tests, the preimmunization serum from the monkey was available for comparison with the postimmunization serum. In other instances, a known normal serum was employed in the control titration. All the serums were inactivated at 56°C for 30 minutes prior to being mixed with the virus suspensions.

The significant data pertaining to the cross-neutralization tests are summarized in Table 1. The virus titers obtained in the presence of the various serums have been expressed as the reciprocal of the logarithm of the 50-percent mortality endpoint, which was calculated by the method of Reed and Muench (*3*). The results indicate an immunological relationship between the Trinidad strain and each of the two type strains with which the Trinidad strain was compared. Taking the results as a whole, it would seem that the Trinidad strain is somewhat more closely related to the dengue-2 strain than to the dengue-1 strain. However, the data are not sufficiently clear-cut to permit a final decision in the matter.

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References and Notes

1. The studies and observations on which this paper is based were conducted by the Trinidad Regional Virus Laboratory with the support and under the auspices of the Government of Trinidad and Tobago, the Colonial Development and Welfare Scheme, and the Rockefeller Foundation.
2. A. B. Sabin and R. W. Schlesinger, *Science* 101, 640 (1945); R. W. Schlesinger and J. W. Frankel, *Am. J. Trop. Med. Hyg.* 1, 66 (1952).
3. L. J. Reed and H. Muench, *Am. J. Hyg.* 27, 493 (1938).

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Characteristic Electrophoretic Patterns of Plasma Proteins of Orders of Amphibia and Reptilia

Immunological methods have been used for many years to study the phylogenetic relationships of animals (*1*). Electrophoresis would seem to offer another useful technique for such studies, since the electrophoretic patterns of the plasma proteins of an animal are characteristic, and distinct differences are found between patterns of closely related forms (*2*).

Paper electrophoretic patterns of the plasma proteins of amphibians and reptiles were determined under uniform conditions as part of a study of the comparative biochemistry of these forms. In each electrophoretic run, a parallel sample of human plasma served as a standard reference. All protein separations were carried out on an LKB paper-electrophoresis unit. A sodium barbital buffer of *p*H 8.6 with an ionic strength 0.05, containing 15 percent glycerol by volume, was used. A linear rate of movement of protein fractions was achieved by overcoming evaporation with the aid of the glycerol (*3*). Six 1-in. strips of Munktell No. 20 S filter paper were dipped into the buffer. Excess buffer was expressed from the paper with a rubber film roller. Seven hundred milliliters of buffer were added to each electrode vessel, and the two buffer surfaces were leveled with a siphon. The ends of the cassette holding the wetted strips were placed in the vessels. Following a 3-hour preliminary equilibration a potential of 170 v was imposed across the strips for an additional 3 hours. Two hundredths of a milliliter of fresh plasma was then spotted across each strip and fractionated for 17 hours at room temperature (26 to 28°C), 170 v, and 4 ma. Each strip was subsequently dried and stained with bromphenol blue (*4*). The optical density at 2-mm. intervals along strips made translucent with mineral oil was measured with a Beckman DU spectrophotometer at 590 mμ.

To date, we have examined about 1200 plasma patterns from more than 800 specimens of more than 100 kinds of amphibians and reptiles. Since genetic and physiological factors are known to influence plasma-protein composition, we have attempted to recognize such effects. Differences in the patterns due to sex, age, starvation, season, and geographic variation have been found (*5*).

The patterns of the various vertebrate orders that were studied seem to possess certain general diagnostic characteristics, provided that allowance is made for variations cited. In Fig. 1, a typical pattern from one species of each amphibian and reptilian order is shown adjacent to a "tentative key." The key is also in agreement with patterns obtained by

