2. Child III, C. G., *ibid., Comment,* 1957, v146, 690.

3. Child III, C. G., *Hepatic Circulation and Portal Hypertension,* Saunders, Phila., 1954.

4. Olivier, L., Stirewalt, M. A., *J. Parasitol.,* 1952, v38, 19.

5. Rodriguez, H. F., Garcia-Palmieri, M. R., Rivera, J. V., and Rodriguez-Molina, R., *Gastroenterology,* 1955, v29, 235.

6. Bibawi, E., ElDeeb, A. A., Mahfouz, M. M., *Am. J. Trop. Med. and Hyg.,* 1955, v4, 913.

7. Lichtenberg, F., *Am. J. Path.,* 1955, v31, 757.

Received December 27, 1957. P.S.E.B.M., 1958, v98.

Geographic Variation in Plasma Protein Patterns of Snakes. (23953)

HERBERT C. DESSAUER AND WADE FOX

Departments of Biochemistry and Anatomy, Louisiana State University School of Medicine, New Orleans

We have reported(1) that electrophoretic patterns of plasma proteins of a number of species of reptiles and amphibians show distinct geographic variation. Some of these protein differences occur between currently recognized geographic races, whereas other differences appear to be independent of morphological variation. To our knowledge only 3 other examples of intraspecific variations in the pattern of plasma proteins have been reported. Deutsch and McShan(2) found distinct differences between patterns of the channel catfish and the white catfish which were considered at that time to be of the same species. Later studies, however, suggest that these two forms should be classified as different species(3). Smithies(4) demonstrated the presence of genetically determined differences between patterns of Caucasian and African races of man. Recently, Zweig and Crenshaw(5) have shown that 2 races of the turtle, *Pseudemys floridana,* can be differentiated by their plasma patterns. The present paper describes the nature of geographic variations found in plasma patterns of several species of snakes (common kingsnakes, racers, common water snakes, and common garter snakes).

Materials and methods. Snakes were either collected personally or obtained from naturalists in other sections of the United States. Locality of capture and scientific and common names(6) were recorded as exactly as possible for each animal. After a fast of one week, specimens were anesthetized with ether and bled from a cardiac puncture. The condition of the primary and secondary sex organs was noted; animals were fixed in formalin, catalogued, and saved for deposit in the La. State Univ. Museum of Zoology. Blood was collected in heparinized tubes and centrifuged at 4°C. Plasma was separated immediately from cells and an aliquot of plasma was subjected to paper-electrophoresis on the same day that the animals were bled. In each electrophoretic run, a sample of human plasma served as a standard reference. Two-hundredths ml of plasma was applied across a 1 inch strip of Muncktells 20 S filter paper and fractionated in barbital buffer (pH 8.6, ionic strength 0.05, 15% glycerol v/v) for 17 hrs at 170 v with an LKB paper-electrophoresis unit operating at 26 to 28°C. Details of the electrophoretic procedure have been published(1). After electrophoresis, each strip was dried and stained with bromphenol blue. The stained strips were made translucent with mineral oil and scanned at 590 mμ with Beckman DU spectrophotometer. Optical densities were plotted against distance along the strip, and the area under the resulting curves was used to calculate percentages of protein fractions. In order to eliminate possible variabilities due to the apparatus, samples of all populations being compared were rerun simultaneously on parallel strips. Fresh samples were not always available on the same day and frozen plasma (stored at −20°C), which produced patterns nearly identical to the fresh, was substituted.

