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Lack of biochemical genetic differences between pallid and shovelnose sturgeon (*Scaphirhynchus albus* and *S. platorynchus*)

Stevan R. Phelps

Fred W. Allendorf

University of Montana
Department of Zoology
Missoula, MT 59812

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Abstract

Pallid (*S. albus*) and shovelnose (*S. platorynchus*) sturgeon throughout the Mississippi River drainage were electrophoretically examined at 52 gene loci. None of these loci displayed differences between these two species. Three polymorphic loci had similar gene frequencies in both species. These species may not be reproductively isolated.

Introduction

The taxonomic status of the two Scaphirhynchus species, albus and platorynchus, has been debated since the beginning of this century. Forbs and Richardson (1905) divided these fish into separate genera, Parascaphirhynchus albus and Scaphirhynchus platorynchus. Berg (1911 and 1948) supported the placement of albus into the genus Scaphirhynchus. The most convincing evidence of congeneric status for these sturgeon was presented by Baily and Cross (1954). They concluded that S. albus and S. platorynchus were readily separable and well-marked species using morphometric and meristic characteristics. They discounted the placement into separate genera, concluding that it would obscure their similarity characterized by several fundamental features compared to other acipenserids. Carlson (Missouri Dept. of Conservation, personal comm.) has recently questioned the species designation of the pallid and shovelnose sturgeon*. Nine sturgeon that he collected from the Mississippi and Missouri Rivers appear to be hybrids on the basis of morphological and meristic characteristics. This observation prompted two questions: (1) Can these species hybridize? (2) How genetically different are the two species in the genus Scaphirhynchus? Documenting the answers is important since S. albus is considered threatened throughout its range.

Methods

Shovelnose sturgeon were collected from the lower Tongue-Yellowstone River drainage and the Missouri River in Montana, the Mississippi and Missouri Rivers from the State of Missouri, and the Chippewa River from Wisconsin (Figure 1). Pallid

*A species is a group of organisms which actually, or potentially, share a common gene pool (Mayr 1964). Through the use of electrophoresis, these sturgeon species should be distinguishable on a genetic basis; they each should contain distinct alleles at certain loci in their respective gene pools. Hybrids between these two species could be identified if they contain alleles unique to both species.

suspected
sturgeon and hybrids were collected from several locations in the Mississippi and Missouri Rivers from the State of Missouri. Tissue samples of muscle, liver, and eye were examined for all individuals and, in addition, heart, intestine and brain were examined from 6 pallid and 5 shovelnose sturgeon to maximize the number of gene loci surveyed (Table 1). The number of loci reported is a conservative estimate. The designation of loci coding for individual enzymes was difficult due to the duplicated genome of these sturgeon (Ohno, et al., 1969). Determining whether a locus is duplicated or not is dependent on the presence of genetic variation at that locus. Enzyme structures (monomer, dimer, etc.) were inferred from the isozyme patterns seen and information on these enzymes from other fish. The variable patterns at four loci could not be resolved. These and other questionable loci may be able to be resolved with breeding data. Breeding studies are necessary to confirm the genetic basis of the isozyme variation. The three loci that are reported to be genetically variable show isozyme patterns similar to those reported in other fish, and conform to a simple genetic model.

The horizontal starch gel electrophoresis techniques used followed those of May, et al. (1979). Staining followed the methods of Allendorf, et al. (1977). The genetic nomenclature used is that of Allendorf and Utter (1979). (See Appendix A for detailed explanation of the electrophoretic process written by May, 1975).

Results

Scaphirhynchus albus and S. platorynchus were genetically identical at all 52 loci examined. They share common allelic variants at 3 loci (Pgi, Pgm-1, Pgm-2) and displayed the same isozyme patterns at four other variable enzymes in which the genetic basis of the observed variation could not be adequately resolved. The lack of breeding data and the apparent duplicate nature of some of these enzyme loci complicate the interpretation of the isozyme patterns (Slyn'ko 1976). Nevertheless, these data suggest that there may be gene flow between these species.

The allele frequencies between sampling areas were compared to each other in order to estimate the amount of genetic divergence between populations sampled (Table 2). There are no statistically significant differences between populations. If there was gene flow between S. albus and S. platorynchus from Missouri State, one would expect the gene frequencies in these samples to be closer than those between isolated areas. All the populations sampled, however, have similar gene frequencies at the three ploymorphic loci. There appears to have been enough movement of fish, at least historically, to homogenize the gene frequencies throughout the range examined. There are also no differences in gene frequencies between S. albus and the S. platorynchus. This information further supports that there is interbreeding between these fish.

Disucssion

The results seem to contradict the current taxonomic status of these sturgeon; there are two morphologically distinct species with no detectable genetic differences. There are at least three possible explanations of this: (1) S. platorynchus and S. albus represent a morphological polymorphism within the same species; (2) nonrandom mating and selection against intermediate morphological forms; (3) these two species have recently diverged and have not yet accumulated any genetically detectable differences.

The sympatric existence of two morphological types (i.e. morphotypes) within a population may have given rise to taxonomic separation by traditional ichthyological systematics. However, this approach of classification may not reflect reproductive relationships (Allendorf, 1979). Two extreme situations have been documented: sibling species in which morphologically similar groups are reproductively isolated (Ryman et al., 1979), and a single random mating population in which the morphological differences are simply a phenotypic polymorphism in a single species (Sage and Selander, 1975).

