slower rate than mouse embryo cells. Failure to reach a comparable rate of multiplication may be a characteristic of this cell line or could depend on nutritional conditions in our experiments, since fluids were changed only weekly and a low concentration of horse serum was used. Furthermore, media used for L cells contained less bicarbonate than that employed with mouse embryo cells, and it was recently reported(10) that virus yield is dependent on bicarbonate concentration of the medium.

Through analysis of host-virus relationships with polyoma virus so far studied, it appears that capability to induce tumors in animals is not necessarily correlated with ability to multiply *in vitro* in cells from the same animals nor with ability to produce CPE in the same cells. Moreover, a correlation does not exist between ability to multiply and CPE. For instance, polyoma virus induces tumors in mice(11) and multiplies in mouse cells *in vitro* with CPE(1). On the other hand, in rats(12) and rabbits(13) tumors are produced, but neither virus growth nor CPE takes place *in vitro*. In hamsters, tumors are induced(14), multiplication of virus takes place *in vitro,* but no CPE is detectable(15). Then, when multiplication *in vitro* occurs, it may result in killing of infected cells (CPE) or in production of virus without cell degeneration, as shown in our experiments with L cells and by Vogt and Dulbecco in hamster tissue cultures(15). Therefore, 3 different kinds of relationships between polyoma virus and cells exist: malignant transformation, multiplication with CPE, and virus production with survival of infected cells. Whether the type of response depends on predetermined and genetic characteristics of cells or on modifiable conditions is now under investigation.

Summary. Propagation of SE polyoma virus in 4 different cell systems *in vitro* has been studied. Mouse embryo cells support multiplication of this virus with CPE. Growth occurs to a lesser extent in L cells without CPE, while it does not take place in HeLa and rabbit cells.

1. Eddy, B. E., Stewart, S. E., Berkeley, W., PROC. SOC. EXP. BIOL. AND MED., 1958, v98, 848.

2. Stewart, S. E., Eddy, B. E., Gochenour, A. M., Borgese, N. G., Grubbs, G. E., *Virology*, 1957, v3, 380.

3. Rabson, A. S., Legallais, F. Y., PROC. SOC. EXP. BIOL. AND MED., 1959, v100, 229.

4. Kilham, L., Olivier, L. J., *Virology,* 1959, v7, 428.

5. Reed, L. J., Muench, H., *Am. J. Hyg.,* 1938, v27, 493.

6. Youngner, J. S., PROC. SOC. EXP. BIOL. AND MED., 1954, v85, 202.

7. Eagle, H., *J. Exp. Med.,* 1955, v102, 595.

8. Hanks, J. H., Wallace, R. E., PROC. SOC. EXP. BIOL. AND MED., 1949, v71, 196.

9. Bader, J. P., Morgan, H. R., *J. Exp. Med.,* 1958, v108, 617.

10. Eddy, B. E., Stewart, S. E., *J. Publ. Health,* 1959, v49, 1486.

11. Stewart, S. E., Eddy, B. E., Borgese, N., *J. Nat. Cancer Inst.,* 1958, v20, 1223.

12. Eddy, B. E., Stewart, S. E., Stanton, M. F., Marcotte, J. M., *ibid.,* 1959, v22, 161.

13. Eddy, B. E., Stewart, S. E., Kirschstein, R. L., Young, R. D., *Nature,* 1959, v183, 766.

14. Eddy, B. E., Stewart, S. E., Young, R., Mider, G. B., *J. Nat. Cancer Inst.,* 1958, v20, 747.

15. Vogt, M., Dulbecco, R., in press.

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Biochemical Polymorphisms in Animals: Haptoglobins and Transferrins. (25714)

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Ford(1) has defined polymorphism as "the occurrence together in the same habitat of 2 or more discontinuous forms of a species in such proportions that the rarest of them cannot be maintained merely by recurrent mutation." Biochemical polymorphic traits of men