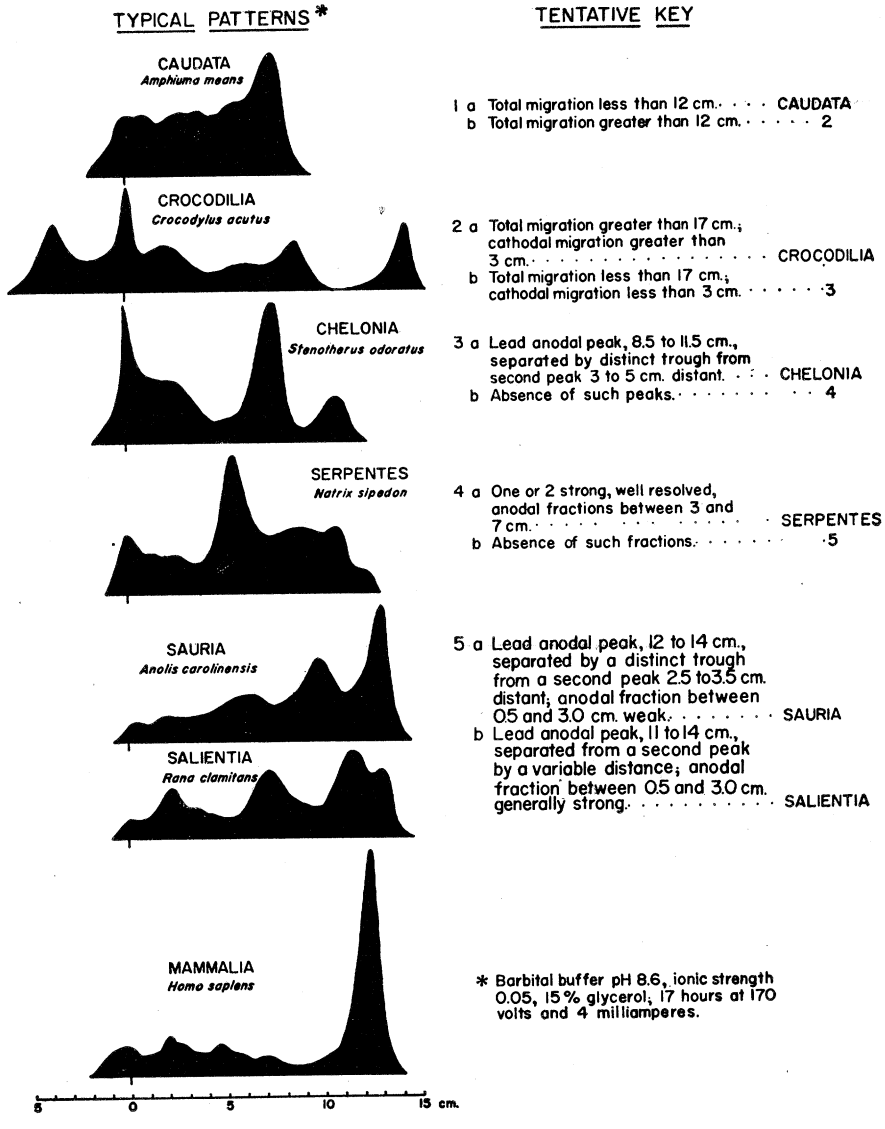

Fig. 1. Characteristic plasma protein patterns of the orders of Amphibia and Reptilia. Plasma samples were applied at the points indicated by the short line below each pattern. The anode is to the right of this mark.

other workers (*2, 6*). Data such as total migration, movement toward the anode or cathode or both, and the presence or absence of specific fractions have been used as taxonomic characteristics. With this key, it should be possible to assign an amphibian or reptile to its proper order on the basis of the plasma pattern of the specimen. The key is based on patterns from 87 species distributed among the families as follows (*7*).

(Caudata) Proteidae, 1; Ambystomidae, 3; Salamandridae, 3; Amphiumidae, 1; Plethodontidae, 6. (Salientia) Bufonidae, 3; Hylidae, 5; Microhylidae, 1; Ranidae, 5. (Chelonia) Chelydridae, 1; Kinosternidae, 2; Emydidae, 5; Trionychidae, 1. (Crocodilia) Alligatoridae, 2; Crocodylidae, 1. (Sauria) Iguanidae, 4; Anguidae, 3; Xantusidae, 1; Teidae, 1; Scincidae, 2. (Serpentes) Colubridae, 30; Crotalidae, 7.

Since there are many major gaps in our preliminary survey of the Amphibia and Reptilia, it is very likely that this key will have to be altered as more data are accumulated. Thus far, however, these studies suggest that paper electrophoretic analysis is useful in revealing characteristics of plasma proteins that may serve as another means of illustrating basic similarities and differences between taxonomic groups.

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References and Notes

1. G. H. F. Nuttall, *Blood Immunity and Blood Relationships* (Cambridge Univ. Press, Cambridge, 1904).
2. H. F. Deutsch and W. H. McShan, *J. Biol. Chem.* 180, 219 (1949).
3. E. Valmet and H. Svensson, *Science Tools* 1, 3 (1954).
4. R. J. Block, E. L. Durrum, G. Zweig, *Manual of Paper Chromatography and Paper Electrophoresis* (Academic, New York, 1955).
5. A report of this work is in preparation.
6. T. L. Gleason and F. Friedberg, *Physiol. Zool.* 26, 95 (1953).
7. K. P. Schmidt, *A Check List of North American Amphibians and Reptiles* (Univ. Chicago Press, Chicago, ed. 6, 1953).