The morphological differences observed between these fish may be maintained in spite of some gene flow between these species. Assuming that electrophoretically

detectable genetic variants are selectively neutral, only one reproductively successful migrant between populations each generation is sufficient to maintain similar gene frequencies between these "species". Isolating mechanisms such as assortative mating, due to the size difference between the two species, may limit gene flow. Differences in natural selection pressures brought about by habitat preferences may also be an important factor in maintaining morphological differences. These phenotypic differences could be maintained by selection in spite of enough gene flow between morphotypes to keep gene frequencies similar.

The inability to detect any isozyme differences may be due to recent speciation. When gene flow between two populations cease, changes in gene frequencies occur as a result of genetic drift or differential selection. Even though these sturgeon are primitive fish, the rate of their protein evolution should be the same as to other vertebrates (Wilson et al., 1978). If these fish are reproductively isolated, it is unlikely that there are no observable gene frequency differences. The identification of morphological intermediates further discounts the recent speciation hypothesis.

The apparent lack of complete reproductive isolation between S. platorynchus and S. albus has important evolutionary and management implications. Did the reproductive isolation develop allopatrically, and the incomplete or subsequent breakdown of isolating mechanisms through recontact? One possible allopatric model is an ancestral population being split into a lacustrine and fluvial ecotypes. Being enclosed into a large glacial lake allowed the development of S. albus into a large, more piscivorous fish. The other portion of the population which now represents S. platorynchus remained in a lotic habitat.

The river sturgeon may have diverged sympatrically. This morphological polymorphism may have come about by the development of two life history strategies. The shovelnose sturgeon represents a small, more quickly maturing, shorter-lived race.

The pallids, on the other hand, developed into a large, long-lived, later-maturing form. This variation in life history strategies may have allowed for the successful survival of these ancient fish.

The genetic population structure of the S. platorynchus populations indicates some important ecological aspects. The similar gene frequencies over a large geographical range is characteristic of a random mating population. This similarity may also come about by the presence of discrete populations having substantial gene flow between them. There has been insufficient time since these areas were isolated from each other by barrier dams for the gene frequencies to drift apart. The larger and more stable the reproductive population size is, the slower genetic drift will occur.

Conclusion

Perhaps S. albus and S. platorynchus are not species; further data are needed. The genetic basis of additional electrophoretic polymorphisms must be understood. Most importantly, sympatric samples of both species from more than one location are needed. The genetic basis of the size differences and the determination of the ability of these fish to produce the alternate morphotype is also important. By these genetic and other ecological research, the status of the S. albus gene pool can be resolved.

The interbreeding of these sturgeon should not lessen the concern of the status of the S. albus gene pool. They represent a valuable genetic resource which is presently endangered. If the survival of this large river sturgeon is in jeopardy, it is important that the biological basis of this 'stock' be understood.

Literature Cited

Allendorf, F. W. 1979. Genetic species versus ecological species: Implications for managmenet of fish stocks. Abstracts of papers at the 109th annual meeting of the Amer. Fisheries Soc.

Allendorf, F. W. and F. M. Utter. 1979. Population genetics. In: Fish physiology, Vol. 8, Eds., Hoar and Randall. Academic Press, N.Y. p. 407-454.

Allendorf, F. W., N. Mitchell, N. Ryman, and G. Stahl. 1977. Isozyme loci in brown trout (Salmo trutta L.): Detection and interpretation from population data. Herditas 86:179-190.

Bailey, Reeve M., and Frank B. Cross. 1954. River sturgeons of the American genus Scaphirhynchus: Characters, distribution, and synonymy. Mich. Acad. of Sci., Arts, and Letters, Vol. XXXIX: 169-208.

Berg, L. S. 1911. Faune de la russie et des pays limitrophes. Poissons (Marsipobranchii et pisces), Vol. 1. St. Petersburg. 337 pp. Cited in Baily and Cross 1954. -1948. Fresh water fishes of the U.S.S.R. and adjoining countries, Vol. I. Acad. Sci. U.S.S.R., Moscow. 466 pp.

Forbes, Stephen A., and Robert E. Richardson. 1905. On a new shovelnose sturgeon from the Mississippi River. Bull. Ill. St. Lab. Nat. Hist., 7:37-44, pls. 4-7.

May, B. 1975. Electrophoretic variation in the genus Oncorhynchus: The methodology, genetic basis, and pratical application to fisheries research and management. M.S. Thesis, U. of Wash. 95 p.

May, B., J. E. Wright, and M. Stoneking. 1979. Joint segregation of biochemical loci in Salmonidae: Results from experiments with Salvelinus and review of the literature on other species. J. Fish. Res. Bd. Can. 36:1114-1128.

Mayr, E. 1963. Animal species and evolution. Harvard Univ. Press. Cambridge.

Ohno, S., J. Muramoto, C. Stenius, L. Christian, W. Kittrell, and N. Atkin. 1969. Microchromosomes in holocephalian, chondrostean and holostean fishes. Chromosoma (Berl.) 26:35-40.

Ryman, N., F. W. Allendorf, and G. Ståhl. 1979. Reproductive isolation with little genetic divergence in sympatric populations of brown trout (Salmo trutta). Genetics 92:247-262.

Sage, R. D. and R. K. Selander. 1975. Trophic radiation through polymorphism in cichlid fishes. Proc. Nat. Acad. Sci. U.S.A. Vol. 72, No. 11, p. 4669-4673.

Slyn'ko, V. I. 1976. Multiple molecular forms of malate dehydrogenase and lactate dehydrogenase in russian sturgeon (Acipenser güldenstäoti BR.) and great sturgeon (Huso huso L.). Akademia Nauk. Biol. Ser. Doklady 228(1-6):201-204.