Results. Plasma protein patterns of snakes characteristically exhibit 2 distinct groups of anodal fractions which are separated by an

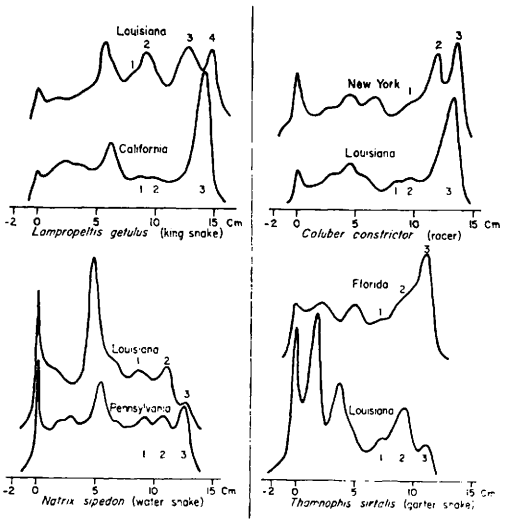

FIG. 1. Paper-electrophoretic patterns of plasma proteins of geographic variants of 4 species of snakes. Plasma was applied at 0 cm; anode is to the right. In pattern of La. specimen of *T. sirtalis* peak at 2 cm is caused by a protein fraction which appears in plasma of snakes during estrus. Only the numbered peaks (Fast Group fractions) are used in geographical comparisons.

area of low protein concentration (Fig. 1). The slower migrating proteins (Slow Group) exhibit one or two well resolved bands between 3 and 7 cm from starting line. The fast migrating proteins (Fast Group) consist of 3 or 4 bands between 7 and 15 cm from the starting line.

The pattern of Slow Group proteins is highly variable even in specimens from a single population. The quantity of these proteins increases with age. They contribute only a small percentage to the total plasma protein of young animals(7). In females of viviparous species such as *Natrix* and *Thamnophis*, not only does the total plasma protein increase at height of estrus, but a new fraction, which may include as much as 20% of total plasma protein, appears in the Slow Group(8). Such a fraction can be seen at the 2 cm position in the pattern of the Louisiana specimen of *T. sirtalis* (Fig. 1). Hemoglobin in plasma of hemolyzed samples migrates with Slow Group proteins(9). In contrast to the variability of the Slow Group, patterns of Fast Group proteins are relatively uniform in specimens from a single population. They are much less affected by such factors as age, sex and hemolysis. For these reasons we believe that Fast Group proteins are more useful than Slow Group proteins in studies of geographic variation. In the comparisons of populations (Table I) we avoided using the Slow Group proteins by computing the percentage of Fast Group fractions relative to total Fast Group protein only.

A striking example of geographic variation in plasma protein is found in the patterns of the common kingsnake, *Lampropeltis getulus* (Table I, Fig. 1). A single protein band as the lead fraction is characteristic of Calif. specimens, whereas in kingsnakes from the southeastern United States the protein in the same region of the strip resolves into two bands. Plasma patterns of the two well known color variants of the California kingsnake are identical. Only minor differences were noted between patterns of kingsnakes from Ga., Ark., La., and Miss. The pattern of the blue racer, *Coluber constrictor*, from New York is similarly characterized by two well resolved lead fractions, whereas only one fraction is present in the same region of the strip in patterns of other racers sampled (Table I, Fig. 1).

Plasma patterns of common water snakes, *Natrix sipedon*, captured in different areas often exhibit quantitative differences in Fast Group fractions of similar mobility. Fast Group proteins of this species characteristically resolve into 3 fractions. In the patterns of the La. and Pa. specimens (Fig. 1) these fractions appear on the anodal side of the trough at 7.5 cm. In the lower Mississippi Valley, the region in which the races *pleuralis, fasciatus, confluens* and *clarki* (?) intergrade, populations living in close proximity often exhibit different patterns (Table I). For example, the pattern of a population from Lutcher, La., differs from that of a population from Westwego, La., which is only 30 miles distant and on the opposite bank of the Mississippi River. The pattern of the specimen from Bay St. Louis, Miss., is markedly different from that of the animal from Wesson, Miss., 150 miles to the north. The pattern of the Gulf marsh snake, *N. s. clarki*, differs in fraction mobility and other characteristics from *N. s. confluens* found in an adjacent but less saline environment.

TABLE I. Relative Migrations of Fast Group Fractions and Their Percentage of Total Fast Group Proteins.

