fied cholesterol, and showed a diminution in the further esterification of cholesterol upon incubation, than the pre-adsorbed plasma. Although the meaning of this is unclear at this time, the apparent removal of some cholesterol esterase activity by tricalcium phosphate gel, a known heparin and prothrombin adsorbent, raises the possibility of some relationship between these substances. Unfortunately determinations of total cholesterol before and after adsorption were not done.

Summary. Tricalcium phosphate gel removes all or the major portion of post-heparin clearing factor activity from plasma but does not decrease the activity of human pancreatic lipase. It is therefore a useful agent in identifying heparin lipoprotein lipase. Endogenous plasma lipolytic activity is completely or nearly completely removed after gel adsorption in almost all subjects in whom it is present, affording further evidence of its similarity to or identity with post-heparin factor. Plasma cholesterol ester and cholesterol esterase is also apparently decreased in the post-adsorptive sample.

1. Nilsson, I. M., and Wenckert, A., *Acta Med. Scand.*, 1954, v150, Supp. 297.

2. Nikkila, E. A., and Pesola, R., *Acta Chem. Scand.*, 1956, v10, 144.

3. Engelberg, H., *Am. J. Physiol.*, 1955, v181, 309.

4. ———, *J. Biol. Chem.*, 1956, v222, 601.

5. Quick, A. J., *The Physiology and Pathology of Hemostasis,* Lea and Febiger, Philadelphia, 1951.

6. Borgstrom, B., *Acta Physiol. Scand.*, 1925, v25, 328.

7. Shore, B., PROC. SOC. EXP. BIOL. AND MED., 1955, v88, 73.

8. Robinson, D. S., *Biochem. J.*, 1956, v62, 18 P.

9. Korn, E. D., *Science,* 1956, v124, 489.

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Paper Electrophoresis of Animal Hemoglobins. (23234)

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The discovery of an abnormal hemoglobin in sickle cell disease has stimulated intensive electrophoretic study of human hemoglobin (1-4). An interest in comparative aspects of erythrocyte turnover(5) led us to undertake electrophoretic characterization of hemoglobins of a variety of animal species. A wide spectrum of migration patterns was found, with some species demonstrating as many as 3 electrophoretically separable hemoglobins.

Materials and methods. Blood was obtained from active adult animals (and 3 calves) (Table I) by cardiac or venipuncture and either oxalate or heparin used as anticoagulant. (Penguin blood was taken from an animal severely ill with aspergillosis.) Human blood was drawn from several healthy adult males, from patients with sickle cell disease(6), and from an individual with hemoglobin C disease(7). Blood cells were washed 3 times with 0.9% NaCl, lysed with 2-3 volumes of distilled water, and centrifuged to separate out particulate (stromal) elements. 0.005-.020 ml samples of hemoglobin solutions (stored frozen until electrophoresis) were stripped on filter paper and placed in a Spinco electrophoresis cell(8) containing barbital buffer of .01 ionic strength, pH 9.2. Voltage was maintained constant at 270 volts for a 5 hour period of electrophoresis. Under these conditions the average current across each 3 cm wide paper strip was .5 m amp. Human A hemoglobin, and usually S and C hemoglobins as well, were run simultaneously in the same cell with animal hemoglobins. Human A hemoglobin

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TABLE I. Animal hemoglobins subjected to electrophoresis. Figures in parentheses indicate number of individuals tested.