Wilson, A. D., S. S. Carlson, and T. J. White. 1977. Biochemical evolution. Ann. Rev. Biochem. 46:573-639.

Figure 1. Sturgeon sampling areas

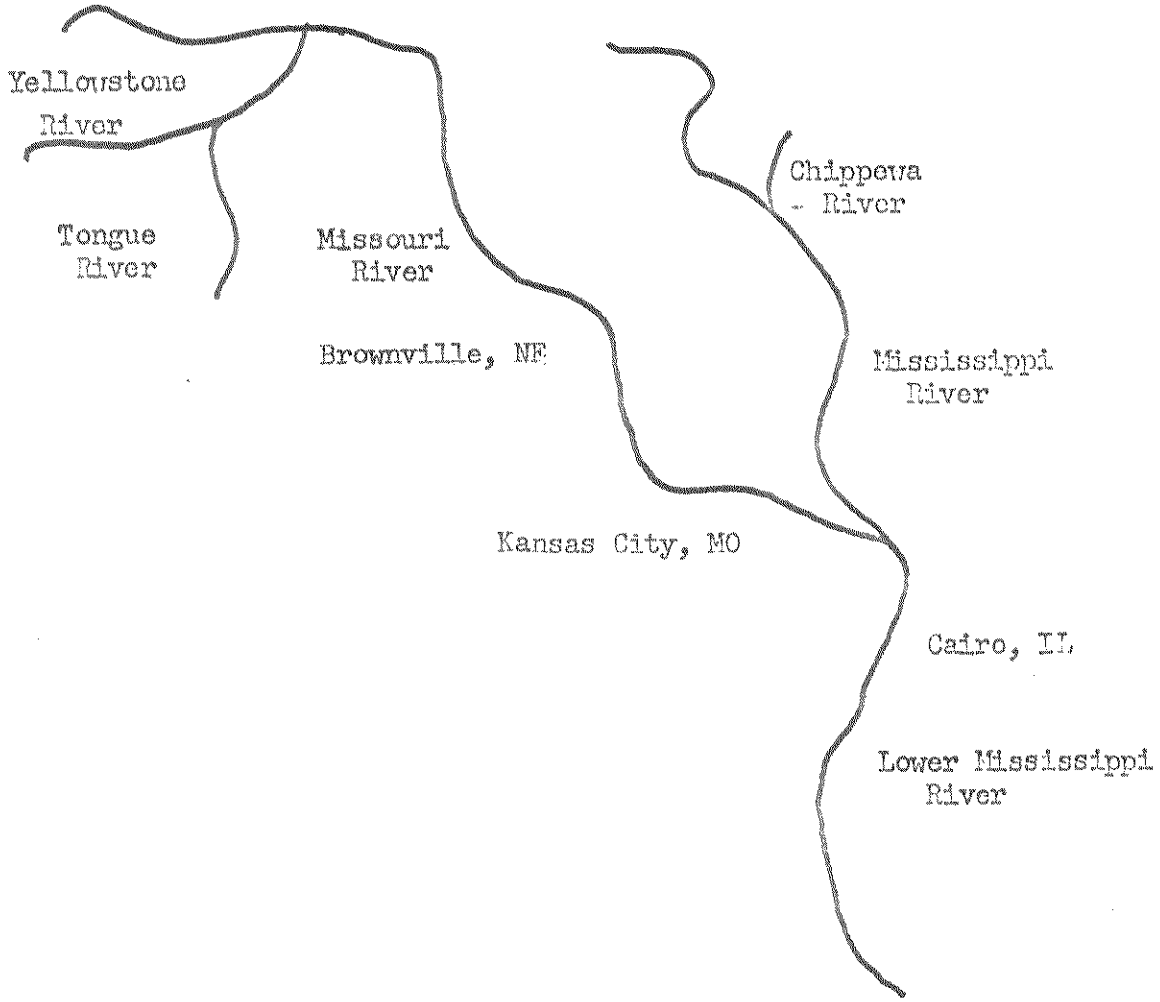


Table 1. Designation of loci coding for different enzymes in *Scaphirhynchus*

Tissues. B = brain, E = eye, H = heart,
I = intestine, L = liver, M = muscle

Enzyme	Locus Designation	Buffer System	Tissue	Polymorphic
AAT	1	AC,RW	all	no
	2	RW	all	no
	3	AC,RW	all	} yes
	4	AC,RW	all	
ADH	no activity			
AK	1	AC	H,L,M	yes?
	2	AC	M	no
	3	AC	H,M	no
AGP	1	AC,RW	L	no
	2	AC,RW	L	no
	3	AC,RW	H	no
ALD	no activity			
CPK	1	AC,RW	M	no
	2	AC,RW	L	no
	3	AC,RW	B,E,H,I	no
DIA	no activity			
EST	1	AC,RW	all	no
	2	AC,RW	all	no
FUM	no activity			
GAP	1	AC,RW	H,M	?
	2	AC,RW	H,L,M	no
	3	AC,RW	E	no
GDH	1	AC,RW	M	no
	2	AC,RW	L	no
	3	AC,RW	H	?
GUS	1	AC,RW	B,H,I,L,M	no
G6PDH	1	AC,RW	B,E,H,L	no
	2	RW	L	no
HK	no activity			

Table 1 continued. Designation of loci coding for different enzymes in Scaphirhynchus

