by polyacrylamide gel electrophoresis into seven areas of activity by Savage et al. (1970a), and into eight bands by Savage (1972). In both studies, two to four isoenzymes were observed per sera. Separation of quail akp on agar gel yielded six different banding patterns (Maeda et al., 1972); two or three bands per sera were observed.

Savage et al. (1971) separated chicken serum akp into five distinct bands using polyacrylamide gel. Individual serum samples displayed one to four isoenzymes. The separation of chicken serum akp by starch gel electrophoresis has yielded only a single "fast" or "slow" isoenzyme per sample. The advantage of polyacrylamide gel over both starch and agar gels was reported by Tamaki and Tanabe (1970). In their study, starch and agar gel zymograms consisted of two patterns containing a single "slow" or one "slow" and one "fast" band when plasma was used. Polyacrylamide gel zymograms also consisted of two patterns which displayed either two "fast" or two "slow" bands.

The best comparisons of polyacrylamide gel with other electrophoretic supporting mediums in the separation of akp has been in papers dealing with human subjects. In their

paper comparing starch gel with polyacrylamide gel, Green et al. (1972), reported that polyacrylamide gel would separate the liver, bone and intestinal sources of akp isoenzymes. Similar separation with starch gel required heat treatment of the serum in order to differentiate the liver and bone isoenzymes.

Smith et al. (1968) also reported a better resolution of human akp isoenzymes using polyacrylamide rather than starch gels. In their comparison of serum and tissue extracts they were able to separate isoenzymes corresponding to kidney, liver, bone and intestinal sources using polyacrylamide gel but did not obtain similar separation using starch gel. In both of these papers, the polyacrylamide gel required a lesser volume of serum containing fewer units of enzyme activity than did starch gel.

Johnson et al. (1972) utilized polyacrylamide gel to separate liver, bone, bile, intestinal and placental isoenzymes and normally observed two or three isoenzymes per sera.

By use of cellulose acetate, Soto (1972) reported that three specific forms of alkaline phosphatase exist in humans: intestinal, placental and non-placental non-intestinal. Normal zymograms contained two peaks of enzyme activity when scanned colorimetrically.

OBJECTIVES

The primary objective of this study was to develop a reliable, repeatable assay procedure for turkey serum alkaline phosphatase using disc electrophoresis on polyacrylamide gel.

In the second phase of the study an attempt was made to associate alkaline phosphatase zymograms or banding patterns, with reproductive ability in turkeys.

In addition, the differences in zymogram types due to sex, breed, diet and reproductive state were investigated.

METHODS AND MATERIALS

General

Fifteen milliliter blood samples were obtained by brachial-venous puncture from 103 turkeys selected from the Michigan State University flock maintained for the "vibrator" condition (Coleman et al., 1960). Sampling was performed on two occasions: one week after a forced molt (at zero egg production) and when the birds were in peak egg production. The birds were force molted by removing the feed and water for two days and decreasing the day length to eight hours of artificial light per day. The eight hour artificial light day was maintained for two months at which time the day length was increased to fourteen hours per day. The peak egg production sample was taken six weeks after the fourteen hour artificial light day was initiated. Average age at the first sampling was 15 months $\pm$ 2 months. This "vibrator" flock was composed of 21 Broad Breasted Bronze (BBB) females, 42 Broad Breasted White (BBW) females, 16 BBB males and 24 BBW males.

A second flock of 46 BBW turkey hens was also sampled during peak egg production. These birds were of the

Nicholas egg-laying strain. Their age at sampling was 42 weeks.

"Vibrator" hens were held in individual cages and the males in a litter floor pen. The Nicholas strain was housed in two bird cages.

All "vibrator" turkeys were fed "ad libitum" a standard turkey breeder ration consisting of 17.84% protein, with 3.06% fat and 1280 kcal. metabolizable energy per pound. Twenty-three Nicholas birds were fed the above ration, the remaining 24 received a ration containing 10% dried poultry anaphage (mechanically dried poultry feces). Protein content of the latter ration was assayed at 17.79%.

Collected blood was placed in a 20 ml. glass tube, stoppered and allowed to clot. In an effort to maintain enzyme activity, blood samples were quickly placed in either an ice bath or a refrigerator.

Blood sampling was consistently performed in the early morning, approximately two hours after the lights had come on.

Clotted blood samples were centrifuged at 10°C for fifteen minutes at 2500 rpm. Serum was harvested and each individual sample placed into three one-dram bottles for storage at -20°C. Throughout the experiment the blood or serum were not subjected to room temperatures for any length of time. All serum samples were processed by polyacrylamide

gel electrophoresis less than one week after they were collected.

In a preliminary experiment, blood samples were collected in a heparinized syringe. The resultant plasma was found to be unsuitable for use in polyacrylamide gel electrophoresis.

Daily egg production records of all hens were kept. The Nicholas strain hens were handled in order to determine which birds were laying. In those cages with two laying birds, egg production was taken as an average of the total eggs produced during the 12 day period prior to taking the blood sample. Egg production per individual was classified into one of four groups: A - zero eggs, B - one to three eggs, C - four to six eggs, D - seven or more eggs.

Semen was collected on three occasions at three day intervals. A one ml. tuberculin syringe was used to measure semen volume. Individual semen production was calculated as the average of the two most similar yields. Semen production per individual was classified into one of three groups: A - 0 to .174 ml., B - .175 to .324 ml., C - .325 ml. or greater.

"Vibrator" turkeys were classified arbitrarily on the basis of how much shaking an individual exhibited (Coleman et al., 1960). Birds had been classified as: Normal, "Vibrator minus" (infrequent and mild shaking of head),

"Vibrators" (moderate shaking of head) and "Vibrator plus" (frequent and intense shaking of head).

Procedure

The procedure used to separate serum proteins was originally described by Davis (1964). A modification of that procedure by Savage (1972) was used to separate alkaline phosphatase (akp) in Japanese quail. Additional changes were made to adapt the procedure to separate Turkey akp isoenzymes as the result of much experimentation. The procedure and apparatus used in this laboratory have proven to be a rapid, repeatable method for electrophoretically separating akp isoenzymes on polyacrylamide gel. Departures from Savage's (1972) technique have served to greatly improve resolution and perhaps decrease some of the drudgery of using polyacrylamide gel electrophoresis. The procedure was as follows:

1. All working solutions were removed from the refrigerator and allowed to reach room temperature.
2. Glass electrophoresis tubes were soaked in column coat solution, shaken out and air dried overnight. The tubes were stoppered with a rubber cap that had also been soaked in the column coat solution.
3. Tubes were inserted into two 12-place gel polymerization racks and secured with adjustment

screws (Figure 1). To insure that all tubes were held perfectly vertical, the rack was levelled using a built-in spirit level.

4. One batch of separation gel was prepared, mixed with a magnetic stirrer and added to each tube in the first rack to a scratch mark 55 mm from the end of the tube. A 5 ml. syringe fitted with a 6.4 cm. X 20 g. needle was used.
5. Tubes were gently tapped with a finger to remove all air bubbles and insure a flat lower gel surface.
6. Separation gel was quickly water layered to permit polymerization and prevent meniscus formation. A 2.54 cm piece of beveled plastic tubing was slipped onto a 6.2 cm x 20 g. needle to facilitate the water layering step. A one ml. glass syringe lubricated with Corning stopcock grease was used.
7. Steps four to six were repeated for the second polymerization rack.
8. Frozen serum samples were removed from a -20°C freezer and allowed to thaw.
9. Separation gel was allowed 30 minutes to polymerize and the water layer removed with a one ml. syringe. Excess water was blotted with a cotton-tipped applicator.
10. Stacking gel was prepared and 0.2 ml. transferred

Figure 1. Polyacrylamide gel (disc) polymerization rack.