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Tissue-Culture Cultivation of Cytopathogenic Agents from Patients with Clinical Hepatitis

The susceptibility of a number of established cell lines of human and animal origin to a variety of selected strains of viruses, as well as to clinical specimens, from various diseases of unproved etiology is being investigated in our laboratories. During the course of these spectrum studies, an icteric serum specimen (MR_1), obtained from a patient presumed to have infectious hepatitis (*1*), apparently induced a destructive effect on an established line of spindle cells from human embryonic testicle (line PD 39T). Subsequently, confirming this original observation, a more striking cytopathogenic effect was observed on the Detroit-6 strain of human epithelial-like cells (*2*) in cultures containing this serum.

Table 1. Isolation of agents in Detroit-6 strain of cells from patients with hepatitis.

Designation	Test material	Data pertaining to cellular cultures			Calculated total dilution of original specimen
		No. of serial passages	Time cytopathogenicity observed (range in days)	Infectivity titer attained	
MR_1	Acute serum	6	6–9	$10^{-4.0}$	$10^{-9.2}$
Sal.	Acute serum	4	6–9	$10^{-1.5}$	$10^{-4.6}$
G. Pas.	Acute serum	6	4–7	$10^{-1.5}$	$10^{-6.0}$
G. Pas.	"Convalescent" serum	1	6–9		$10^{-2.0}$
D. Keb.	Acute serum	5	5–8	$10^{-1.0}$	$10^{-5.2}$
D. Keb.	"Convalescent" serum	1	6–9		$10^{-2.0}$
H. Pom.	Acute serum	7	5–8	$10^{-1.0}$	$10^{-5.3}$
H. Pom.	"Convalescent" serum	1	6–9		$10^{-2.0}$
New	Stool	5	6–9	$10^{-3.0}$	$10^{-5.0}$
Gar.	Stool	6	5–8	$10^{-1.5}$	$10^{-6.0}$
USA	Stool	2	6–9	$10^{-1.5}$	$10^{-3.2}$

A number of spindle and epithelial cell lines of human embryonic origin (skin, lung, liver, adrenal, spleen, uterus, fallopian tube, trachea) (*3*), as well as monkey kidney tissue, have not shown any degenerative change when they were inoculated with MR_1. In further studies with other cell lines, the original serum and fluids removed from the fifth passage in Detroit-6 cells have exhibited a similar cytopathogenic effect toward cultures of human amnion cells (*4*). In addition, after five passages in the Detroit-6 strains of cells, the harvested fluids produced a cytopathogenic effect on cultures of HeLa cells (*5*).

However, transfers beyond three successive serial passages have not been made in either amnion or HeLa cultures, and evidence for actual multiplication is as yet inconclusive. Syverton (*6*), while attempting to establish serum and infectious hepatitis virus in HeLa cell cultures, observed degenerative changes that were not transferable beyond three passages. Further work on use of the HeLa strain and amnion cells for primary isolation and maintenance of similar agents is in progress, but it appears to date that their susceptibility is of a lower order than that of Detroit-6 cells. The testicular line of spindle cells (PD 39T) used originally was lost in passage. Consequently, the studies reported here have been mainly with the Detroit-6 strain, in which the MR_1 agent has been passed serially without difficulty.

Following the repeated confirmation of the observation with MR_1, a number of similar transmissible agents, presumably viral in nature, have been isolated from tubes inoculated with serum and stools of patients (*7, 8*) diagnosed as having clinical hepatitis. The specimens had been stored for variable periods at −70°C until tested. Young cultures of Detroit-6 cells have been used throughout the study, and the cells are washed three times with a maintenance solution consisting of mixture No. 199 (*9*) containing 10-percent horse serum to remove the human serum contained in the growth medium. The primary inoculum has been either 0.5 ml of 1/5 serum dilution or a 10^{-2} dilution of centrifuged supernate in the case of stool specimens.

Following 4 to 14 days of incubation at 36° to 37°C, the cell monolayer becomes disrupted and extended. Groups of small dark granular cells, irregular in size and shape, then aggregate in clumps throughout the culture. No inclusions have been noted, and areas of apparently normal cells are observed that indicate that all cells are not affected. Subcultures to younger cells are usually prepared at intervals ranging from 3 to 10 days, and characteristic cellular changes have been noted after each transfer.

Figure 1 shows the appearance of a control culture as observed in a fresh preparation (upper left) and stained with azure-eosin (upper right). The cytopathogenic changes produced by one of the agents (G. Pas.) is illustrated from a fresh preparation (lower left) and stained with azure-eosin (lower right). These cultures were incubated for 3 days and then were inoculated with virus, and the photographs (*10*) were taken following 8 days of incubation. Controls have consisted in simultaneous passage of serum from individuals with