Mammals
- Man
- Chimpanzee (*Pantroglodytes*) (1)
- Rhesus (*Macaca mulatta*) (4)
- Macaque (" *syrichta*) (1)
- Llama (*Lama glama*) (1)
- Horse (draft) (5)
- Cow (Hereford) (7)
- Calf (") (3)
- Pig (Hampshire, Berkshire) (4)
- Sheep (Hampshire) (6)
- Goat (brush) (2)
- Dog (mongrel) (14)
- Cat (") (7)
- Mouse (white, random bred) (5)
- Rat (Sprague-Dawley, ") (6)
- Guinea pig (Hartley-Beltsville hybrid) (5)
- Rabbit (New Zealand White) (7)
- Hamster (Golden Syrian) (5)

Birds
- Chicken (N. H., mongrel Leghorn) (11)
- Pigeon (white Carneaux) (6)
- Duck (white Pekin) (9)
- Robin (*Turdus migratoria*) (1)
- Penguin (*Aptenodytes forsteri*) (1)

Reptiles
- Turtles:
  - *Terrapene carolina carolina* (5)
  - *Pseudyms floridana* (2)
  - " *rubriventris* (1)
- Black Snake (*Elaphe obsoleta*) (1)
- Water " (*Natrix taxispilota*) (1)

Amphibia
- Toad (*Bufo marinus*) (2)
- Frog (*Rana pipiens*) (7)

Fish
- Carp (*Cyprinus carpio*) (1)

migrated approximately 6 cm in 5 hours, with a variation of as much as 0.5 cm between 2 strips in the same cell. Conclusions in regard to relative mobility were based on multiple runs in which consistent significant differences were observed. Blood from one turtle (*Terrapene carolina*) was incubated *in vitro* with $Na_2Cr^{51}O_4$, which has been found to bind firmly with both human and animal hemoglobin(5). Hemoglobin thus labelled was submitted to electrophoresis and separated into 2 components as described below. The paper strip was run through a densitometer‡ then cut transversely into 1-3 cm pieces bearing varying amounts of pigment,

‡ Spinco Analytrol, Specialized Instruments Corp., Belmont, Calif.

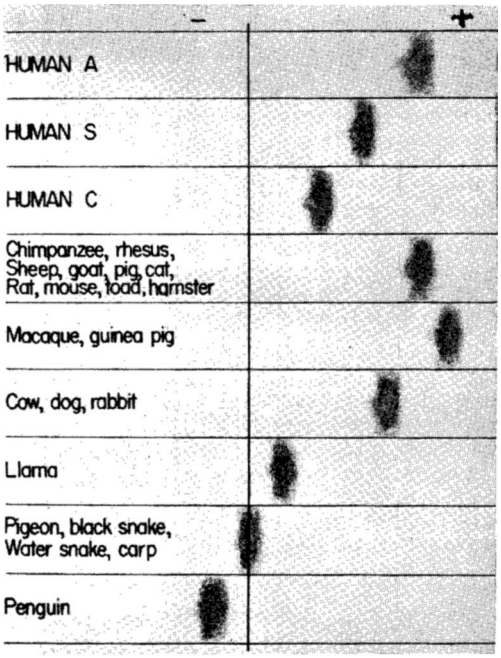

FIG. 1. Diagrammatic representation of electrophoretic patterns of various human hemoglobins and of animal species demonstrating single-component hemoglobins.

which were placed in counting vials and the emissions of chromium^{51} scanned in a well-type scintillation counter.

Results. Mammalian hemoglobins. The pattern of mammalian hemoglobins displaying single electrophoretic components is shown in Fig. 1. Those found to have anodic mobility similar to human A hemoglobin included chimpanzee, rhesus monkey, pig, goat, cat, mouse, rat, hamster, and 4 of 6 sheep. Hemoglobin of guinea pigs and a macaque possessed greater mobility, while that from llama, cow, dog, and rabbit migrated less rapidly than human A hemoglobin.