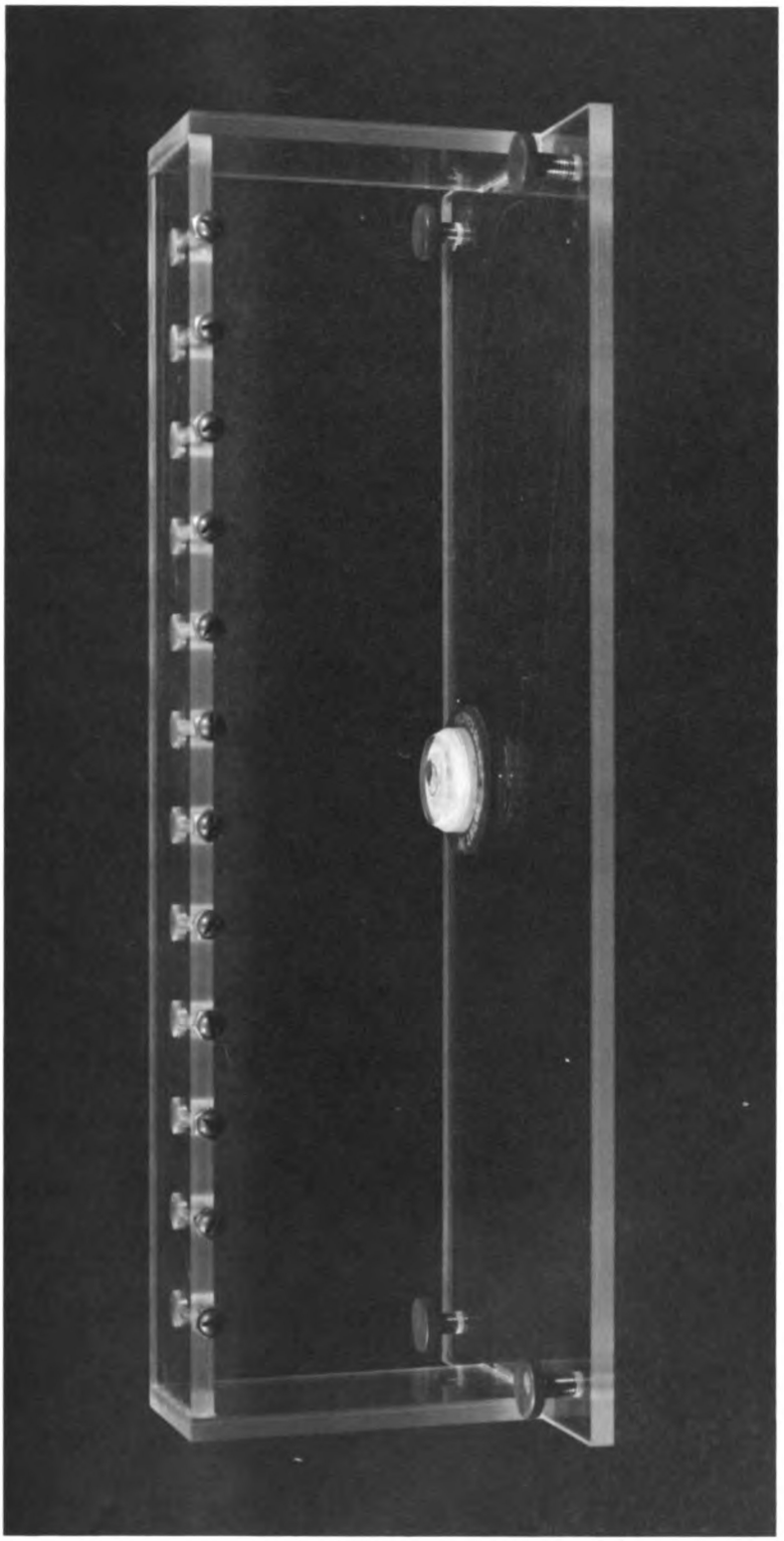


Figure 1

to each tube and the stacking gel was water layered.

11. A fluorescent desk lamp was placed over both racks at a distance of 5 cm. and photopolymerization was allowed to proceed for 45 minutes.
12. Serum samples were prepared by mixing equal volumes of serum with 40 percent sucrose.
13. Tubes with completely polymerized gels were inserted into the rubber grommets (after careful removal of the rubber cap) in the upper buffer reservoir, and then centered between the upper and lower electrodes.
14. Lower reservoir was filled with 3 liters of lower reservoir buffer. A hanging drop of buffer was placed on the end of each separation gel to prevent air bubble entrapment.
15. Upper reservoir was then placed on top of the lower reservoir submerging the lower 3 cm. of the electrophoresis tubes.
16. Upper reservoir was filled with 2 liters of upper buffer and the gels were gently flushed with a buffer filled syringe.
17. A 40 microliter serum sample was carefully layered on top of the stacking gel.
18. A cart carrying the loaded electrophoresis cell

and power supply was then placed in a 4°C cooler.

19. Electrode leads were connected, anode to the bottom.
20. Power supply was switched on and the current adjusted to 25 milliamps for the first five minutes.
21. Current was then adjusted to 85 milliamps, 100 volts, for the remainder of the separation (approximately three hours).
22. Turkey serum was separated until the blue colored tracking dye had migrated to within 13 mm. of the end of the electrophoresis tube.
23. Power supply was switched off and the cart returned to the previous laboratory.
24. Buffer was decanted and the tubes removed from the rubber grommets.
25. A 5 ml. syringe was fitted with a blunted 6.2 cm. X 26 g. needle and was used to remove the gel by "rimming", i.e., injecting water between the well of tube and the gel.
26. Gel was then placed into a glass tube containing 15 ml. of modified incubation mixture and allowed to react for 1.5 hours at 24°C.
27. Stained gels were stored in glass tubes filled with 5 percent acetic acid and allowed to destain for 48 hours before reading.

28. Any serum samples showing diffuse banding or light staining were diluted 1:1 with distilled water, centrifuged at 8500 rpm, 10°C, for fifteen minutes and reseparated.

Apparatus

Electrophoretic cell (Figure 2) was constructed of 3 mm. thick plexiglas. The lower buffer reservoir was 17 cm. square and 5 cm. deep. A plexiglas tube 15 cm. long and 5 cm. in diameter was mounted in the center of the upper reservoir to hold the electrodes. The upper buffer reservoir was 17 cm. square and 3 cm. deep (Figure 3). The two electrodes (Figure 3-A) consisted of No. 19 platinum wire mounted into two grooves 85 mm. apart on the plexiglas tube. Twenty-four holes were drilled in the upper reservoir, equally spaced and equidistant from the central electrodes. Electrical grommets (Figure 3-B) were fitted into each hole to hold the electrophoresis tubes. The glass electrophoresis tubes were 75 mm. in length with 5 mm. inner diameter. The upper reservoir was fitted with L-shaped plexiglas "legs" to facilitate loading and unloading and also to secure it to the lower reservoir.

Power supply consisted of a Heath Regulated H.V. Power Supply Model IP-17. A Corning Model 12 pH meter was used to make all pH measurements. A Sorvall RC-12 automatic

Figure 2. Polyacrylamide gel (disc) electrophoresis cell.

