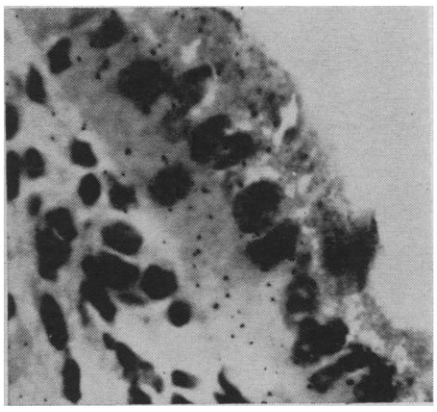

Fig. 2. Autoradiograph of intestine (parallel to autoradiographs of Fig. 1). The radioactivity is present in the epithelium of the villus, but at a lower concentration than in the proximal convoluted tubule of the kidney.

Tissues from control mice (injected with unlabeled methotrexate), processed similarly, showed no significant chemography.

In the liver, the parenchymal cells were uniformly labeled, whereas the Kupffer cells and connective tissues were unlabeled. Grain counts on AR-10 stripping film autoradiographs prepared by the Appleton technique were carried out on 300 randomly selected parenchymal cells: an area 6 × 6 μm over the nucleus was examined, and a similar area over the cytoplasm of the same cell. The mean grain count over the nucleus was 4.0 ± 0.12 (S.E.) grains per unit area; that over cytoplasm was 1.4 ± 0.07 grains per unit area. Background counts away from the tissue were below 0.1 grain per unit area. In the liver cells, therefore, the concentration of enzyme appears to be higher in the nucleus than in the cytoplasm.

In the kidney, the proximal convoluted tubules were heavily labeled: the grain densities over these cells were the highest seen in the tissues examined. No other structure in the kidney was significantly labeled (Fig. 1). In the proximal convoluted tubules, nuclei often appeared to be labeled, but the highest grain densities were seen over the cytoplasm of the apical half of the cell.

In the duodenum, labeling was limited to the epithelial cells (Fig. 2), which were radioactive both in the crypts of Lieberkuhn and on the villi. As in the kidney, nuclei sometimes appeared to be labeled, but the highest grain densities occurred over cytoplasm in the apical region of the cells.

It appears possible, then (on the basis of the evidence cited earlier for the specificity of methotrexate binding), to study the distribution of folate reductase in tissues by radioisotope cytochemistry and to compare its concentration in different sites on the basis of relative grain densities. The mere fact that localization of bound methotrexate can be revealed thus provides the basis of a new cell-tracing technique. This could be a valuable alternative to the use of ^{3}H-thymidine in studies of cell migrations and lifetimes.

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5 January 1966

Antigenic Correspondence of Serum Albumins among the Primates

Abstract. *The relative cross-reactivities as judged by the quantitative precipitation reaction of 22 primate serum albumins were determined with pooled antiserums against human serum albumin. The antigenic correspondences to human serum albumin of all but three serum albumins fell into narrow ranges, and the groups defined by these ranges are distinct taxonomic categories. The values of the cross-reactivity ranges are consistent with the presumed phylogenetic relationships to man on the assumption that the evolutionary modification of protein structure has been progressive and divergent.*

Recent research correlating protein structure with systematics or phylogenetics has taken two directions. One is the analysis of primary protein structure (*1*, *2*). The other, immunochemical correspondence, while applied around the turn of the century by Nuttall (*3*), was refined and developed by Boyden and his colleagues (*4*, *5*). In recent years more strictly quantitative methods and more informative qualitative techniques have been used (*6–9*).

Our study was undertaken to provide and evaluate data obtained by quantitative precipitation on the cross-reactivity of antigens from a relatively large number of species in the same order of mammals. Serum albumins of primates were selected for several reasons. (i) Human serum albumin (HSA) is relatively well characterized immunochemically (*10*) and physicochemically (*11*). (ii) It is commercially available in a sufficiently pure state for immunization of rabbits. (iii) Serums from many living species of the Primates are obtainable. (iv) The understandable interest of biologists in the systematics and evolution of this order has provided information on the comparative anatomy of living and fossil forms, which is important for the interpretation of antigenic correspondence.