A number of species disclosed 2-component patterns (Fig. 2). Hemoglobins of 5 draft horses gave identical separations of double bands slightly faster than human A. While each of 7 adult Hereford steer presented single hemoglobins moving more slowly than A (Fig. 1), hemoglobins from 2 of 3 Hereford calves (estimated age 2-6 months) contained 2 components, one equal in anodic mobility to that of adult steer hemoglobin, the other slower (Fig. 2). The third calf

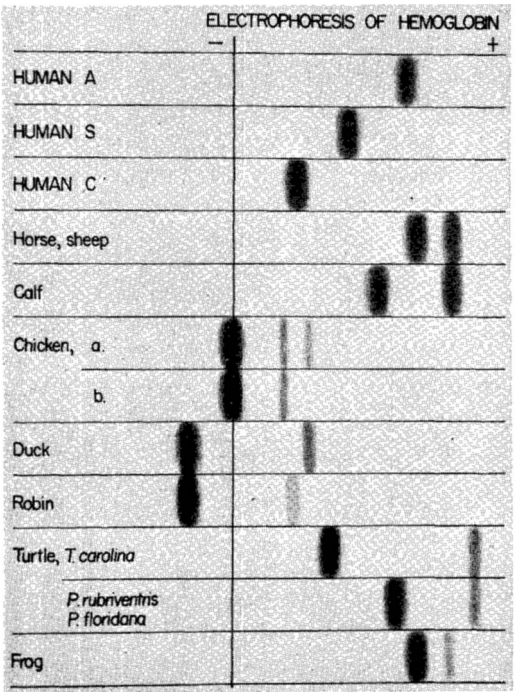

FIG. 2. Diagrammatic representation of electrophoretic patterns of various human hemoglobins and of animal species demonstrating multiple component hemoglobins.

hemoglobin had the pattern of adult steer. Two of 6 Hampshire sheep hemoglobins tested each showed 2 components, one with mobility of A hemoglobin, the other faster (Fig. 1, 2).

Avian hemoglobins. Hemoglobin from each of 6 New Hampshire chickens separated into 3 components; the major band (approximately 70%) migrated little if any distance from point of origin, while 2 faint lesser bands moved toward the anode (Fig. 2, a). One of 5 mongrel Leghorns also displayed 3 hemoglobin components; the others had but 2, lacking the more rapid of the components moving toward the anode (Fig. 2, b). Hemoglobin from each of 9 ducks separated into a major component (85%) migrating toward the cathode and a lesser component (15%) directed toward the anode. Robin hemoglobin also proved separable into a major positively charged component and a lesser negatively charged moiety (Fig. 2). Penguin and pigeon hemoglobins travelled as single bands, the former moving slowly to the negative pole, the latter displaying no significant deviation from the point of origin (Fig. 1).

Reptile hemoglobins. Hemoglobins of each of 3 species of turtle—*Terrapene carolina, Pseudyms floridana,* and *Pseudyms rubriventris*—possessed 2 widely separated bands migrating toward the positive electrode, one component somewhat more rapidly moving than human A hemoglobin, the other more slowly moving (Fig. 2). Hemoglobin of one turtle (*Terrapene carolina*), labelled *in vitro* with radioactive chromium^{51} before electrophoresis, was found to have 2 peaks of radioactivity which coincided with visible pigment peaks. Specific activities of the 2 hemoglobin components were nearly equal (Fig. 3). The black snake and water snake were found to have single component hemoglobins which moved but little from the origin point (Fig. 1).

Amphibian and piscian hemoglobins. Each of 2 toads presented single hemoglobins with mobility and direction similar to that of human A (Fig. 1). In 7 frogs, there was a