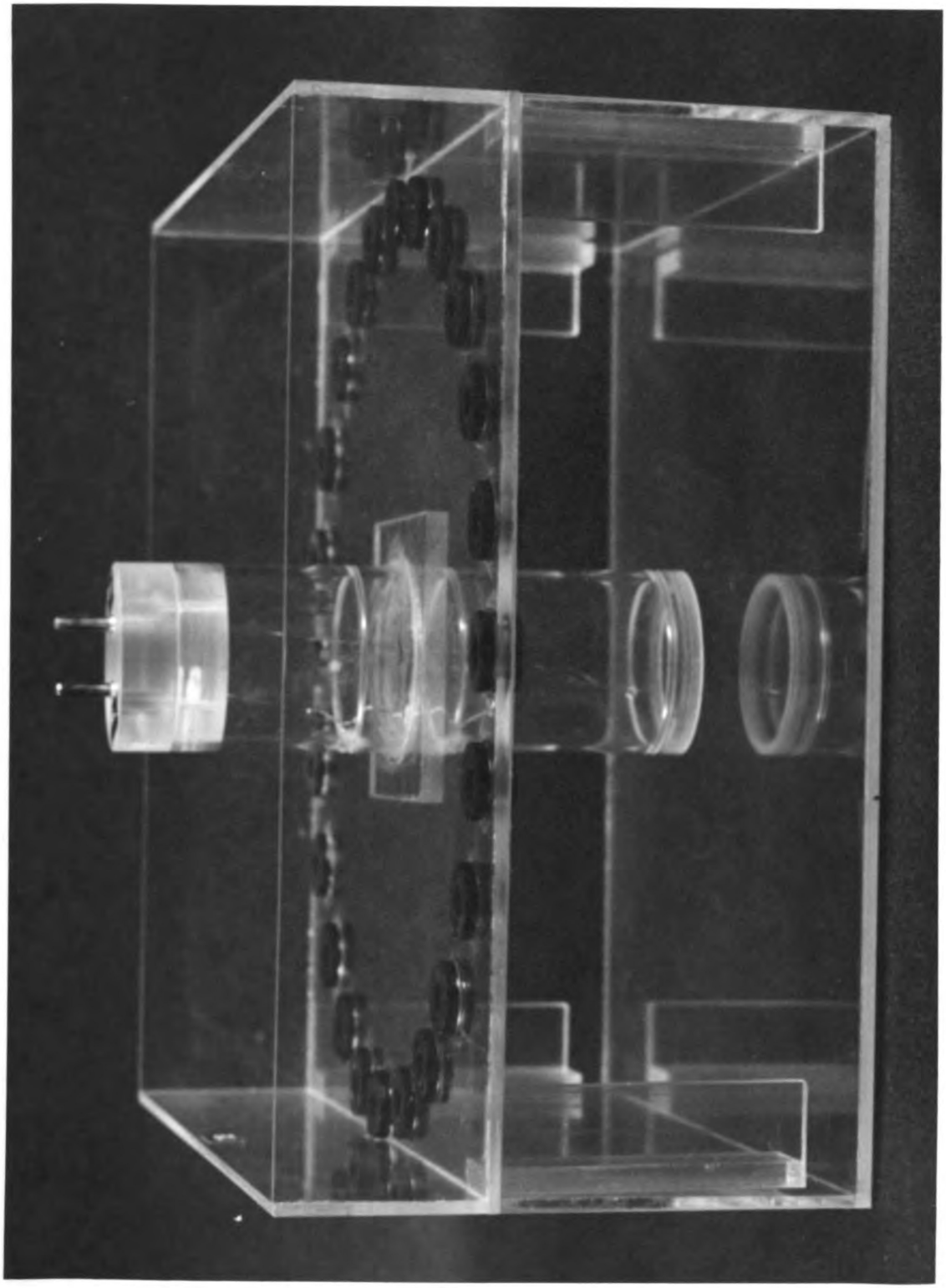


Figure 2

Figure 3. Upper buffer reservoir, polyacrylamide gel (disc) electrophoresis cell.

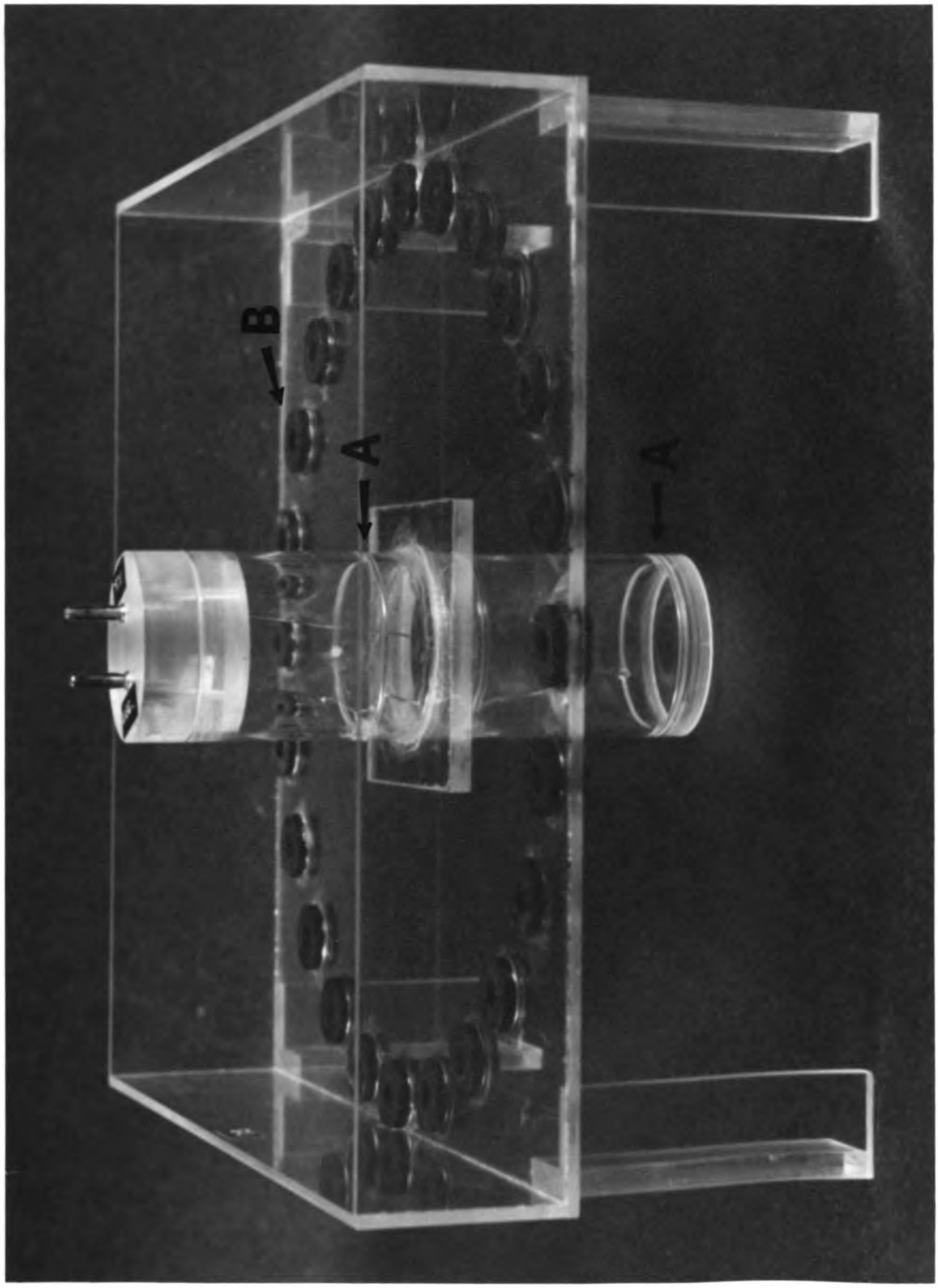


Figure 3

refrigerated centrifuge was used to spin down all serum samples.

Chemical Formulations

1. Stock Solutions (Davis, 1964; Savage, 1972)

A		B	
1 N HCl	approx. 48 ml.	1 M H_3PO_4	25.6 ml.
Tris	36.3 gm.	Tris	5.7 gm.
TEMED	.46 ml.	TEMED	.46 ml.
Water to	100 ml.		
titrate to pH 8.9 with HCl		titrate to pH 6.9	

C		D	
Acrylamide	30 gm.	Acrylamide	10 gm.
Bis	.8 gm.	Bis	2.5 gm.
Water to	100 ml.	Water to	100 ml.

E		F	
Riboflavin	4.0 gm.	Sucrose	40 gm.
Water to	100 ml.	Water to	60 ml.

2. Terms

   A. Tris = 2-Amino-2-(hydroxymethyl)-1,3-propanediol

   B. TEMED = N,N,N',N'-Tetramethylenediamine

   C. Bis = N,N'-Methylenebisacrylamide

   D. Water = deionized distilled water (fresh)

3. Other Solutions

   A. Ammonium Persulfate solution (AP)

Ammonium Persulfate	.14	gm.
Water to	100	ml.

   B. Bromophenol Blue Solution (BPB)

Bromophenol Blue	.03	gm.
Water to	100	ml.

C. Column Coat Solution

Tween 20 (Polyoxyethylene(20) Sorbitan Monolaurate - 3 ml.
Ethylene Glycol - 3 ml.
Water - 94 ml.

D. Upper buffer, modified (Buchler Instruments, 1966)

Tris - 38.4 gm.
Glycine - 7.0 gm.
BPB - 2.0 ml.
Water - 2.0 liters
(titrate to pH 8.85 with Tris)

E. Lower Buffer (Buchler Instruments, 1966)

Tris - 29.0 gm.
11.4 N HCl- approx. 11.5 ml.
Water to 2.0 liters
(titrate to pH 8.07 with HCl)

F. Gel Storage Solution

Glacial Acetic Acid - 70 ml.
Water - 930 ml.

G. Incubation Mixture, modified (E. C. Apparatus Co., St. Petersburg, Florida)

Alpha-naphthyl acid phosphate - 100 mg.
4-Amino-diphenylamine diazonium salt - 250 mg.
Magnesium sulfate · 7 H_2O - 260 mg.
Maleic acid - 120 mg.
Tris - 12 gm.
Water to 400 ml.

4. Working Solutions

Separation Gel

Solution A - 5 ml.
Solution C - 5 ml.
AP - 10 ml.

Stacking Gel

Solution B - 1 ml.
Solution D - 2 ml.
Solution E - 1 ml.
Solution F - 3 ml.

Photographic Technique

Polyacrylamide gels to be photographed were placed in 20 ml. test tubes filled with gel storage solution. The test tubes were placed on a sheet of white glass and light was transmitted from below by means of a 15-watt fluorescent lamp.

Exposure time was set at 1/15 (one-fifteenth) seconds at F-8 using Panatomic-X film. Prints were made on number three contrast paper.

PROCEDURAL EVALUATION

Upon attempting to separate the isoenzymes of turkey akp little progress was made when using the disc electrophoresis procedures that were described in several of the papers reviewed. A confusing array of buffer systems, gel systems, sample preparations and staining procedures have been reported. The initial experimentation in this laboratory consisted of making many comparisons among those papers dealing with polyacrylamide gel electrophoresis in order to find an optimum procedure for separation of turkey serum akp. As the laboratory investigation was conducted in a series of steps the discussion will proceed accordingly.