The order Primates can be subdivided into at least four major divergent groups with a considerable range in characteristics from primitive to modern (*12*). The Prosimii or "pre-monkeys" include the tree shrews, lemurs, pottos, and tarsiers; the Ceboidea are comprised of the New World or South American monkeys; the Cercopithecoidea or Old World monkeys include the African and

Asian monkeys; and the Hominoidea include the great apes (Pongidae) and man (Hominidae).

Crystalline HSA (Pentex, lot 3) was used for all immunizations. It contained no detectable impurities by microimmunoelectrophoretic analysis of 100 μg, when used with two different "broad-spectrum" antiserums against human serum.

Male rabbits (New Zealand White) were used for antibody production. Two series of three injections on alternate days were separated by a two-week rest period. Each series consisted of a total of 17.5 mg of HSA in physiological saline. Nonproducing animals or low producers were eliminated on the basis of test bleedings. The remaining animals were given 20 mg of antigen in 2 ml of complete Freund's adjuvant distributed among several sites. Blood was collected by 25 ml bleedings at 1, 2, and 3 weeks, and by exsanguination 5 weeks after the final injection.

Antiserum pool II was prepared from bleedings of five rabbits in which there was detectable antibody to a single (always the same) α_1-globulin. Sodium azide was added to a concentration of 0.1 percent, and the serum was stored in 10-ml lots at −20°C. When the antiserum pool was absorbed with the crystalline albumin it still gave the α_1 reaction by immunoelectrophoretic analysis of whole human serum. By quantitative precipitation with the absorbed antiserum a maximum impurity reaction was obtained by the addition of approximately twice the amount of whole human serum required for equivalence of the albumin reaction with unabsorbed antiserum. The dissolved precipitate had an optical density at 280 mμ of 0.005 or approximately 1.5 percent of the reaction of crystalline albumin at twice equivalent antigen.

The reference test antigen was prepared by pooling equal volumes of 28 human serums obtained from a commercial blood bank. Serum samples of the wide variety of species including the two nonprimate species were obtained from several sources (*13*). All samples except rhesus serum, which was freshly drawn and frozen at the Rockefeller University, were received in frozen or lyophilized state.

The quantitative precipitin procedure used was similar to that described by Kabat (*14*). Dilute solutions of whole serum were used as test antigens for comparative cross-reactivity of serum albumins. This was required since many samples were too small to permit adequate purification of the albumin. The amount of antiserum in each reaction was 0.25 ml, and the quantities of antigen added were initially recorded as volumes of whole serum—since no method for evaluating albumin concentration proved reliable. New points were supplemented, if required, to assure a continuous reaction curve, particularly near the equivalence zone.

Reaction mixtures were prepared in duplicate with sodium phosphate buffer, *p*H 7.2, ionic strength 0.1; total volume was 2.5 ml. Reactions were incubated for 4 days at 5°C. The precipitates were collected by centrifugation (5°C, 1400 *g*, 2 hours). They were washed twice with 2.5 ml of buffer and dissolved in 2.5 ml of 0.25*M* acetic acid. These solutions were read in a Zeiss (PMQ II) spectrophotometer at 280 mμ. If assays were doubted for any reason they were repeated. Reliable assays were repeatable within 2 percent.

The data for each precipitin curve were normalized to antigen equivalence (*15*), since absolute quantities of antigen employed were not known. The serum volume used to obtain equivalence was assigned a value of 1.0 and actual or interpolated precipitate absorbancies for antigen volumes representing convenient fractions or multiples of antigen equivalence were obtained from original curves. These values were then plotted against their respective antigen equivalence fractions as percentages of the absorbancy of the human equivalence reaction.

Table 1 presents the correspondences in percentages of the several serum albumins to HSA at 0.4 and 1.0 antigen equivalence. In the last column are tabulated the correspondences calculated as percentage of curve area between 0.15 and 6.0 antigen equivalence. The latter calculation is comparable to the analytical methods of Boyden and DeFalco (*4*), and the results may be compared with those obtained by Gemeroy for a selection of primate serums (*8, 16*). The test method used by Gemeroy, turbidimetric analysis after a relatively brief incubation during which the second stage of the reactions could hardly have achieved equilibrium, may account for his lower correspondences to HSA [chimpanzee (*Pan*), 70 to 78 percent; orangutan (*Pongo*), 62 to 73 percent; rhesus (*Macaca*), 35 to 41 percent; *Cebus*, 10 to 26 per cent; *Lemur*, 6 to 13 percent; bovine serum albumin, 4 percent]. Samples of orangutan serum were not available for our study.