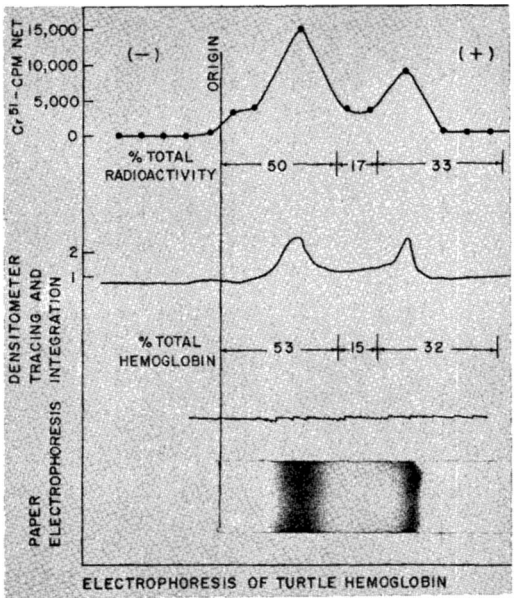

FIG. 3. Paper electrophoresis pattern, densitometer tracing, and radioactivity assay of turtle hemoglobin (*T. carolina*) labelled with radioactive chromium *in vitro*. The 2 electrophoretically separable components appear to have been labelled proportionately as indicated by comparable specific radioactivity of each peak. (Specific radioactivity = % total radioactivity/% total hemoglobin.)

heavy band moving faster than A hemoglobin, closely preceded by a faint spot suggestive of a second faster-moving component. When samples were run for periods of 8 hours, 2 components were more clearly separable (Fig. 2). In the case of carp hemoglobin there was rather wide diffusion about the line of origin, but no evidence of multiple bands (Fig. 1).

Discussion. Landsteiner and co-workers (9) determined electrophoretic mobilities of hemoglobins from several animal species, using Tiselius apparatus. The present study confirms their finding of similar mobilities in hemoglobin of the rabbit, dog, and sheep, somewhat greater mobility of horse and guinea pig hemoglobin, and slower migration of chicken hemoglobin. An ever-increasing number of reports concerned with the electrophoresis of animal hemoglobins has appeared in recent years(10-18).

Several groups have now reported the finding of 2 components in the electrophoresis of chicken hemoglobin(10-12). In the present study separation of the hemoglobin from all 6 New Hampshire birds and one Leghorn into 3 components and from 4 other Leghorns into 2 components indicates species differences in hemoglobin composition in this fowl. Our finding of double-component hemoglobins in the duck and robin and of single-component hemoglobin in the pigeon is confirmed by Dunlap, Johnson, and Farner(12). These workers studied the blood of 21 species of birds and, with the exception of the pigeon, found 2 hemoglobin components in all.

In the case of the Hereford cow, it would appear likely that temporary persistence of a fetal form of hemoglobin(19) accounts for the finding of double hemoglobin components in the calf and single components in adult animals. Study of the oxygen dissociation of fetal and maternal blood has demonstrated a developmental adult change in hemoglobin not only in the cow, but in a variety of other species including the goat, rabbit, sheep, chicken, frog, and turtle(20). Cabannes and Serain(14) found 15 of 80 bovine hemoglobins to possess 2 components and, excluding genetically transmitted variations, suggested that the more rapid of these represented an abnormal hemoglobin associated with illness in the donor. It would appear possible to us that these 2-component bovine hemoglobins represented instances of persisting fetal together with adult type hemoglobin.

In some of those species in which there are individual differences in the composition of hemoglobin from adult animals, there is evidence which indicates that the variations in hemoglobin types are genetically determined. Harris and Warren and their co-workers(18) observed a transmission of hemoglobin types in Scottish blackface sheep consistent with the view that these are determined by 2 alleles each responsible for the formation of one kind of hemoglobin. Similar variations in the hemoglobins of many hundred cattle, sheep, goats, horses, and mules were reported by Cabannes and Serain(16) who, however, did not present genetic studies in their animals. From the studies of hemoglobin patterns in inbred strains Ranney and Glueck-sohn-Waelsch(17) propose genetically based differences in mouse hemoglobin. Blood smears from each of the types observed disclosed no morphologic differences of the erythrocytes.

Svedberg and Hedenius(21) suggested that the formation of dimers and trimers might account for individual hemoglobins with 2 or more sedimentation constants in some species of reptiles and amphibians, and it might be argued that electrophoretic heterogeneity of turtle and frog hemoglobin could be due to such double molecules. Such conjugates were not observed in other classes of vertebrates (including birds) and it would appear more likely to us that differing molecules rather than dimer formation account for the finding of multiple hemoglobin components.