Tissues. B = brain, E = eye, H = heart,
I = intestine, L = liver, M = muscle

Enzyme	Locus Designation	Buffer System	Tissue	Polymorphic
IDH	1	AC,RW	all	no
LAP	1	AC	I	no
LDH	1	AC,RW	H,I,L	?
	2	AC,RW	H,I,L	?
	3	AC,RW	B,E,H,M	} yes
	4	AC,RW	B,E,H,M	
	5	AC,RW	B,E,I,H	} yes
	6?	AC,RW	B,E,I,H	
MDH	1	AC,RW	all	no
	2	AC,RW	all	yes
ME	1	AC	H,I,L,M	no
	2	RW	H,I,L,M	?
	3	RW	H	no
PEP	1	AC,RW	all	no
	2	AC,RW	all	no
PGI	1	RW	H,L,M	yes
	2	RW	H,L,M	no
	3	RW	H,L,M	no
PMI	1	AC,RW	all	no
	2	AC	all	no
PGM	1	AC,RW	all	yes
	2	AC,RW	all	yes
	3	AC,RW	B,E,I,L	?
6PGDH	1	AC	all	no
SDH	1	AC,RW	L	no
SOD	1	RW	H,L,M	yes
XDH	1	RW	H,L,M	no
GENERAL PROTEIN	4 sharp identifiable bands, muscle tissue, RW buffer			

1 CONT.

Table List of enzymes used in the study.

AAT	Aspartate aminotransferase
*ADA	Adenonsine deaminase
ADH	Alcohol dehydrogenase
AGP	α - Glycerophosphate dehydrogenase
AK	Adenylate kinase
ALD	Aldolase
CPK	Creatine phosphokinase
DIA	Diaphorase
EST	Esterase
*FDP	Fructose -1,6 diphosphotase
FUM	Fumerase
G6PDH	Glucose-6-phosphate dehydrogenase
GAPDH	Glyceraldehyde-3 phosphate dehydrogenase
GDH	Glutamate dehydrogenase
*GLYDH	Glycerol dehydrogenase
*GPT	Glutamate pyruvate transaminase
GUS	B-glucoronidase
HK	Hexokinase
IDH	Isocitrate dehydrogenase
LAP	Leucine aminopeptidase
LDH	Lactate dehydrogenase
MDH	Malate dehydrogenase
ME	Malic enzyme
MPI OR PMI	Mannose-6-phosphate isomerase
*NP	Nucleoside phosphorylase
PEP	Peptidase
6PGDH	6-Phosphogluconate dehydrogenase
PGI	Phosphoglucose isomerase
*PGK	Phosphoglycerate kinase
PGM	Phosphoglucomutase
*PK	Pyruvate kinase
SDH	Sorbitol dehydrogenase
SOD	Superoxide dismutase
*SUCDH	Succinate dehydrogenase
*TPI	Triosephosphate isomerase
XDH	Xanthine dehydrogenase

* NOT USED

Table 2. Allele frequencies at Pgi, Pgm-1 and Pgm-2

Location	Pgi*	Pgm-1*	Pgm-2*	Number of genes examined
		S. platorynchus		
Cairo, IL	.90	.87	.98	40
Kansas City, MO	.89	.94	1.00	18
Lower Mississippi, MO	.78	.95	.98	40
Missouri River, MT	.87	.97	.99	76
Tongue River, MT	.88	.97	1.00	70
Brownville, NE	.92	.96	.98	50
Chippewa River, WI	.74	.96	.98	60
		S. albus		
pooled samples	.81	.96	.96	26
		Hybrids		
pooled samples	.88	1.00	1.00	8

*Frequency of the common (100) allele

Appendix A

BASIC EXPLANATION OF THE ELECTROPHORETIC PROCEDURE (MAY, 1975)

Sample Preparation

Tissues that were not tested at the time of collection were frozen at -40°C for variable lengths of time. Some proteins were stable during freezing for long periods of time (e.g., PGM, LDH, and TO) while others tended to lose activity rapidly (e.g., AAT, IDH, and ADH). In general, muscle enzymes were more stable than liver enzymes.

Tissue extracts from muscle, brain, heart, liver, eye, and intestine were prepared by combining equal amounts of tissue and water in 12 x 75 mm glass tubes and squashing this mixture with a glass rod. This process breaks the cell membranes and releases the soluble enzymes contained in that tissue into the water. The extracts were then centrifuged for 5 minutes at 1000 x g to separate the soluble proteins form the broken tissues.

Gel Preparation

A form for the starch gel was prepared by clamping four plexiglass strips to a 10 1/2" X 7" X 1/4" glass plate (Figure 1). The vertical strips measured 7" X 3/4" X 1/4" and the horizontal strips measured 8 1/2" X 3/4" X 1/4". Thicker strips were used (3/8" or 1/2") when thicker gels were required.

The gels were prepared using 35 grams of hydrolyzed potato starch (Electrostarch-Electrostarch Co., Madison, Wis.) in 250 ml of the appropriate buffer. One quarter of the buffer was used to suspend the starch in solution in a 1000 ml erlenmeyer flask. The remainder of the buffer was heated to boiling and then added to the unheated starch-buffer suspension while constantly swirling the flask. This mixture was heated to boiling and kept at boiling temperature for a few moments while

occasionally swirling the flask to prevent burning the starch. The mixture was degassed under the vacuum of a water aspirator for approximately one minute to remove any air bubbles. The starch was then poured into the form described above and allowed to cool to room temperature before using.