The normalized curves of correspondence values, averaged for the albumins from each major taxonomic group, are plotted (semilogarithmic) in Fig. 1. Albumin curves for gibbon (*Hylobates*) and night monkey (*Aotes*) are plotted individually, and their values are not averaged with their respective taxonomic groups. Figure 2 illustrates the curves of the prosimian families and the nonprimate species.

The correspondence values in Table 1 fall into distinct ranges charasteristic

Table 1. Immunochemical correspondence values of primate serum albumins.

Species *	Fraction antigen equivalence 0.4	Fraction antigen equivalence 1.0	Curve area (%)
Hominoidea (Superfamily)			
Homo sapiens	100	100	100
Gorilla gorilla	100	98	92
Pan troglodytes	99	96	97
Hylobates lar (3) †			
Hylobates lar Pileatus (3)	81	82	79
Cercopithecoidea (Superfamily)			
Macaca mulatta	77	82	82
Colobus polykomos abyssinicus	83	80	78
Papio doguera (3)	72	78	75
Cercocebus galeritis agilis	72	78	79
Erythrocebus potas	80	76	76
Cercopithecus aethiops	74	76	79
Ceboidea (Superfamily)			
Aotes trivirgatus	74	77	74
Ateles paniscus	64	65	58
Saimiri sciurea	54	62	60
Tamarin tamarin	55	60	54
Lagothrix lagotricha	58	60	61
Cebus apella	48	52	45
Prosimii (Suborder)			
Lemur fulvus collaris (3)	33	40	37
Lemur macaco	30	38	34
Lemur fulvus fulvus	35	37	37
Tupaia glis	29	36	31
Perodictus potto	28	34	31
Galago crassicaudatus crassicaudatus	26	31	28
Propithecus verrauxi coquereli (3)	23	23	22
Insectivora (Order)			
Hedgehog	16	16	17
Artiodactyla (Order)			
Pig‡	7	10	8

* Higher taxa arranged in order of assumed decreasing phylogenetic relationship to man. Species within groups are arranged according to values obtained at antigen equivalence. † Numbers in parentheses indicate number of different samples tested. Data presented are average values. ‡ Crystalline bovine serum albumin, also representing artiodactyla, gave equivalence cross-reactions of approximately 15 percent. This value is very similar to that obtained by Weigle in a reciprocal reaction.