Area of capture	No. of animals	Subspecies	Relative migration*† 1	2	3	4	% of fast group proteins† 1	2	3	4
			Lampropeltis getulus							
Calif.	3	*californiae*	67 ± 2	77 ± 2	104 ± 3		12 ± 1	14 ± 2	74 ± 3	
Ga.	4	*getulus*								
Ark.	2	*holbrooki*	59 ± 3	70 ± 2	95 ± 4	112 ± 4	17 ± 4	24 ± 4	49 ± 6	20 ± 6
La.	21	"								
Miss.	2	"								
			Coluber constrictor							
N. Y.	1	*constrictor*	69	88	102		25	37	38	
Fla.	1	*priapus*								
Miss.	2	"	57 ± 4	71 ± 3	95 ± 4		12 ± 4	21 ± 7	67 ± 8	
La.	14	*flaviventris*								
Ark.	2	"								
Calif.	2	*mormon*	61 ± 1	70 ± 3	96 ± 1		14 ± 3	5 ± 1	81 ± 3	
			Natrix sipedon							
Pa.	1	*sipedon*								
N. Y.	2	"	64 ± 3	76 ± 4	87 ± 3		37 ± 6	28 ± 3	35 ± 4	
Mass.	2	"								
Ill.	2	*sipedon*	64 ± 2	75 ± 3	85 ± 1	93 ± 2	25 ± 2	25 ± 3	21 ± 3	29 ± 2
Mo.	2	*pleuralis*	66 ± 4	80 ± 2	91 ± 3		34 ± 3	18 ± 4	49 ± 2	
Miss.										
Wesson	1	*pleuralis* (?)	65	75	90		38	17	46	
Bay St. Louis	1	*confluens*(?)	65	80	93		55	21	24	
La.										
Lutcher	6	*confluens*	68 ± 2	85 ± 4	96 ± 3		41 ± 9	47 ± 8	12 ± 3	
Westwego	9	*confluens*	67 ± 3	81 ± 3	94 ± 4		54 ± 5	37 ± 8	9 ± 5	
Other areas	9	*confluens*	68 ± 4	83 ± 4	95 ± 4		50 ± 5	39 ± 6	11 ± 5	
Theriot	4	*clarki*	70 ± 3	80 ± 3	90 ± 2		30 ± 4	35 ± 2	35 ± 3	
Fla.	7	*pictiventris*	64 ± 4	79 ± 4	91 ± 4		31 ± 3	21 ± 3	48 ± 3	
			Thamnophis sirtalis							
La.	4	*sirtalis*	67 ± 6	85 ± 5	102 ± 3		29 ± 2	54 ± 3	17 ± 2	
Minn.	6	*sirtalis*	65 ± 4	85 ± 3	98 ± 2		25 ± 3	39 ± 3	36 ± 3	
Fla.	5	*sirtalis*	63 ± 3	84 ± 3	97 ± 3		24 ± 4	33 ± 5	43 ± 2	
N. Y.	2	*sirtalis*	69 ± 4	84 ± 4	97 ± 4		30 ± 7	37 ± 7	34 ± 9	
Mass.	10	"								
Ore.	4	*concinus*	68 ± 4	80 ± 5	95 ± 3		26 ± 6	38 ± 5	36 ± 5	
Wash.	5	"								
Calif.										
San Joaquin	6	*fitchi*	74 ± 4	95 ± 5			25 ± 4	75 ± 5		
San Francisco	6	*tetrataenia*	70 ± 5	93 ± 4			22 ± 4	78 ± 5		

* Distance which fraction migrates × 100/distance which reference human plasma albumin migrates (avg of 12.7 ± .8 cm). † Mean ± stand. dev.

The Fast Group proteins of the common garter snake, *Thamnophis sirtalis,* also generally resolve into 3 fractions. In the pattern of the eastern garter snake, *T. s. sirtalis* (Fig. 1), the trough which separates Slow from Fast Group proteins appears at 6 cm. Although La. and Fla. populations are currently recognized as members of the same subspecies, their patterns differ both in rate of migration and in relative amounts of Fast Group fractions. Other regional differences occur within this subspecies (Table I). Of the west coast garter snakes, the pattern of *T. s. concinus* from Ore. and Wash. appears more similar to those of the eastern subspecies than to those of two Calif. races, *T. s. fitchi* or *T. s. tetrataenia.* Patterns of *fitchi* and *tetrataenia* differ from other subspecies of *T. sirtalis* but are quite similar to each other.