Electrophoresis

The prepared gel was cut approximately 3 cm from the cathodal end. Filter paper wicks (3 X 8 mm) were dipped in the extracts and placed vertically in the gel cut. Forty wicks could be placed on a single gel in this manner (Figure 2). A wick containing a dye marker (diluted red food coloring) was placed at the end of the gel to determine the rate of protein migration. Disposable, absorbant cloths (e.g., Handi-wipes) were used to conduct the electric current from the tray buffer to the appropriate ends of the gel (Figure 3).

The wicks were removed after ten to fifteen minutes of electrophoresis and the two sections of the gel were placed firmly together. An ice pack on a glass plate was placed on top of the gel and the appropriate voltage applied until the dye marker had migrated 3 to 8 cm from the cut (origin); optimal migration distances for a given buffer system varied among proteins.

Staining Procedures

After completion of electrophoresis, the gel was sliced horizontally (Figure 4) into four or more sections, depending on the thickness of the gel. This was done by sequentially placing pairs of one-sixteenth inch plastic strips on the sides of the gel and drawing monofilament sewing thread through the gel. The slices were placed into individual staining trays with the side of the slice nearest the center of the gel facing upward.

Enzymes catalyze specific biochemical reactions. It is therefore possible to visualize the location of a particular enzyme on the starch gel by supplying the appropriate substarte and cofactors and by involving a product of the enzymatic reaction in a color producing reaction. The colored product becomes deposited on the gel forming a visible band where a particular enzyme has been electrophoretically localized. As an example, the staining reaction for the enzyme lactate dehydrogenase is diagrammed in Figure 5.

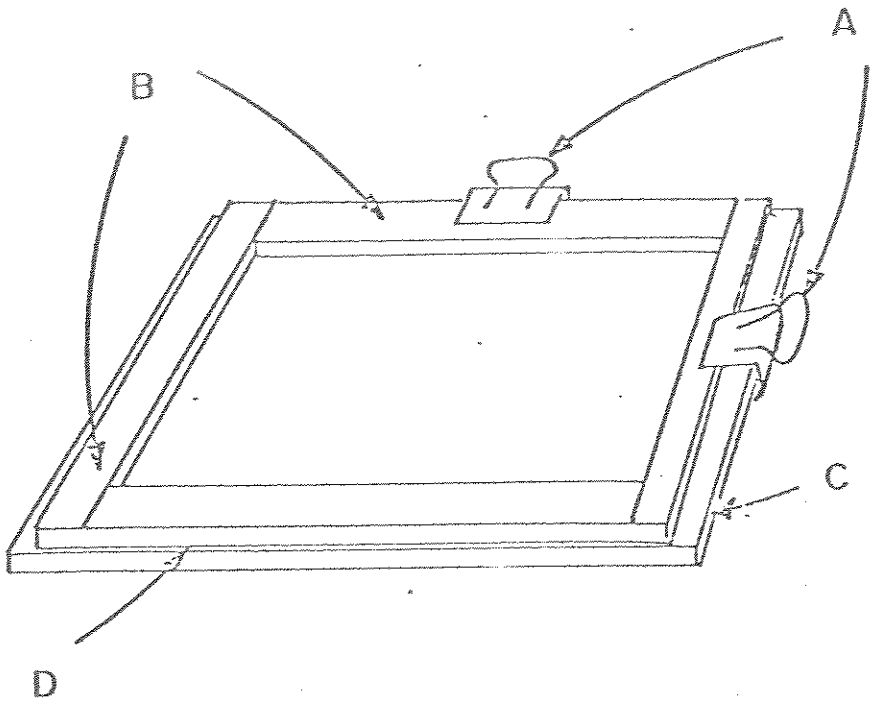
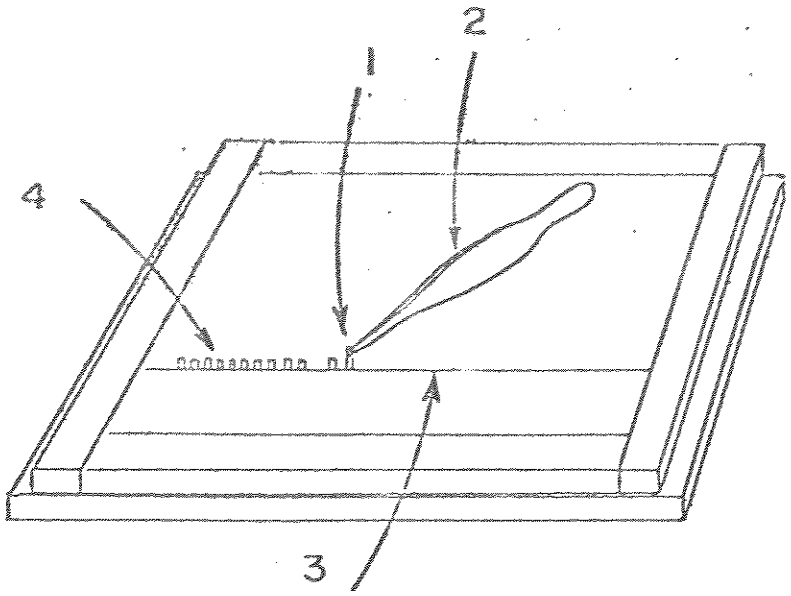


Figure 1.--Form for starch gel.

A - The plexiglass strips are firmly held in place with paper clamps.
B - Plexiglass strips. Vertical - 7" x ¼" x 3/4";
Horizontal - 8½" x ¼" x 3/4".
C - Glass plate - 10½" x 7" x ¼".
D - Firm contact between the glass plate and plexiglass strips is made by moistening strips with water.