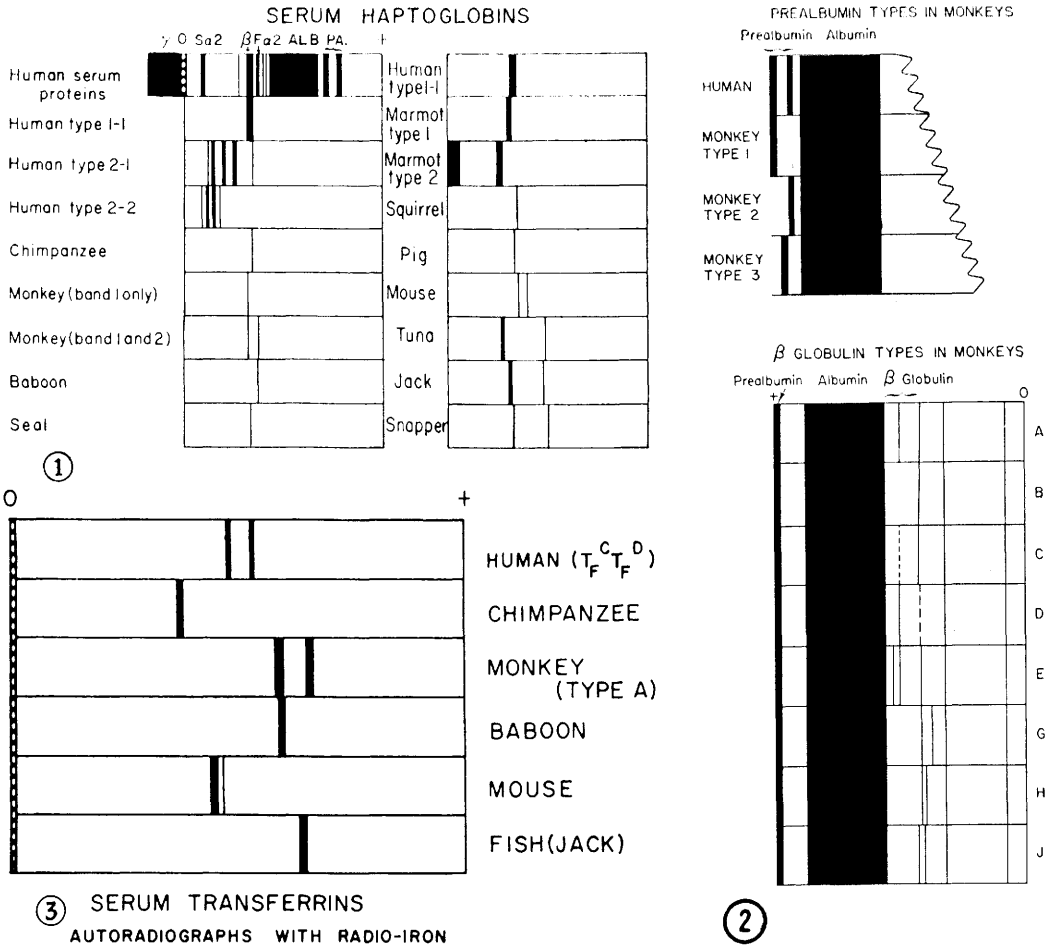

FIG. 1. Diagrams of positions of haptoglobin bands in several animals (see Table I for numbers studied). The excess hemoglobin band is not shown. Position of fast-moving bands in 3 fish is not consistent. Relative positions of human serum proteins shown in the first panel. γ, gamma globulin; O, origin; Sa2, slow alpha-2-globulin; ALB, albumin; PA., pre-albumin; +, positive pole. Nomenclature is that of Smithies(3).

FIG. 2. Diagram of monkey serum protein pattern after starch gel electrophoresis in borate buffer and amido black staining (see text). Origin is on right and positive pole to left. Gamma globulins are not shown. The 8 different patterns of beta globulins are indicated. Autoradiographs made with serum containing radioiron to demonstrate position of transferrin bands were made, but are not shown in order to conserve space. Radioiron binds to each of the β-globulin bands, including the slow one in type G, but not to the slow moving band in type E. The polymorphism in the position of the fast-moving prealbumin is also shown diagrammatically. Iron does not bind to the prealbumin(7,12).