Discussion. Previous studies of geographic variation have dealt primarily with morphological differences between populations of a species which replace each other geographically (allopatric populations). More recent

studies reveal that physiological differences between such populations are prevalent(10) although less obvious. Our data show that modifications of plasma proteins are also present. These modifications are revealed by variations in position, number and quantity of protein fractions in paper-electrophoretic patterns of plasma.

Individuals of populations from a limited geographic area tend to have very similar plasma protein patterns, whereas those from widely separated areas usually have different patterns. Kingsnakes and racers are good examples of species in which geographic differences are reflected as variations in the position and number of fractions. In these species the lead protein migrates as a single peak in some populations but as two peaks of different mobility in other populations.

Quantitative variations in fractions of similar mobilities are well illustrated by a comparison of patterns of different populations of water snakes. In *Natrix sipedon*, for example, populations within the subspecies *N. s. confluens* have small, but probably significant, differences in the relative quantity of certain fractions. Even greater differences occur between characteristic subspecies (*N. s. confluens*, *N. s. sipedon* and *N. s. pictiventris*). Dissimilarities between the pattern of *N. s. clarki* and those of other races of *N. sipedon* are of sufficient magnitude to raise a question as to the justification of its being classified as a race of this species. Further sampling of the species and a better understanding of the significance of the patterns are necessary, however, before the technic of paper-electrophoresis can be utilized as a valid basis for taxonomic decisions.

It would be premature to assert that most geographic differences in plasma proteins are genetically determined. Several observations, however, favor this hypothesis: patterns of Fast Group proteins show little seasonal variation, only minor differences with age and insignificant differences between the sexes. Further, a Fast Group protein pattern may be either distributed uniformly over a large area or may be quite variable within a small area. Since electrophoretic patterns thus display the same type of variation and distribution as morphological and physiological characteristics, it seems logical to propose that they are also inheritable and subject to natural selection.

In keeping with the above hypothesis is the interesting parallelism which appeared when patterns of *T. sirtalis* and *N. sipedon* from the delta region of S.E. La. were compared with patterns of their respective counterparts from N.E. states and Fla. In both species the concentration of the fastest moving fraction was much less in the patterns of La. samples than in those of the eastern populations. Further investigation revealed a similar relative fraction difference between La. and Fla. specimens of both *T. sauritus* (ribbon snake) and *N. cyclopion* (green water snake). In view of the fact that these four species are closely related (subfamily Natricinae) it seems plausable to suggest that due to a basically similar genetic potential they have utilized a common means of adapting to the delta environment.

We propose that in adapting to a local environment populations have accumulated genetic mutations which produce minor alterations of the plasma proteins, some of which affect their electric charge. These result in small alterations in electrophoretic mobilities or in the quantities of characteristic electrophoretic fractions. The amount of variation possible appears to be held within certain limits determined by a basic species pattern and consequently closely related species may show similar adaptations.

Summary. Distinct geographic differences between plasma proteins are revealed in kingsnakes, racers, water snakes and garter snakes by variations in the position, number and quantity of fractions of paper-electrophoretic patterns. The faster migrating fractions of the patterns are most useful in such comparisons as slower migrating fractions exhibit much physiologic variation.

We wish to thank the following persons for contributions of specimens: Bayard Brattstrom, W. E. Brode, David Jameson, Ernest Liner, Charles Martinez, William Riemer, Charles Shaw, and Richard Zweifel.

1. Dessauer, H. C., Fox, W., *Science*, 1956, v124, 225.

2. Deutsch, H. F., McShan, W. H., *J. Biol. Chem.*, 1949, v180, 219.

3. Blair, W. F., Blair, A. P., Brodkorb, P., Cagle, F. R., Moore, G. A., *Vertebrates of United States*, McGraw-Hill, N. Y., 1957.