Buffer Systems

A suitable buffer system appears to be a crucial component of polyacrylamide gel electrophoresis. A single buffer can be used in both upper and lower reservoirs (continuous) or different buffers can be used (discontinuous). Hjerten et al. (1965) recommended a continuous buffer

system for optimal resolution of the protein species being separated. In addition to the chemical constitution of the buffer of buffers used, an alkaline pH (approximately 9 ± 1 pH unit) and molar concentration ranging from .05 to .30 are necessary for separation of alkaline phosphatase. The maximum activity of akp was reported to occur in a .30 molar buffer (Babson et al., 1966). A number of continuous buffer systems recommended by E. C. Apparatus Co., St. Petersburg, Florida were investigated: Tris-Citrate (pH 7.0), Tris-Maleic Acid (pH 9.0), Tris-Glycine (pH 8.3) and a phosphate buffer (pH 7.0). A Tris-Borate buffer (pH 9.5) described by Green et al. (1972) was also used. Turkey serum akp subjected to polyacrylamide gel electrophoresis using a continuous buffer system consistently failed to migrate. The zymograms normally observed consisted of one or two bands in close proximity to the gel origin. In all cases of akp electrophoretic separation with a continuous buffer system, Cyanogum-41 gel (E. C. Apparatus Company, St. Petersburg, Florida) was used along with the incubation mixture described in this report (Taswell and Jeffers, 1963). The activity of akp has been reported to be inhibited by borate (Wilcox, 1966) and Glycine (Smith et al., 1968). However, borate has been successfully used in the electrophoretic separation of akp (Bamford et al., 1965; Chiandussi et al., 1962; Epstein et al., 1967; Smith et al., 1968). Glycine was successfully incorporated into

the upper buffer used by Savage (1972). In the present paper, the use of glycine in a discontinuous buffer system yielded the best resolution of turkey akp isoenzymes. A slight modification of the upper reservoir buffer used by Savage (1972), resulted in a shorter separation time and sharper bands. The modification consisted of adding additional Tris to attain a pH of 8.85.

Gel System

The initial polyacrylamide gel investigated was Cyanogum-41 (C-41) and mixed in concentrations of 5, 7, and 10 percent. The result in all cases was diffuse banding at the gel origin. The use of a 3 percent C-41 "stacking" gel had no effect on akp migration or band sharpening. A probable cause for the poor enzyme resolution with C-41 may be the high percent (5.0) of cross-linking agent (Bisacrylamide) in the gel. The gel systems described by Smith et al. (1968), Green et al. (1972), and David (1964), when used with their recommended buffers, did not yield satisfactory results. The gel system described by Davis (1964), was modified to produce a five percent acrylamide gel with two and one-half percent Bisacrylamide without success. The achievement of good isoenzyme separation was realized in the present system in which the "separation" gel contained 7.5% acrylamide and 2.6% bisacrylamide.

The importance of using a "stacking" gel has generally been denied in the literature (Smith et al., 1968; Epstein et al., 1967; Green et al., 1972; Hjerten et al., 1965). It has been the experience in this laboratory that the sample concentrating effect of the "stacker" gel at the interface of the two gels will improve the resolution of akp isoenzymes.

Staining Procedure

Due to the non-specific nature of alkaline phosphatase a great number of substrates have been reported in the literature. The substrates investigated in this laboratory included: Sodium phenolphthalein diphosphate, Sodium phenolphthalein monophosphate, p-Nitrophenol phosphate, Sodium beta-naphthyl acid phosphate and Sodium alpha-naphthyl acid phosphate. The enzyme substrates were used in combination with each of the following dye-couplers: 4-amino-diphenylamine diazonium salt, Fast Red TR salt, Fast Blue BB salt (Gurr) and Fast Blue RR salt. The combination of Sodium alpha-naphthyl acid phosphate and 4-amino-diphenylamine diazonium salt used in a stain buffer of pH 9.2 proved optimal (Taswell and Jeffers, 1963).

Chicken intestinal alkaline phosphatase (Sigma Chemical Company) was used as a standard to compare histological staining techniques. A number of different stain procedures

(referenced in List of References) were able to demonstrate akp activity of this standard when dissolved in either distilled water, normal unheated serum and/or heated serum (60°C for 15 minutes).

The staining buffers reviewed contained Magnesium and/or Manganese for enzyme activation but the optimum pH of the stain buffer was reported by Babson et al. (1966), to be a function of substrate species and concentration. The specific staining procedures of a number of workers were used unsuccessfully and consequently discarded (Green et al., 1972; Maeda et al., 1972; Brown and Manley, 1970; Smith et al., 1968; Savage, 1972; Kaplan and Rogers, 1969; E. C. Apparatus Co., 1971).

Sample Preparation

Throughout the literature both serum and plasma alkaline phosphatase have been separated by electrophoresis. As was indicated earlier, plasma was found to be unsuitable for polyacrylamide gel electrophoresis. The absence of enzyme migration in plasma samples could possibly be attributed to the presence of fibrinogen. It has been reported by Maurer (1972) that fibrinogen will migrate much slower in particular electrophoresis gels than would be expected due to its molecular weight. The fibrinogen molecule is triangularly shaped and apparently blocks the pores

in polyacrylamide gel. Starch gel was reported to be unaffected by human fibrinogen (Warnock, 1966).

In this study, caution was taken to preserve enzyme activity by cooling the blood samples and storing the serum samples at -20°C. Gutowska et al. (1943) reported that the activity of akp in plasma will decrease gradually corresponding to the length of time held at room temperature. It was recommended by Sanger et al. (1966), that chicken serum be assayed for akp activity as soon as possible. Taswell and Jeffers (1963) observed that human serum could be held for long periods of time at -20°C without appreciable loss of enzyme activity.

Individual serum samples were divided into three aliquots to facilitate repeated separations of those sera displaying unsatisfactory enzyme banding. Thawing of serum samples and subsequent refreezing was reported by Rendel and Stormant (1964), to decrease the akp activity in sheep serum by fifteen to forty percent; in addition, the decomposition of the isoenzymes resulted in slower migration rates in starch gels.

No attempt was made during this experiment to standardize the amount of enzyme activity contained in each 40 µl serum sample undergoing electrophoretic separation. In the case of Wilcox (1966), chicken serum was diluted with saline to produce equal enzyme activity in all the serum

samples being separated on starch gel. He observed that actual banding intensity varied from very faint to very dark in samples of similar akp activity. In addition, he discovered that increased dilution of serum samples resulted in decreased enzyme mobility in the starch gel.

The activity of human akp was reported by Fishman and Ghosh (1967) to increase when the serum was diluted with water. In some cases an eighty percent increase was observed. In the present paper the isoenzyme banding patterns of pure serum and diluted serum were compared. Turkey serum was diluted in ratios of 1:1, 1:2 and 1:3 with distilled water and "stacking" gel buffer (stock solution B). Dilution normally resulted in fainter banding intensity while isoenzyme mobility did not appear to change. As was mentioned earlier, dilution of turkey serum 1:1 with distilled water was discovered to increase resolution of the isoenzyme bands in those sera displaying poor resolution. It has been proposed by Hjerten et al. (1965), that the dilution of serum will sharpen the zones of enzyme activity during polyacrylamide gel electrophoresis due to a decreased conductivity of the serum sample.

RESULTS

A total of thirteen distinct patterns (zymograms) of turkey alkaline phosphatase were observed. Isoenzyme bands were observed in thirteen different regions of the polyacrylamide gels. The isoenzyme possessing the highest mobility was classified as band number one, as suggested by Brewer (1970). The remaining bands were numbered in the order of decreasing mobility with the slowest moving band assigned number thirteen.

In Figure 4, a photograph is shown of each zymogram type observed in this study accompanied by a schematic drawing to help illustrate the faint bands. Each band is numbered as indicated by the arrows. Zymograms were "read" and classified according to zymogram type on two different occasions. It was observed that individual gels could be accurately classified with a repeatability of approximately 96.3%.

The data, presented in Table 1, shows each zymogram type observed at zero and peak egg production expressed as a percentage of the total number of each zymogram type observed. Data for this table was obtained from the

Figure 4. Turkey serum alkaline phosphatase zymograms.