FIG. 3. Starch gel electrophoresis (tris buffer) of animal and human serum which has been equilibrated with radioiron (see text). Diagram of contact print of autoradiograph showing relative position of transferrin bands.

and animals are being discovered with increasing frequency. In the present paper preliminary data on 2 such systems are presented. Haptoglobins are a family of serum proteins which bind hemoglobin(2). Smithies(3) has shown by means of starch gel electrophoresis that there are 3 main haptoglobin types determined by 2 autosomal allelic genes Hp^1 and Hp^2 (Fig. 1). The presence of polymorphism for beta-globulins has been shown in some human populations(4), cows(5), sheep(6), and other animals(7) by means of starch gel electrophoresis. Beta-globulins appear equivalent to "transferrin," the serum protein which binds iron. In humans, transferrins are genetically controlled at a single locus, Tf, by at

least 3 alleles Tf^B, Tf^C and Tf^D(4).

Methods. Starch gel electrophoresis was by the method of Smithies(3). All studies were done using both conditions for serum proteins (borate buffer pH 8.6-8.9) and discontinuous (tris) buffer system of Poulik(8). Haptoglobins were determined by adding approximately 120 mg/100 ml of human hemoglobin solution to serum and staining for hemoglobin bound to the haptoglobins using benzidine and hydrogen peroxide after completion of electrophoresis. Fe^{59} (as $FeCl_3$) was obtained from Abbott Labs (Oak Ridge, Tenn.). Aliquots of aqueous solution were added to serum and equilibrated at 4°C for 24 hours. Final concentration of Fe^{59} in serum was approximately 3×10^{-5} molar. For radio-iron studies, the gel was split in half, one half used for autoradiography, and the other stained for protein with amido black.

Results. Haptoglobin patterns of 208 lower primates have been studied. These include 170 monkeys (*Macaca mulatta*), 4 chimpanzees (*Pan toglodytes*) and 34 baboons (*Papio*). In none of these was a pattern resembling a human type 2-2 or 2-1 seen. All of them had a band in approximate position of type 1-1 haptoglobin (Fig. 1). In approximately 1/3 of the monkeys, there was a second band (band 2) traveling faster than the common one. After addition of excess of hemoglobin, migration of band 2 became slower and merged with the slower moving band. A similar phenomenon is seen in serum of seals(7). Samples of sera, drawn at one month intervals, were available from 10 monkeys. Position of haptoglobin band 1 was identical in both specimens; the fast-moving band (band 2) was, however, absent or considerably decreased in some animals in the second specimen. This suggests that this band is not persistent during life. Fast-moving bands, similar to monkey band 2, were seen in chimpanzee, baboon, and some other animals. None of the other animals (Table I) tested had a pattern similar to human 2-2 or 2-1, but many had a haptoglobin band with approximately the same migration as human type 1-1. Several unusual varieties were seen (*i.e.*, marmot type 2) and some animals had bands migrating faster than the haptoglobin type 1-1 band. This was in nearly all cases excess hemoglobin, but fast-moving haptoglobin bands could not be ruled out in every case, since some sera used were hemolyzed. Mice had a band in the position of human 1-1 band as well as a fast-moving band. There was some variation from strain to strain in position of fast-moving bands and in amount of hemoglobin bound.

TABLE I. Animals Studied. Numbers examined for haptoglobins are also given. For further information on animals 4, 5, and 6, see reference(12).

Species	No. studied
1. Chimpanzee *(Pan toglodytes)*	4
2. Monkey *(Macaca mulatta)*	170
3. Baboon *(Papio)*	34
4. Seal *(Callorhinus ursinus)*	30
5. Arctic marmot *(Marmota caligata broweri)*	4
6. Ground squirrel *(Citellus parryii barrowensis)*	8
7. Domestic pig *(Sus scrofa)*	5
8. Mouse *(Mus musculus)*	10
9. Tuna (fish) *(Cymnosarda nuda)*	1
10. Jack (fish) *(Caranx sexfasciatus)*	3
11. Snapper (fish) *(Lutjanus vaigiensis)*	1

Beta-globulins. In a study of sera of 143 monkeys, several distinct beta-globulin patterns have been found (Fig. 2). The difference between type B and type C seems a quantitative one, and these 2 types have been grouped together. Distribution of types was as follows: type A, 51; type B and C, 67; type D, 8; type G, 5; type H, 7; type J, 4. The distinction between types J and H was sometimes difficult. In addition, there was one sera of type E. A diagram of autoradiograph of monkey type A, compared to human transferrin type CD (genotype Tf^C Tf^D) is shown in Fig. 3. A description of monkey autoradiographs is given in caption of Fig. 2. The same patterns were seen using borate or tris buffer, but better resolution was obtained with the latter, particularly if the gel buffer was diluted to half strength.