In those species in which 2 component hemoglobins are of regular occurrence in adult animals (horse, birds, turtles, frog) it would seem likely that both components represent normal forms of hemoglobin. Perhaps in some there is continued production of a fetal type hemoglobin into adult life. Convincing evidence linking the presence of multiple hemoglobins with hematologic disease in animals is (still) lacking.

Summary. Differences have been found

both in the number of components electrophoretically separable from individual animal hemoglobins and in the mobility of these components relative to human A, S, and C hemoglobins. Under the conditions employed, with the exception of the horse, 2 of 3 calves, and 2 of 6 sheep, all of which possessed 2 components, the (adult) mammalian hemoglobins studied were found to have single components. Some displayed anodic mobility similar to that of human A hemoglobin (chimpanzee, rhesus monkey, pig, sheep, goat, cat, mouse, rat, hamster), others had greater mobility (macaque, guinea pig), and some possessed lesser mobility (llama, cow, dog, rabbit). In the Hereford steer, it would appear that temporary persistence of fetal hemoglobin accounts for the finding of 2-component hemoglobin in the calf and single-component hemoglobin in adults. Hemoglobin from several New Hampshire chicken and one Leghorn was found to have 3 electrophoretic components; that from other Leghorns, ducks, and a robin showed 2 components. Penguin and pigeon hemoglobin had single components. Hemoglobin prepared from each of 3 species of turtles possessed 2 widely separated bands. Black snake, water snake, and toad had one-component hemoglobins. Frog hemoglobin gave a 2-component pattern. With few exceptions the number of individual hemoglobins tested in any species has to date been small. It is likely that the study of larger numbers of animals will uncover additional examples of heritable variations in hemoglobin types.

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1. Itano, H. A., *Science,* 1953, v117, 89.

2. Singer, K., *Am. J. Med.,* 1955, v18, 633.

3. Chernoff, A. I., *New Eng. J. Med.,* 1955, v253, 322.

4. Itano, H. A., Bergren, W. R., and Sturgeon, P., *Medicine,* 1956, v35, 121.

5. Rodnan, G. P., Ebaugh, F. G., Jr., and Fox, M. R. S., *Blood,* 1957, v12, 355.

6. Pauling, L., Itano, H. A., Singer, S. J., and Wells, I. C., *Science,* 1949, v110, 543.

7. Thomas, E. D., Motulsky, A. G., and Walters, D. H., *Am. J. Med.,* 1955, v18, 832.

8. Motulsky, A. G., Paul, M. H., and Durrum, E. L., *Blood,* 1954, v9, 897.

9. Landsteiner, K., Longsworth, L. G., and van der Scheer, *J. Science,* 1938, v88, 83.

10. Johnson, V. L., and Dunlap, J. S., *ibid.,* 1955, v122, 1186.

11. Sydenstricker, V. P., Oliver, R., Chandler, B. M., and Sydenstricker, O., PROC. SOC. EXP. BIOL. AND MED., 1956, v93, 396.

12. Dunlap, J. S., Johnson, V. L., and Farner, D. S., *Experientia,* 1956, v12, 352.

13. Hamoir, G., *Nature,* 1953, v171, 345.

14. Cabannes, R., and Serain, C., *Compt. rend. soc. biol.,* 1955, v149, 7.

15. ———, *ibid.,* 1955, v149, 1193.

16. ———, *ibid.,* 1955, v149, 1103.

17. Ranney, H. M., and Gluecksohn-Waelsch, S., *Ann. Human Genet.,* 1955, v19, 269.

18. Evans, J. V., King, J. W. B., Cohen, B. L., Harris, H., and Warren, F. L., *Nature,* 1956, v178, 849.

19. Ross, J., and Romyn, C., *J. Physiol.,* 1938, v92, 249.

20. McCutcheon, F. H., *Scient. Monthly,* 1952, v74, 297.

21. Svedberg, T., and Hedenius, A., *Biol. Bull.,* 1934, v66, 191.

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