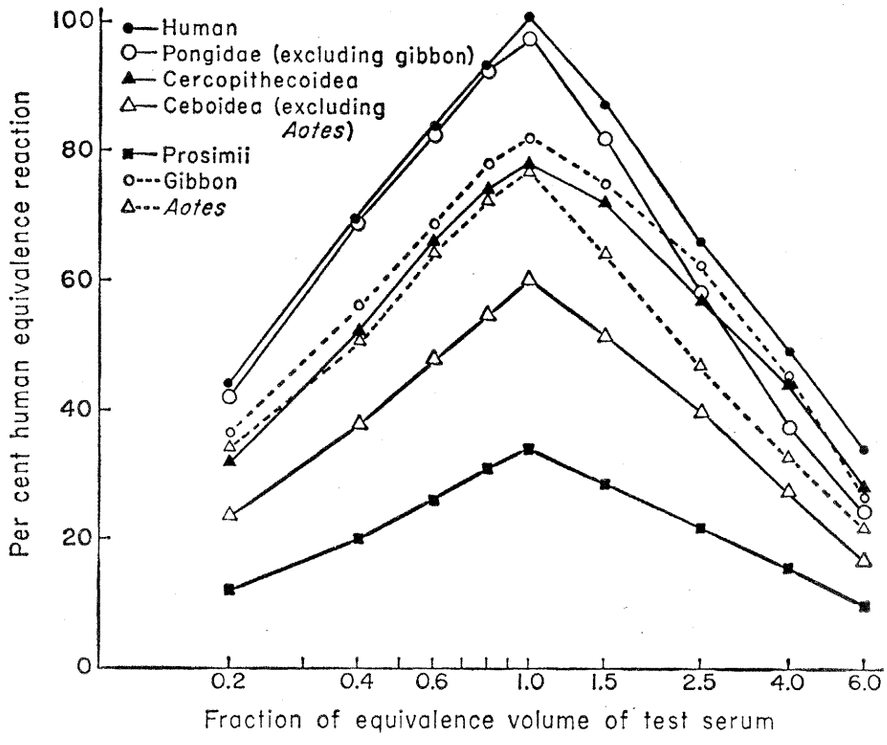

Fig. 1. Average values for antigenic correspondence of serum albumins of species in major primate taxa (——). Individual species which vary significantly from their group average (- - -). The volume of antigen (test serum) which yields maximum precipitate with 0.25 ml antiserum is defined as the equivalence volume.

for each major taxonomic subdivision. The albumins of three species differ significantly from other members of their respective groups in their reaction with antibodies to HSA. Gibbon (*Hylobates*) gives a much lower value than the other great apes, chimpanzee (*Pan*), and gorilla; the value for night monkey (*Aotes*) in the Ceboidea is relatively high for the group; and *Propithecus* is low among the Prosimii. The analysis of homologous antigens from a sizable number of related species is expected to reveal exceptions to the general pattern of results. They are in many respects more interesting since they may suggest correlations with other evolutionary modifications of the species in question.

Proteins of gibbon and orangutan (see the results of Gemeroy) are less closely related to those of man than are the homologous proteins of gorilla and chimpanzee. Goodman found this true in the case of serum albumins, transferrins, and ceruloplasmins (*17*). The primary structures of hemoglobins from the adult human, chimpanzee, and gorilla are quite similar but that of orangutan gives somewhat different peptide patterns ("finger prints") by electrophoretic-chromatographic analysis (*18*). Gibbon hemoglobin appears similar to that of human, differing significantly in only one tryptic peptide of the β-chain (*2*).

According to Simpson, evidence from fossils suggests that gibbon has probably been distinct from the other apes since early Oligocene (35 million years) and certainly since the Miocene (28 million years). Orangutan is morphologically and adaptively similar to living chimpanzees (*19*). Indeed serological evidence and karyological evidence (*20*), seem to be the only basis for the separation of *Pongo* from *Pan* and *Gorilla*. Although it is generally accepted that *Homo* is most closely related to *Pan* and *Gorilla* ". . . *Homo* represents an anatomical and adaptive complex very radically different from that of any other known animal . . ." (*19*). The close correspondence of the albumins of men and the African apes found in this study might suggest that there has been convergent or parallel evolutionary modification. There seem to be no other examples, however, of convergent characters between pongids and hominids (*19*). The alternative is that there have been only slight changes in the antigenic structure of albumins in these lines of descent for over 20 million years. Goodman has proposed that the advanced development of the highly efficient hemochorial placenta, particularly in man, by increasing the possibility for isoimmunization of the mother by fetal serum antigen, would exert a severe selective pressure against the survival of mutant genes which introduce new antigenic determinants into the population (*21*). This would result in a decrease in the rate of evolutionary change of antigenic structure.

The higher-than-expected correspondence of *Aotes* albumin to HSA is also more likely to be due to arrested modification than to convergence, but the qualitative data of Goodman (*22*) on the determinants involved are not decisive in this regard, nor would it seem possible to invoke the placental theory to explain a decreased rate of modification. *Aotes* differs from most of the other Ceboidea in having certain primitive features of the hand (*12*), but it shares this characteristic with tamarins whose albumin cross-reacts within the range of the other New World monkeys.

The difference between the albumins of the two families of lemurs represented in this study, Lemuridae (*Lemur*) and Indriidae (*Propithecus*), is also difficult to explain on the basis of available information about other distinctive traits. Indeed there is no reason to suppose that quantitative analysis of other protein antigens would confirm that *Aotes* and *Propithecus* are exceptions to the general pattern of antigenic correspondences.