The radioiron binding of 4 chimpanzees, 7 baboons, 6 strains of mice, and a fish (snapper) were also studied (Fig. 3). No intraspecies variations were seen. There is a single band in chimpanzees, baboons, and fish which, in chimpanzees and mice, migrated slower, and in monkeys, baboons, and fish, migrated faster than human transferrins. These studies do not rule out a polymorphism in these species, and Dr. J. Buettner-Jansuch

has kindly informed us that he observed variation in baboon beta-globulin patterns.

There are 3 positions of pre-albumin band of monkeys (Fig. 2), and this may also constitute a polymorphism(7). This protein binds radiothyroxine(12).

Discussion. In comparing inherited proteins in different species, it cannot be assumed that proteins that have the same mobility on starch gel electrophoresis, or that bind the same material (*i.e.*, hemoglobin and iron) are the same substances. Although such proteins may be very similar, it is necessary to compare them more explicitly by physical and chemical means before their equivalence can be assumed. Such studies are in progress.

Haptoglobin polymorphism in humans does not occur in monkeys studied, all those tested having a protein with migratory properties similar to that of human type 1-1 haptoglobin. This implies that the Hp^2 gene is rare in monkeys and probably also in other animals tested. In terms of some of the concepts on development of polymorphisms(9), this may indicate that if a mutation to the Hp^2 gene occurred in monkeys, it offered no advantage in internal and external environment then obtaining, and it did not increase in the population in subsequent generations. On the other hand, when the human gene pool experienced this mutation, conditions favored its spread in the population, and it increased in frequency until balanced by other forces.

Examples analogous to this have been seen in other polymorphic systems, such as the trait associated with urinary excretion of beta aminoisobutyric acid (BAIB)(10). On the other hand, polymorphisms present in humans have been found to exist in lower primates. Fisher, Ford, and Huxley have shown that dimorphism for taste of phenylthiocarbimide is present in chimpanzees and probably other primates(11), and it appears to be present in monkeys (*Macaca mulatta*) as well (Blumberg, unpublished observation).

In transferrins, polymorphism exists in primates but appears to involve proteins with different physical properties than human transferrins. It will be of interest to compare these proteins chemically and physically and to determine the evolutionary significance of their differences.

Summary. Two genetic polymorphic systems, haptoglobins and transferrins, have been studied in various animal species. Haptoglobin polymorphism, present in human populations, has not been found in other primates so far tested. A polymorphism for transferrins exists in monkeys, but it includes proteins different from those found in human polymorphism.

1. Ford, E. B., *Genetics for Medical Students,* 1956.

2. Jayle, M. F., Boussier, G., *Exposes Ann. Biochim. Med.*, 1935, v17, 157.

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

4. Smithies, O., Hiller, O., *Biochem. J.*, 1959, v72, 121.

5. Hickman, C. G., Smithies, O., *Proc. Genetics Soc. Canada,* 1957, v2, 39.

6. Ashton, G. C., McDougall, E. I., *Nature,* 1958, v182, 945.

7. Blumberg, B. S., Allison, A. C., Garry, Barbara, *J. Cell. Comp. Physiol.,* 1960, in press.

8. Poulik, M. D., *Nature,* 1957, v180, 1477.

9. Ford, E. B., *ibid.,* 1957, v180, 1315.

10. Gartler, S. M., Firschein, I. L., Gidaspow, T., *Acta Genet. Separatum,* 1956/7, v6, 435.

11. Fisher, R., Ford, E. B., Huxley, J., *Nature,* 1959, v144, 750.

12. Blumberg, B. S., Robbins, J., *J. Clin. Invest.*, 1959, v38, 988.

Received January 19, 1960. P.S.E.B.M., 1960, v104.