4. Smithies, O., *Biochem. J.*, 1955, v61, 629.

5. Zweig, G., Crenshaw, J. W., *Science*, 1957, v126, 1065.

6. Conant, R., Chairman, Committee on Herpetological Common Names, Soc. of Icthy. and Herp., *Copeia*, 1956, No. 3, 172.

7. Dessauer, H. C., Fox, W., *Fed. Proc.*, 1956, v15, 242.

8. Dessauer, H. C., Fox, W., Gilbert, N. L., PROC. SOC. EXP. BIOL. AND MED., 1956, v92, 299.

9. Dessauer, H. C., Fox, W., Ramirez, J. R., *Arch. Biochem. and Biophys.*, 1957, v71, 11.

10. Prosser, C. L., *Biol. Rev.*, 1955, v30, 229.

Received January 2, 1958. P.S.E.B.M., 1958, v98.

Typing of ECHO Viruses by a Complement Fixation Technic. (23954)

PEKKA HALONEN,* LEON ROSEN AND ROBERT J. HUEBNER

U. S. Dept. of Health, P.H.S., Nat. Inst. of Allergy and Infectious, Diseases, N.I.H., Bethesda, Md.

Typing of ECHO viruses(1) by standard neutralization tests is an inconvenient and laborious procedure because of large number of prototypes. Recent findings, that complement-fixing (CF) antigens for ECHO viruses could be prepared consistently from monkey kidney tissue cultures and that these antigens reacted type specifically with ECHO antisera prepared in guinea pigs and monkeys(2), suggested the possibility of typing ECHO virus isolates by means of a CF technic. This preliminary report describes simple methods which have been successful in typing 28 ECHO virus isolates by such a procedure.†

Methods. CF antigens were prepared by inoculating each of 4 rhesus kidney tissue culture tubes containing 1 ml of the following: lactalbumin hydrolysate 0.5%, calf serum 2%, Earle's solution 97.5%, with 0.1 ml of undiluted fluid from the original or an early monkey kidney passage of the ECHO isolate. After complete degeneration of tissue had taken place, the tubes were frozen and thawed, and pooled tissue culture fluid from these tubes was used as antigen without centrifugation. Of 28 ECHO virus isolates tested, 15 were from a Washington, D.C. welfare institution(3), and 13 were from other sources in Washington, D.C., and from other localities in U.S. and England (ECHO type 9).‡ All were typed originally by neutralization tests except the ECHO type 10 isolates. The latter, however, produced the unique cytopathogenic effect characteristic of the prototype strain of ECHO 10 virus. The CF typing tests for most strains were done without prior knowledge of the neutralization test data. ECHO antisera used in CF tests had been prepared in monkeys(4).§ Homotypic and heterotypic CF titers of these sera against CF antigens of ECHO prototype strains prepared in monkey kidney tissue culture are published elsewhere(2). Dilutions of monkey sera are shown in Table I and in each instance represented at least 4 units of antibody. These dilutions were determined by the desirability of conserving serum and the fact that 4 units of antibody have been used successfully for many years in this and other laboratories as the best working dilutions for purposes of titrating viral and rickettsial antigens. Complement fixation tests were performed by standard technic in this laboratory(2).

Results. Representative results of CF typing tests are shown in Table I. Each ECHO antigen reacted only with homologous antiserum, except the ECHO 8 antigens, which reacted with both type 1 and 8 antisera, and to

* Rockefeller Foundation Fellow and Visiting Scientist, N.I.H. Present address: University of Helsinki, Finland.

† The valuable assistance of H. C. Turner in performing complement fixation tests and H. Fox in preparing antigens is gratefully acknowledged.

‡ The authors are indebted to Dr. Karl Habel for supplying these 13 ECHO isolates.

§ The sera were obtained from Nat. Foundation for Infantile Paralysis.