One of the more interesting results of this investigation is the correspondence value of the tree shrew (*Tupaia*). There is still some disagreement among systematists concerning the proper relationship of the tree shrew to the Primates. Some hold the view that its resemblances to the insectivores outweigh its primate affinities (*23*). The high correspondence of *Tupaia* to HSA, which is within the range of other prosimians and twice the value of the insectivore (hedgehog) included in the series, provides further support to the view that the tree shrew is a true primate. *Tupaia* alone among the prosimians, however, has evolved a hemochorioplacenta. If the isoimmunization theory of evolutionary arrest is correct, and if it could apply to the tree shrew, then it is pos-

sible to hold that the high cross-reaction of the albumin is not a valid indicator of the degree of phylogenetic relationship of this organism to man, and that the antigenic correspondence observed is only coincidentally equivalent to the prosimians. This question could probably be resolved by quantitative and qualitative analysis with antiserums to certain prosimian and insectivore albumins.

The many variables in the immune response and in immunochemical reactions must be considered in the interpretation of cross-reaction data. In addition, the variables of evolution must be considered in relating immunochemical correspondence to phylogenetic relationships.

1) The specificity of antibodies depends on two factors: the multiplicity of distinct determinants against which antibodies are formed, and the heterogeneity of antibodies to each determinant with respect to binding affinity. The extent to which determinant groups are shared by a cross-reacting antigen and the reference antigen, and the net relative affinities of the antibodies for each common antigenic site will determine the amount of antibody in the precipitate. Other factors which affect the relative specificities of antiserums are differences in the response of individual animals to immunization, the length of time immunization is prolonged (*24*), and the phylogenetic relationship of the animal producing antibodies and the species of the antigen donor.

Antiserums from hyperimmunized animals were employed for our experiments in order to assure measurable cross-reactivity of nonprimate albumins. The high cross-reactivity of the albumins from the chimpanzee and gorilla may be due in part to the decrease in specificity resulting from hyperimmunization.

Pooling antiserums assures a sufficient quantity of reactant for a large study and minimizes aberrant results due to variation of response among the individual rabbits. One of 18 rabbits produced antiserum with no detectable antibody to nonalbumin serum components. A pool of bleedings (pool I) from this rabbit was used for gel diffusion studies and a few quantitative precipitation experiments. Pools I and II contained 3.7 and 3.6 mg of antibody per milliliter, respectively, but they differed markedly in their combining ratios (R is the ratio of antibody to antigen, by weight, in the precipitate at equivalence) giving the values $R_{(\text{pool I})} = 4.6$ and $R_{(\text{pool II})} = 6.4$. Pool I gave significantly higher correspondence values than pool II with gibbon albumin and with *Galago* albumin. Values significantly lower than pool II values were obtained with rhesus (*Macaca*) and baboon (*Papio*) albumins.

Table 2. Averages and ranges of immunochemical correspondence to HSA of serum albumins from major subdivisions of primates. Members of same genus were averaged and used once in determining the average list in table. Values are for equivalence reaction.

Taxa*	Av.	Range	Divergence time † ($\times 10^6$ yr)
Hominoidea			
Hominidae (*Homo*)	100		
Pongidae (*Pan, Gorilla*)	97	96–98	20–30
Pongidae (*Hylobates lar*) ‡	82	81–83	30–40
Cercopithecoidea	78	76–82	35–45
Ceboidea (excluding *Aotes*) §	60	52–65	45–55
Prosimii (excluding *Propithecus*) ‖	36	31–39	55–65

* Classification according to Fiedler (*28*). † Times elapsed since divergence from hominid line of descent as suggested by G. G. Simpson, personal communication. ‡ Four different individuals. § Value for *Aotes* was 77. ‖ Value for *Propithecus* was 23.

2) The methods employed for antigenic correspondence studies must be considered very critically. Ideally, they should make use of antigens in similar states of preservation and purity, both achieved by the same methods. Under such circumstances the relative amounts of antibody in the quantitative precipitates at equivalence is a measure of antigenic correspondence to the reference antigen or immunogen. When the actual amount of the cross-reacting antigen is unknown, however, and the assay values of the total precipitate are used to determine cross-reactivity, it is necessary to determine the possible error introduced by the assumption that the combining ratios (R) are the same in both reactions. Weigle has shown that R decreases irregularly with the degree of cross-reaction, the decrease being as great as 20 percent for antigen homologs from species in the same order and as much as 45 percent for certain more distantly related antigens (*6*). The operational assumption, however, is that $B_1/B_0 = T_1/T_0$, where B_0 and T_0 are assay values for precipitate antibody and total precipitate for the reference reaction, and B_1 and T_1 are the corresponding terms for the cross-reaction. If $\ddot{B}_1$ is the assumed value of B_1 when