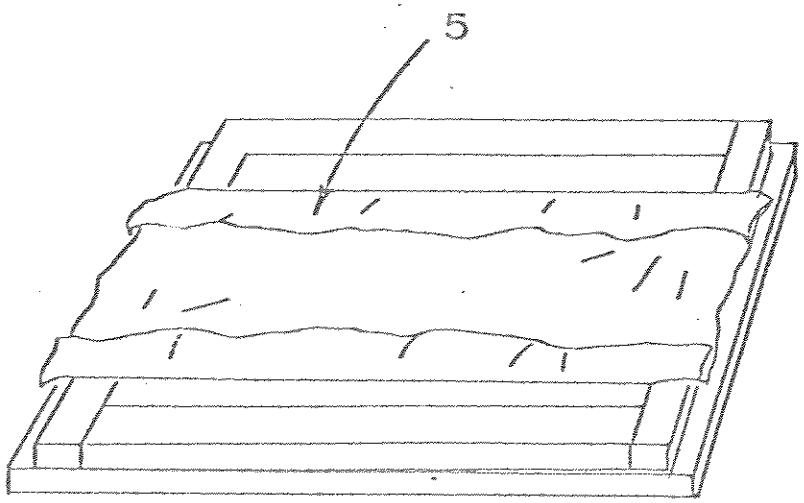


Figure 2.--Placement of samples on starch gel.

1 - Filter paper inserts (Schleicher and Schuell grade S&S No. 470) are saturated with test samples and inserted adjacently in the cut portion of the gel. Each insert holds approximately 0.02 ml of sample.
2 - Forceps.
3 - Gel is cut at three cm with a scalpel.
4 - One to two mm spacing between samples.
5 - A layer of plastic wrap is placed over the gel and folded back to expose 1 cm of gel at each end.

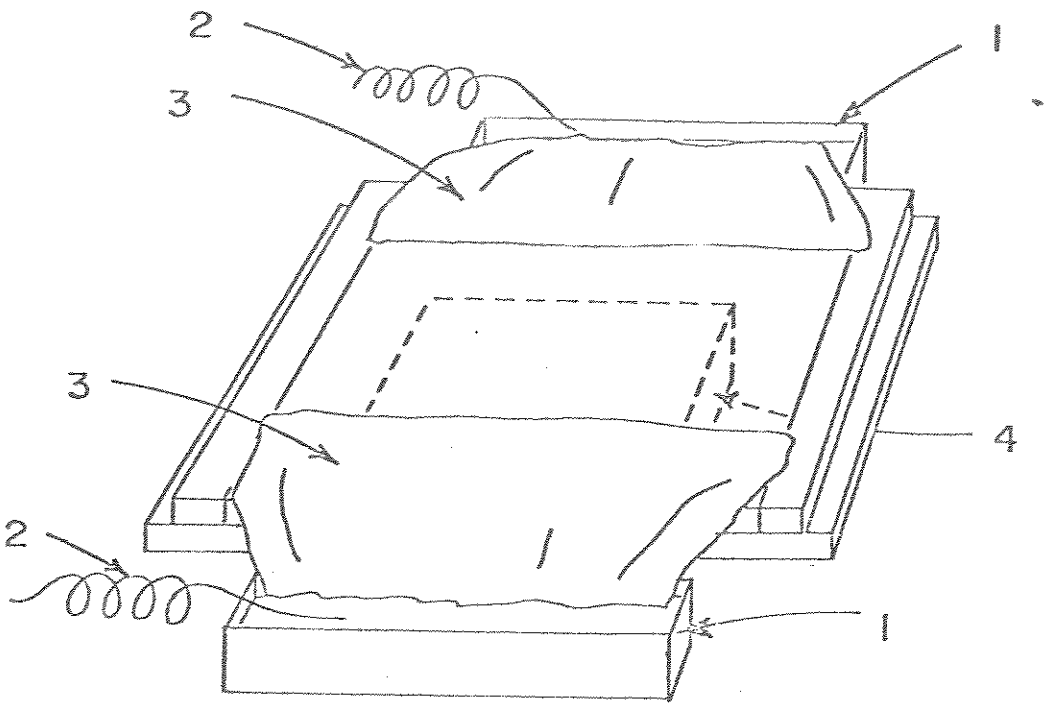


Figure 3.--Preparation of gel for electrophoresis.

1 - Plastic butter dish covers (or similar containers) are filled 2/3-full with the electrode buffer solution.
2 - Platinum electrodes are placed in the buffer and secured with clothes-pins.
3 - An absorbant wick (e.g., Handiwipes) is placed in the buffer solution and firmly pressed to the surface of the gel, covering the exposed surface.
4 - The gel frame is elevated about two inches by any convenient material, placed beneath the glass plate.