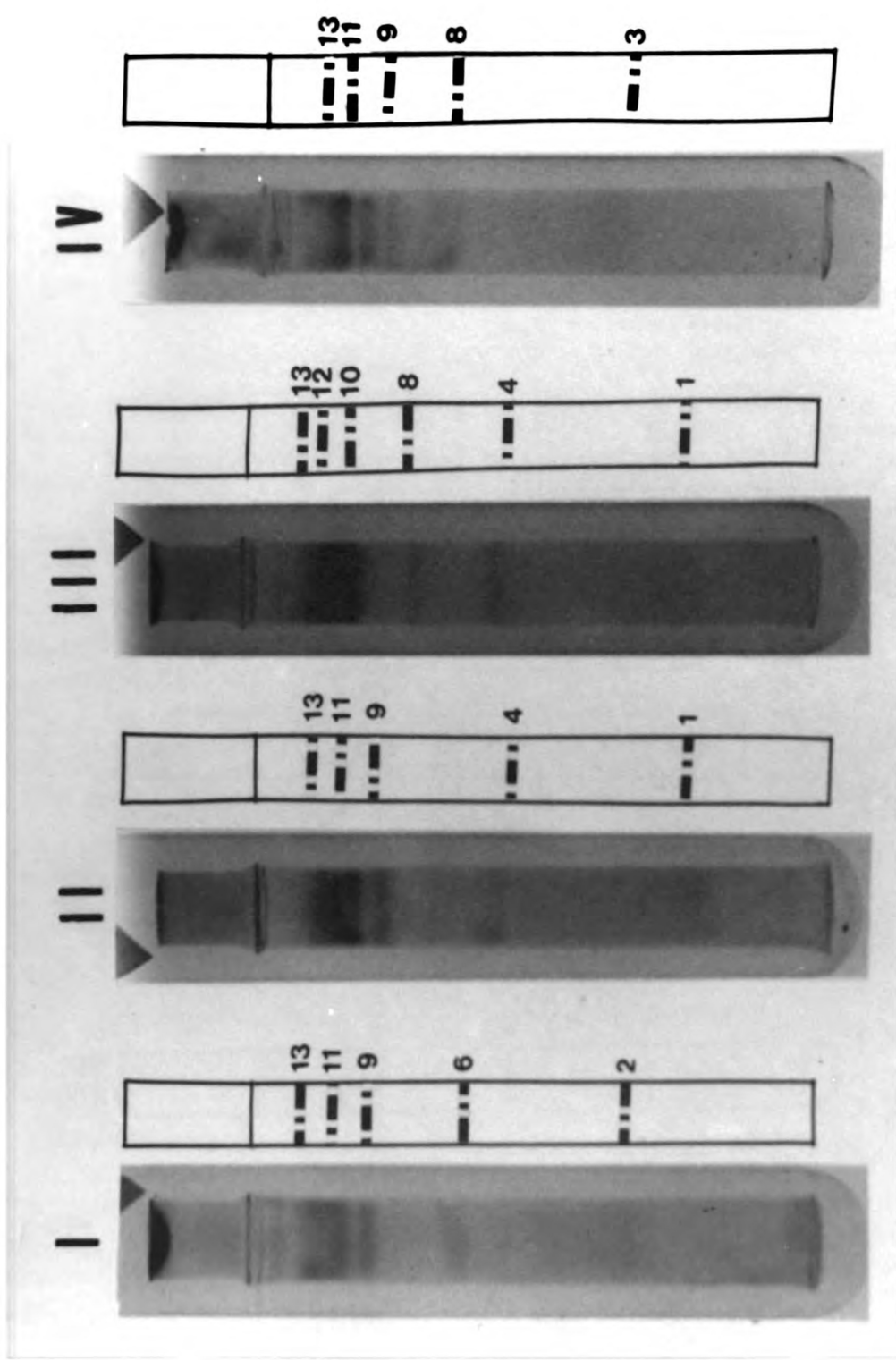


Figure 4 continued

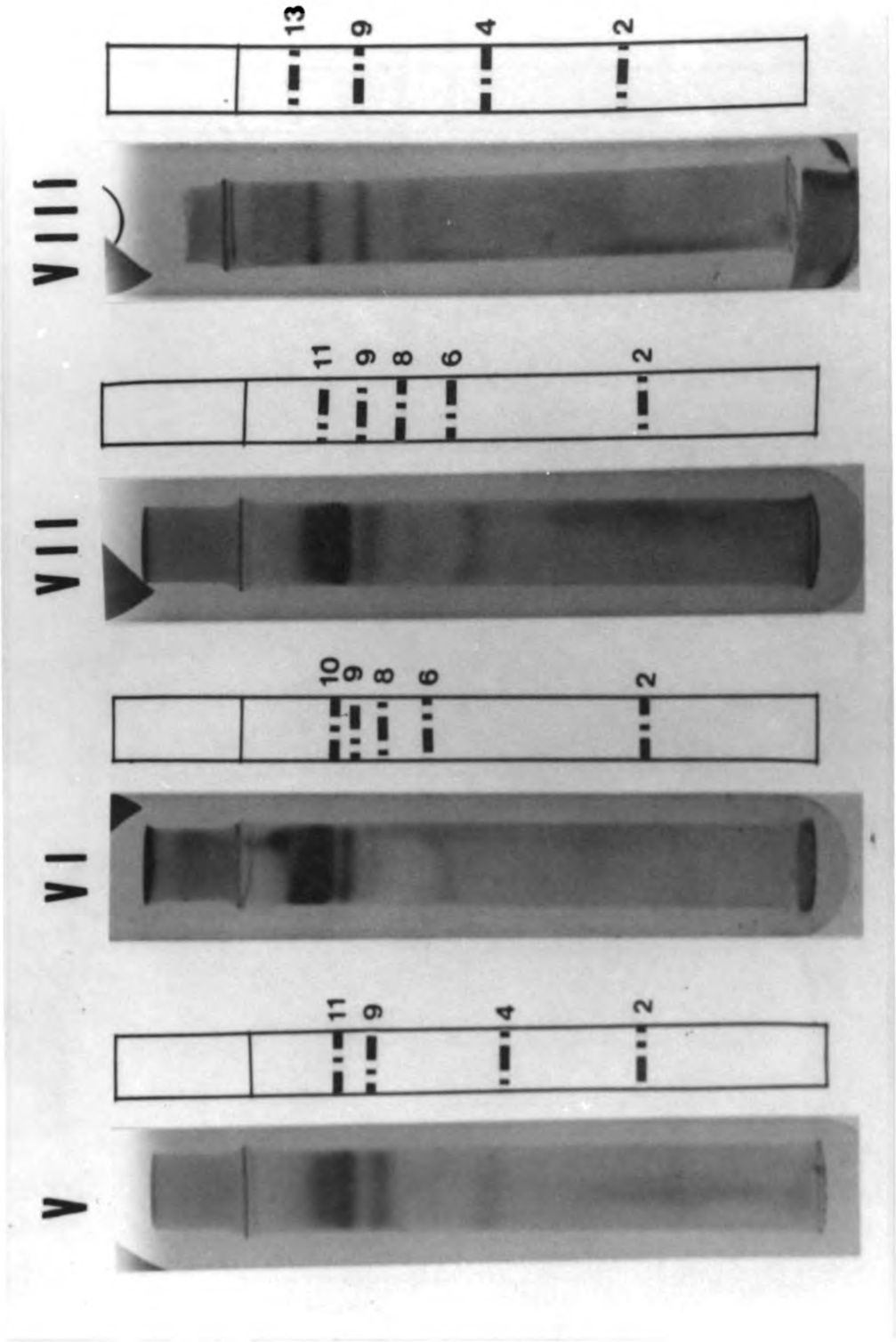


Figure 4--continued

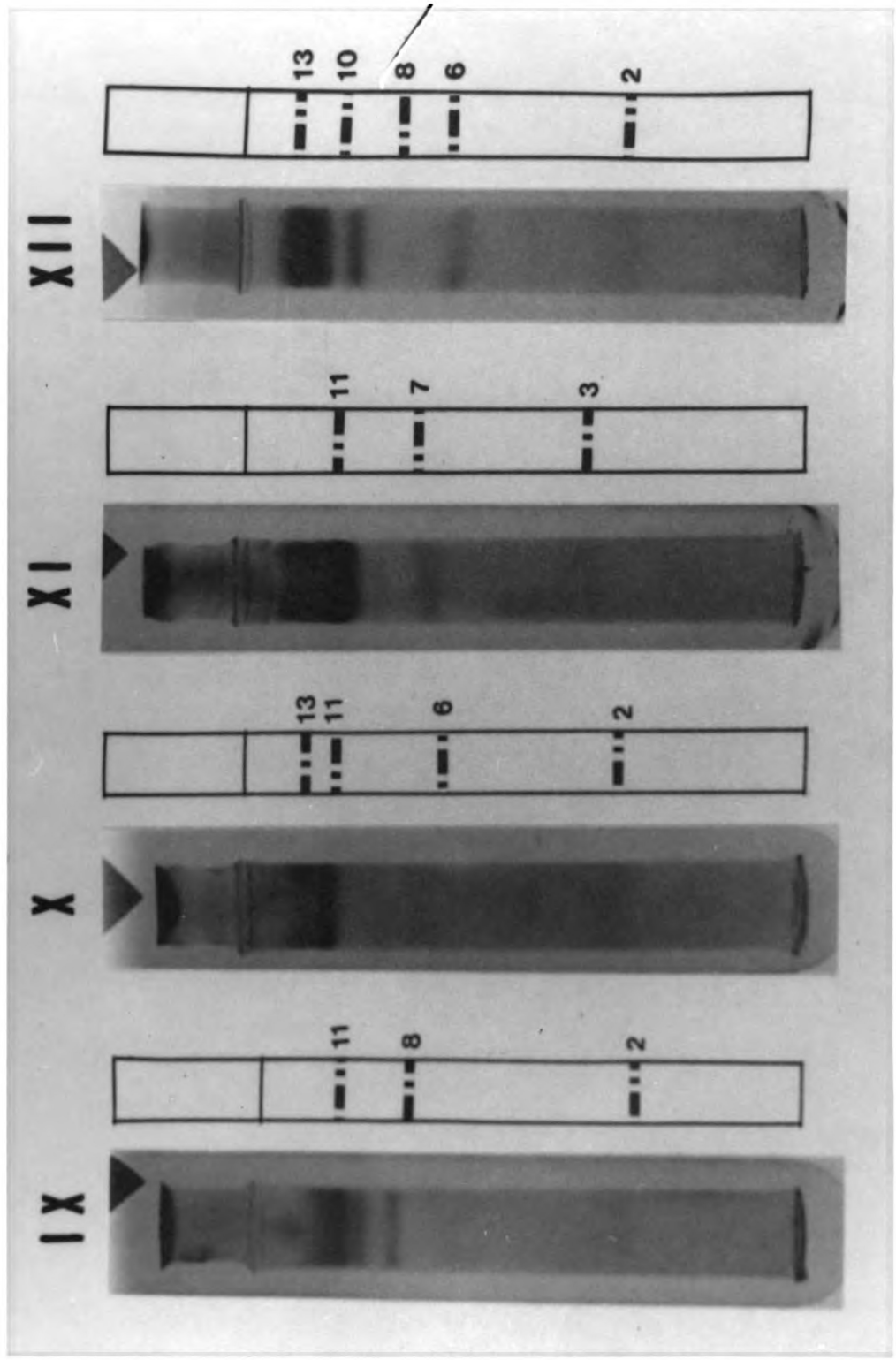
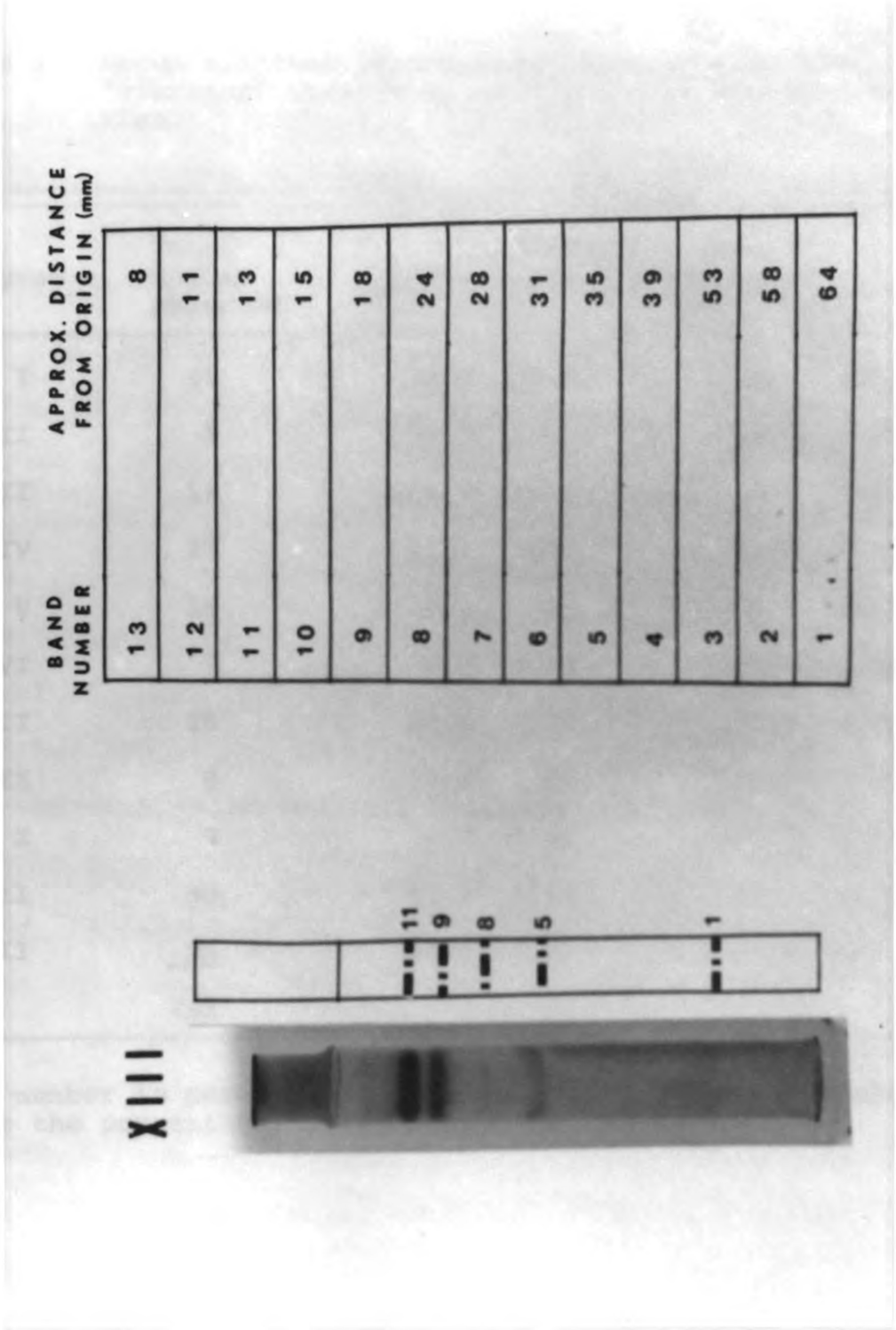


Figure 4--continued



BAND NUMBER	APPROX. DISTANCE FROM ORIGIN (mm)
13	8
12	11
11	13
10	15
9	18
8	24
7	28
6	31
5	35
4	39
3	53
2	58
1	64

Figure 4--concluded

Table 1. Serum alkaline phosphatase isoenzymes in the "vibrator" turkeys at zero and peak egg production.

Zymogram type	Total number observed	Production Level (percentage of Zymograms) zero		peak	
I	45	35.5	(16)[1]	64.5	(29)
II	9	33.3	(3)	66.7	(6)
III	11	100.0	(11)	0.0	(0)
IV	27	74.0	(20)	26.0	(7)
V	15	0.0	(0)	100.0	(15)
VI	7	0.0	(0)	100.0	(7)
VII	25	88.0	(22)	12.0	(3)
IX	8	75.0	(6)	25.0	(2)
X	5	0.0	(0)	100.0	(5)
XII	33	63.3	(21)	36.7	(12)
XIII	16	25.0	(4)	75.0	(12)
	201				

[1]The number in parenthesis indicates the number of samples that the percentage represents.

"vibrator" flock. Zymogram type III was observed only in the zero production period; whereas, zymogram types V, VI, and X were observed only in the peak egg production period.

The data in Table 2 illustrates strain differences for the zymogram types. "Vibrator" birds were observed to lack zymogram types VIII and XI because these particular types were present only in the Nicholas strain. Zymogram types II, V, VI, and IX were not displayed by the Nicholas strain. With one exception, zymogram type X which was observed only in the Broad Breasted White "vibrator" birds, both "vibrator" strains displayed the same zymogram types. In two particular instances, zymogram types I and XII, which involved fairly high numbers of individuals, each "vibrator" strain possessed a reasonably equal percentage of either type.

The data in Table 3 compares the zero production period zymogram types to the peak production period zymogram types of the "vibrator" hens classified as to laying intensity during the peak production period. In the zero production period, zymogram types I, IV, IX, and XII were observed to appear in the birds classified as high intensity layers; whereas, in the peak egg production period zymogram types II, IV, V, VI, IX, and XII were associated with the high intensity layers. Zymogram types II, IV, V, VI, IX, X, and XII were not observed to occur in birds classified as

Table 2. Strain differences in serum alkaline phosphatase isoenzymes.