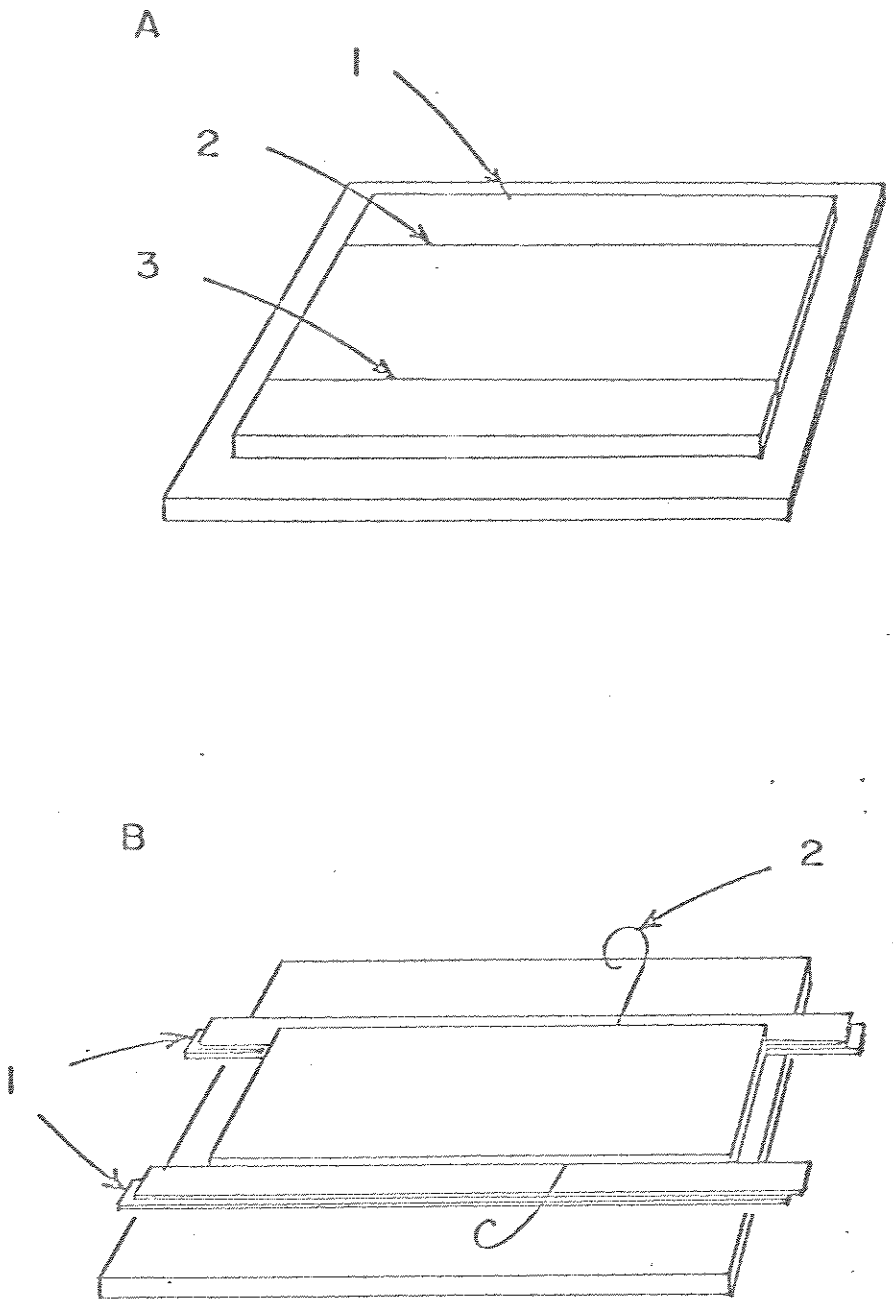


Figure 4.--Slicing procedure for starch gels.

A-1. When electrophoresis is finished, the plexiglass strips are removed and those portions of the gel anodal to the buffer boundary or dye marker are discarded.
A-2. Buffer boundary.
A-3. Origin.
B-1. Sets of plexiglass strips of 1/16" thickness are sequentially placed on each side of the gel.
B-2. The gel is cut by pulling a tightly drawn nylon thread along the surface of the strips.

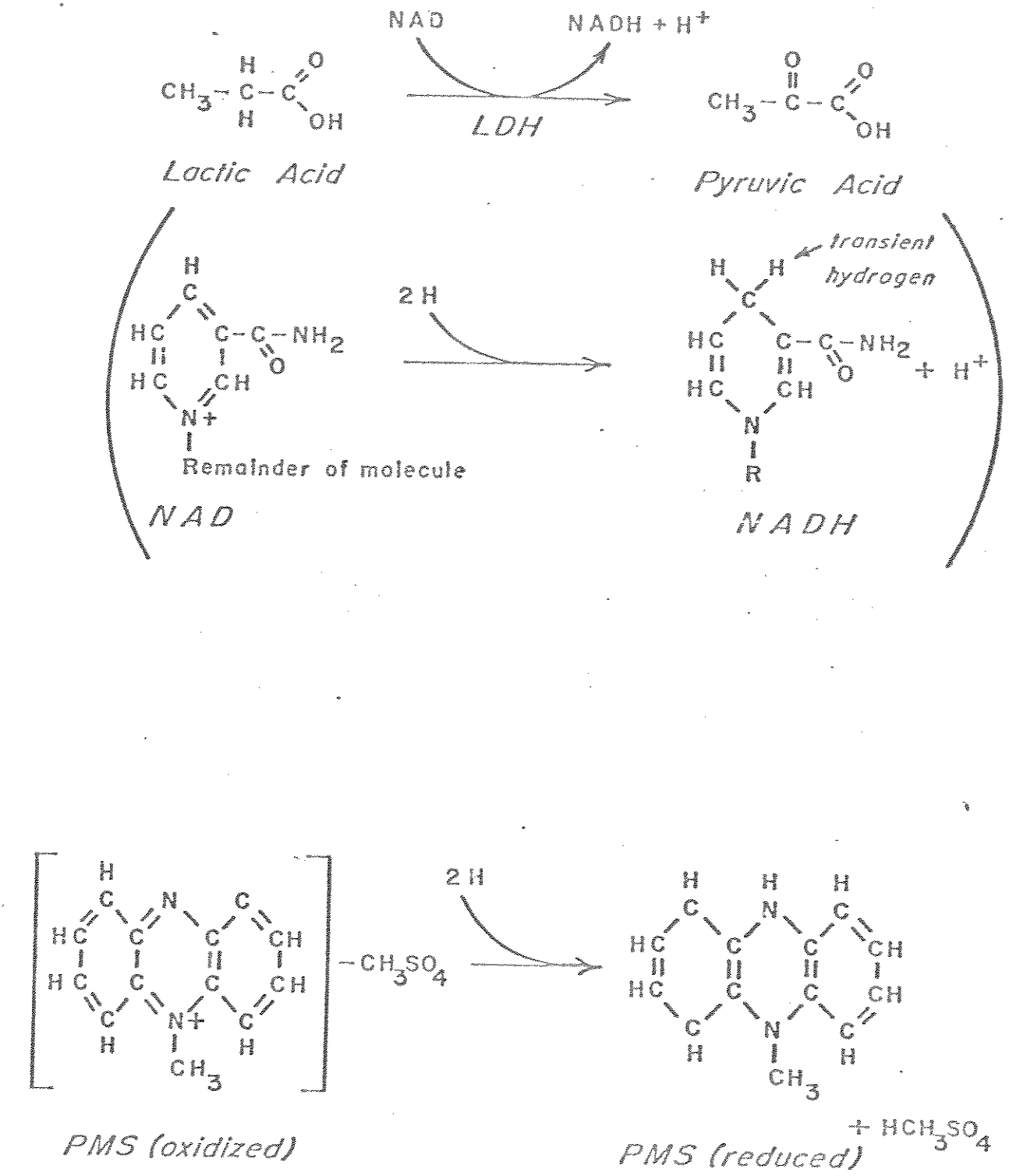


Figure 5.--Staining reaction for the enzyme lactate dehydrogenase. Lactate dehydrogenase (LDH) catalyzes the conversion of lactic acid to pyruvic acid, reducing nicotinomide adenine dinucleotide (NAD) and releasing a H^+ ion into solution. The transient hydrogen of NADH is picked up by an intermediary, phenazine methosulphate (PMS).

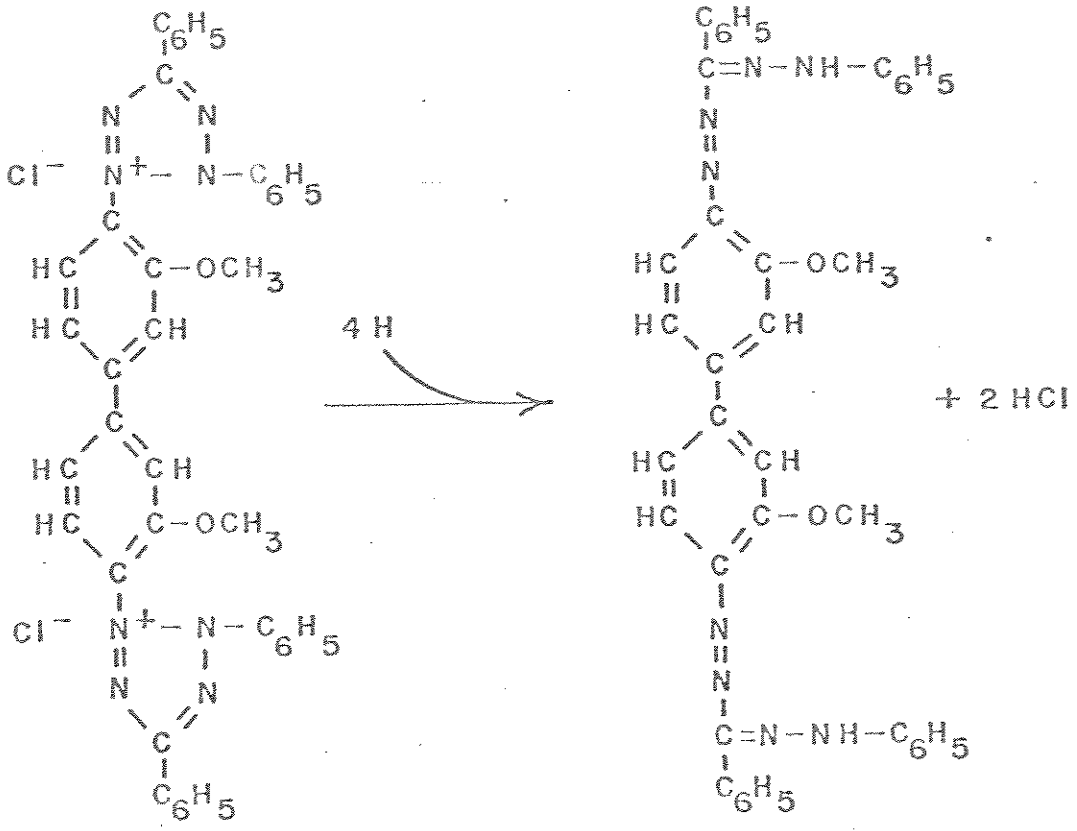


Figure 5.--Continued. PMS then passes on the hydrogen to nitro-blue tetrazolium (NBT), a yellow soluble salt, converting it to a blue insoluble dye. The insoluble blue dye is seen only where the enzyme is present on the gel.