Zymogram type	Total number observed	"Vibrator" female & male BBW[1]		"Vibrator" female & male BBB[2]		Nicholas female BBW	
I	47	51.1	(24)[3]	44.6	(21)	4.2	(2)
II	9	22.2	(2)	77.8	(7)	-	
III	14	50.0	(7)	28.6	(4)	21.4	(3)
IV	31	58.1	(18)	29.0	(9)	12.9	(4)
V	15	60.0	(9)	40.0	(6)	-	
VI	7	71.4	(5)	28.6	(2)	-	
VII	34	55.9	(19)	17.6	(6)	26.4	(9)
VIII	3	-		-		100.0	(3)
IX	8	87.5	(7)	12.5	(1)	-	
X	10	50.0	(5)	-		50.0	(5)
XI	8	-		-		100.0	(8)
XII	42	45.2	(19)	33.3	(14)	21.4	(9)
XIII	19	68.4	(13)	15.8	(3)	15.8	(3)

[1]Broad Breasted White

[2]Broad Breasted Bronze

[3]The number in parenthesis indicates the number of individual samples that the percentage represents.

Table 3. Serum alkaline phosphatase isoenzymes in the "vibrator" turkey hens in relation to laying intensity.

Zymogram type	Laying Intensity (percentage of zymograms)[1]							
	Zero Egg Production Period				Peak Egg Production Period			
	A	B	C	D	A	B	C	D
I	-	1.7 (2)[2]	6.7 (8)	3.3 (4)	1.7 (2)	2.5 (3)	3.3 (4)	8.3 (10)
II	-	-	-	.8 (1)	-	-	2.5 (3)	1.7 (2)
III	.8 (1)	.8 (1)	-	5.0 (6)	-	-	-	-
IV	-	2.5 (3)	3.3 (4)	9.2 (11)	-	.8 (1)	.8 (1)	4.2 (5)
V	-	-	-	-	-	-	5.0 (6)	2.5 (3)
VI	-	-	-	-	-	-	1.7 (2)	4.2 (5)
VII	.8 (1)	-	3.3 (4)	5.0 (6)	-	-	-	.8 (1)
IX	-	-	1.7 (2)	2.5 (3)	-	-	-	.8 (1)
X	-	-	-	-	-	-	1.7 (2)	2.5 (3)
XII	-	.8 (1)	1.7 (2)	.8 (1)	-	1.7 (2)	.8 (1)	1.7 (2)
Total number Zymograms observed	2	7	20	32	2	6	19	32

[1]Laying intensity was determined for a 12 day period and each bird was assigned into one of the following categories: Group A = 0 eggs in 12 days; Group B = 1-3 eggs in 12 days; Group C = 4-6 eggs in 12 days; Group D = 7 or more eggs in 12 days.

[2]The number in parenthesis indicates the number of individual samples that the percentage represents.

non-layers (i.e., zero egg production in the selected 12-day egg production period).

The data in Table 4 shows the zymogram types in relation to egg laying intensity of the Nicholas strain. Zymogram types I, III, and XI were not observed in the birds classified as non-layers. Zymogram type VIII was observed only in birds classified as non-layers, while type I was observed only in birds classified as high intensity layers.

Table 5 shows data for each zymogram type observed in the "vibrator" flock after separation into groups of birds displaying the "Normal", "Vibrator", and "Vibrator-" or "Vibrator+" conditions. No noticeable percentage of difference was observed due to the intensity of vibration.

The data in Table 6 shows the zymogram types of the Nicholas strain in relation to diet. Except for zymogram type VIII, which was observed only in birds that received the standard diet, the birds on both diets had similar percentages for all zymogram types.

The data in Table 7 shows semen volume in relation to zymogram type. Males classified as high semen producers (Group C) represented 30 percent of the total population but were not associated with any particular zymogram type. In addition, no zymogram type appeared consistently in the males classified as low semen producers (Group A).

Table 4. Serum alkaline phosphatase isoenzymes in the Nicholas strain turkeys in relation to laying intensity.

Zymogram type	Laying Intensity (percentage of Zymograms)[1]			
	A	B	C	D
I	-	-	2.2 (1)[2]	2.2 (1)
III	-	2.2 (1)	4.4 (2)	-
IV	4.4 (2)	-	2.2 (1)	2.2 (1)
VII	4.4 (2)	4.4 (2)	6.6 (3)	2.2 (1)
VIII	6.6 (3)	-	-	-
X	6.6 (3)	-	2.2 (1)	2.2 (1)
XI	-	4.4 (2)	4.4 (2)	8.8 (4)
XII	8.8 (4)	6.6 (3)	2.2 (1)	2.2 (1)
XIII	4.4 (2)	-	2.2	-
Total Number Zymograms Observed	16	8	12	9

[1]Laying intensity was determined for a 12 day period and each bird was assigned into one of the following categories:
Group A = 0 eggs in 12 days
Group B = 1-3 eggs in 12 days
Group C = 4-6 eggs in 12 days
Group D = 7 or more eggs in 12 days

[2]The number in the parenthesis indicates the number of individual samples that the percentage represents.

Table 5. Serum alkaline phosphatase isoenzymes in the "vibrator" turkeys in relation to intensity of vibration.

Zymogram type	Vibration Intensity (percentage of Zymograms)[1]		
	Normal	Vibrator & Vibrator-	Vibrator+
I	6.5 (13)[2]	9.5 (19)	6.5 (13)
II	1.5 (3)	1.0 (2)	2.0 (4)
III	2.0 (4)	2.5 (5)	1.0 (2)
IV	5.0 (10)	4.5 (9)	4.0 (8)
V	4.0 (8)	1.0 (2)	2.5 (5)
VI	.5 (1)	1.0 (2)	2.0 (4)
VII	5.0 (10)	4.4 (9)	3.0 (6)
IX	2.0 (4)	1.5 (3)	.5 (1)
X	1.0 (2)	.5 (1)	1.0 (2)
XII	6.0 (12)	8.5 (17)	2.0 (2)
XIII	2.5 (5)	4.4 (9)	1.0 (2)
Total Number Zymograms Observed	71	79	51

[1]Vibration Intensity: Normal=No shaking
Vibrator & Vibrator- = Infrequent and mild to moderate shaking
Vibrator+ = Intense shaking

[2]The number in parenthesis indicates the number of individual samples that the percentage represents.

Table 6. Serum alkaline phosphatase isoenzymes in the Nicholas strain female turkeys in relation to diet.

Zymogram type	Diet (percentage of Zymograms)	
	Standard	10% Poultry anaphage
I	50.0 (1)[1]	50.0 (1)
III	33.3 (1)	66.7 (2)
IV	25.0 (1)	75.0 (3)
VII	66.7 (6)	33.3 (3)
VIII	100.0 (3)	0.0 (0)
X	60.0 (3)	40.0 (2)
XI	37.5 (3)	62.5 (5)
XII	22.2 (2)	77.8 (7)
XIII	66.7 (2)	33.3 (1)
Total Number Zymograms Observed	22	24

[1]The number in parenthesis indicates the number of individual samples that the percentage represents.

Table 7. Serum alkaline phosphatase isoenzymes in the "vibrator" turkeys in relation to semen production.

Zymogram type	Semen Production (percentage of Zymograms)[1]					
	Zero Egg Production Period[2]			Peak Egg Production Period[2]		
	A	B	C	A	B	C
I	-	2.7 (1)[3]	-	5.4 (2)	10.8 (4)	8.2 (3)
II	2.7 (1)	-	5.4 (2)	2.7 (1)	2.7 (1)	-
III	-	5.4 (2)	2.7 (1)	-	-	-
IV	-	5.4 (2)	2.7 (1)	-	-	-
V	-	-	-	-	8.2 (3)	2.7 (1)
VII	2.7 (1)	13.5 (5)	8.2 (3)	2.7 (1)	2.7 (1)	-
IX	2.7 (1)	2.7 (1)	-	-	-	-
XII	10.8 (4)	16.2 (6)	8.2 (3)	8.2 (3)	8.2 (3)	5.2 (2)
XIII	2.7 (1)	2.7 (1)	2.7 (1)	2.7 (1)	13.5 (5)	13.5 (5)

[1]Semen production was determined in the peak egg production period and the volume was: Group A = .000-.174 ml.; Group B = .175-.324 ml.; Group C = .325 or more ml.

[2]Refers to the female time period in Table 3 as the reference point. Semen volumes observed during zero egg production period ranged from zero to very low values.

[3]The number in parenthesis indicates the number of individual samples that the percentage represents.

The data in Table 8 shows the zymogram types of the "vibrator" flock in relation to sex. Zymogram types VI and X were observed in females only as these particular types were absent in the males. Zymogram type XIII appeared exclusively in male "vibrator" birds.

Table 8. Serum alkaline phosphatase isoenzymes in "vibrator" turkeys in relation to sex.

Zymogram type	Percentage of Zymogram Type	
	Females	Males
I	73.9 (34)[1]	26.1 (12)
II	66.7 (6)	27.3 (3)
III	72.7 (8)	27.3 (3)
IV	88.9 (24)	11.1 (3)
V	60.0 (9)	40.0 (6)
VI	100.0 (7)	-
VII	52.0 (13)	48.0 (12)
IX	75.0 (6)	25.0 (2)
X	100.0 (5)	-
XII	31.3 (10)	68.7 (22)
XIII	-	100.0 (16)

[1]The number in the parenthesis indicates the number of individual samples that the percentage represents.

DISCUSSION

Electrophoretic separation of turkey serum akp isoenzymes on starch gel has previously yielded a total of four bands (Stevens and Garza, 1968), with individual samples displaying up to three bands. In this study electrophoretic separation of turkey serum akp on polyacrylamide gel has revealed thirteen different bands of activity with individual samples possessing three to six bands. This increase in isoenzyme numbers is attributed to the use of a more refined electrophoretic technique. Due to the electrophoretic procedure used in this study, the actual composition of each individual band observed in the polyacrylamide gels can not be determined. The existence of thirteen distinct molecular species of alkaline phosphatase possessing similar enzyme activity was demonstrated in this study. Alkaline phosphatase may be similar to other enzymes that are known to exist as isoenzymes in that the bands observed may represent either complete polymers or their component monomers or dimers.

The effects of egg production in chickens and quail upon either akp isoenzymes or total akp activity has not

been clarified in the literature. It has been postulated by previous workers that chicken and quail serum akp is controlled by several genetic and physiological factors (Auchanachie and Emslie, 1934; Savage et al., 1971; Savage et al., 1970a; Savage et al., 1970b; Tanabe and Wilcox, 1960; Tamaki and Tanabe, 1970; Rako et al., 1964; and Stutts et al., 1957).

Rako et al. (1964) and Rao et al. (1969) observed higher levels of akp in prelay hens and reduced levels when the birds were in full egg production. Tanabe (1962) observed that onset of lay would increase serum akp activity. Savage et al. (1970a) reported an increased mobility of quail serum isoenzyme "AKP-90" upon onset of lay. Female quail in egg production were observed by Savage et al. (1970b) to have only "Type II" zymograms while nonlayers and males had only "Type I" zymograms.

In an attempt to find zymograms indicative of laying status the "vibrator" flock was sampled on two occasions; when egg production was zero and during peak egg production. Zymogram type III was only found in the force-molt period (zero egg production) of individuals of either sex. Zymogram type V was observed only in the peak egg production period of individuals of either sex. Since zymograms type III and V were found in either sex they may reflect a particular metabolic state rather than the effect of egg

production. Zymogram type VI and X were found in "high intensity" laying females only and may indicate the influence of both egg production and metabolism. Rako et al. (1969) observed a negative correlation of akp activity with total egg production which seemingly indicates a definite role of akp in egg production. However, Auchanachie and Emslie (1934) reported that the productivity of chickens was not related to plasma akp activity and that the enzyme plays only a minor role in egg production.

The possible effect of laying intensity upon akp activity has also been explored. Gutowska et al. (1943) observed differences in total enzyme activity between good and poor producers. Common (1936) reported that no correlation existed between serum akp and intensity of egg production as measured by trap-nest records for four weeks prior to sampling. In addition, Common (1936) observed that past and present good egg producers exhibited similar enzyme activity.

Egg production was measured over a twelve day period in this study in an attempt to associate zymogram types with laying intensity. Zymogram types II, V, VI and X were observed in "vibrator" hens laying from four to twelve eggs during the measurement period. In contrast, only zymogram type IX was observed in the same group of layers in their zero egg production period. It is interesting to

note that zymogram types VI and X were observed only in females with high egg laying intensity and that these zymograms were separated by several isoenzyme bands on the gel media.

The influence of genetic factors upon akp activity and isoenzymes has been recognized for some time. Stutts et al. (1957) consistently observed different akp activity levels in various inbred lines of chickens. In this study zymogram types II, V, VI, and XI were only observed in "vibrator" birds, conversely, zymogram types VIII and XI were observed only in the Nicholas strain. Engh and Wilcox (1971) noted varying frequencies for a fast band in starch gel electrophoresis among fifteen strains of chickens, however, since all of these strains had been selected for high egg production it would appear that this band was unrelated to egg production. Savage et al. (1970a) suggested that isoenzymes AkP 89 and AkP 90 in Japanese quail were under genetic control. Stevens and Garza (1968) reported that bands 2 and 3 were mutually exclusive in turkeys and that codominance could not exist between these isoenzymes. Studies of the inheritance of chicken serum akp isoenzymes observed using starch gel electrophoresis by Wilcox (1963, 1966) and Law and Munro (1965) has led to the conclusion that serum akp is controlled by a single autosomal loci exhibiting complete dominance. The increase in total number of akp isoenzymes

observed in turkey serum in this study over that number observed by Stevens and Garza (1968) suggests an improvement in akp isoenzyme electrophoresis procedures. This refined technique now offers a better opportunity to investigate the problem of isoenzyme inheritance in turkeys and perhaps quail and chickens as well.

Due to several generations of interbreeding, the "vibrator" flock is assumed to be genetically similar and such individuals would differ only by those genes responsible for the vibrator condition. Inasmuch as most zymogram types are displayed equally among the vibrator groups such results appear to be consistent with the literature.

The minor change in protein source in the diets of the Nicholas strain would not be expected to affect akp zymograms. The only exception to this is zymogram type VIII, observed in birds fed the standard diet. This particular zymogram type was not observed in the "vibrator" flock and its association with a particular zymogram is possibly due to chance because of the low number of individuals involved.

Zymogram types VI and X were restricted to females only and zymogram type XIII was observed in males only. Sex linkage has not been reported in the literature and such results suggest a possible influence of sex hormones instead. Brown and Badman (1961) demonstrated the effects of exogenous gonadal hormones in chicks. High levels of estrogen

and progesterone were observed to decrease total akp activity and these effects may serve to explain the wide variations in total enzyme activity observed in some laying hens.

Zymogram types V, VI, and X were not observed during the zero egg production period. These particular zymograms were observed in the peak egg production period in those "vibrator" hens laying four or more eggs during the twelve day period. Such results may suggest the influence of estrogen upon these zymograms; however, such an idea would be refuted by zymogram type III which is displayed only during the zero egg production period by hens that were both high and low intensity layers.

"Vibrator" males producing high semen volumes (Group C) were not associated with any particular zymogram type(s). Zymogram type V was observed only during the peak egg production period and in both Group B and Group C individuals. Zymogram types III and IV were again observed in both Group B and Group C individuals but only during the zero egg production period. Production of androgen by the males was probably not as low as the production of estrogen by the females during the zero egg production period as some of the males tested yielded very low volumes of semen. This fact may possibly be attributed to the lack of a specific zymogram or zymograms associated with high semen production (Group C).

SUMMARY

Turkey serum akp from the Michigan State University "vibrator" flock and the Nicholas Broad Breasted White egg-laying strain has been separated by polyacrylamide gel electrophoresis into 13 bands of activity or isoenzymes. Following electrophoresis these activity bands were arranged into 13 different patterns or zymograms. The increase in total number of isoenzymes observed over that observed by previous workers is attributed to an improved electrophoretic procedure.

Zymogram type III was observed in the "vibrator" flock only in the zero egg production period while types V, VI, and X were observed in the peak egg production period only. The latter two zymogram types were observed in high intensity layers only.

Zymogram types II, V, VI, and IX were observed only in "vibrator" birds while types VIII and XI were observed only in the Nicholas strain.

In the zero egg production period, zymogram types I, IV, IX, and XIII were observed to appear in the "vibrator" birds

classified as high intensity layers. Zymogram types II, IV, V, VI, IX, X, and XII were not observed in birds classified as non-layers.

Zymogram type I was observed only in high intensity layers of the Nicholas strain while type VIII was observed only in non-layers. Zymogram types I, III, and XI were not observed in non-layers.

The "vibrator" condition did not appear to affect isoenzyme banding pattern.

Semen production levels were not reflected by any particular zymogram types.

Incorporation of 10 percent dried poultry anaphage in the ration did not affect akp zymograms. Several zymogram types appeared in high intensity "vibrator" layers only and several others in high semen producers as well. Such banding patterns may reflect the influence of sex hormone status, egg production or simply a changed metabolic rate as the result of an increased artificial light day.

Zymogram types VI and X were observed in females only while type XIII appeared exclusively in male "vibrator" birds.

RECOMMENDATIONS

Further investigation into the inheritance of particular isoenzymes would yield more substantial evidence of the influence of genetic factors in akp.

Assuming an influence of gonadal hormones upon akp zymograms either an assay of blood hormone levels or direct feeding and/or injection of such hormones may serve to explain the presence of such a great variety of banding patterns.

Identification of tissue sources of akp isoenzymes similar to that accomplished in human medicine may also help to explain the great number of isoenzymes observed in this study and may lead to a greater appreciation of the role of the isoenzymes in certain metabolic conditions and/or disease states.

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