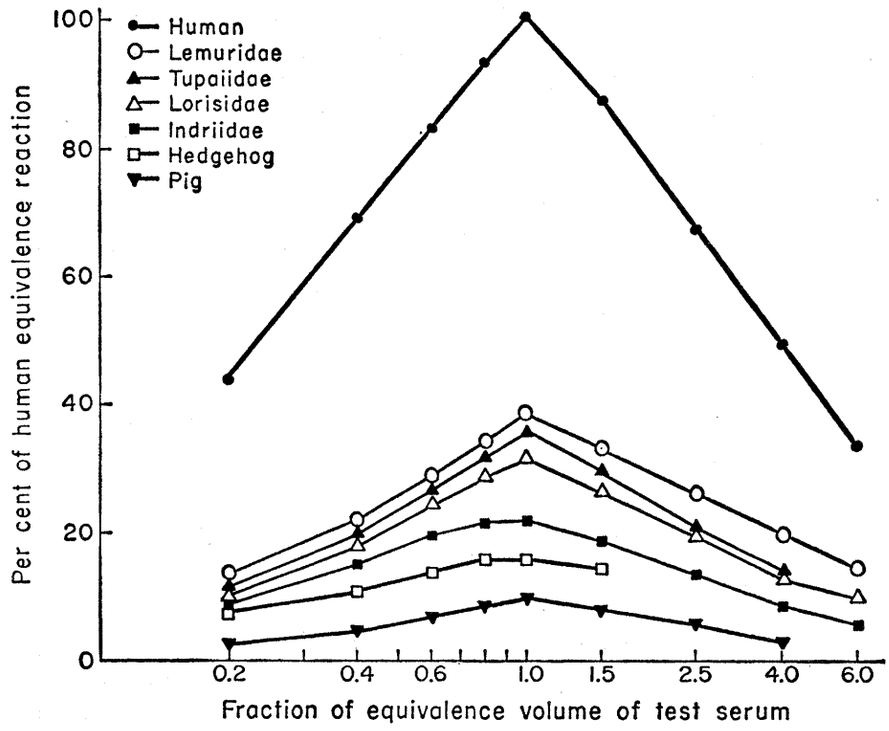

Fig. 2. Values for antigenic correspondence of prosimian families and nonprimate species.

R differs from R_0 then the assumption is stated

$$\frac{\tilde{B}_1}{B_0} = \frac{T_1}{T_0} = \frac{B_1E_b + A_1E_a}{B_0E_b + A_0E_a} \quad (1)$$

where the general reaction equation in terms of the assay is

$$T = BE_b + AE_a \quad (2)$$

A is the precipitate antigen, E_a is the extinction coefficient (E_{280}; 1-cm light path, 1-percent solution) of the antigen and E_b is the extinction coefficient of antibody globulin. For the purpose of this estimation, E_a is considered insignificantly variable among closely related, native antigen homolog and E_b is considered relatively invariable within the antibody population (*25*). The percentage of error (e) in B_1 when R differs from R_0 is expressed by

$$e = \frac{\tilde{B}_1 - B_1}{B_1} \times 100 \quad (3)$$

When $R_0 = B_0/A_0$, $R_1 = B_1/A_1$ and $S = E_b/E_a$, the expression rationalizes to

$$e = \frac{100(R_0 - R_1)}{R_1(1 + R_0S)} \quad (4)$$

If E_{280} is 5.5 for HSA and 15 for antibody, S equals 15/5.5 or approximately 2.7. As in the general case above, we have assumed similar values of the extinction coefficient for all serum albumins. We also assumed that the relation, $R_{(\text{pool II})} = 6.4$, would hold for HSA in whole serum, and on the basis of Weigle's findings that the decrease in $R_{(\text{pool II})}$ would be no more than 20 percent for the cross-reactions of the primate serum albumins. The possible over-estimate, in percent, of antibody precipitated, was approximately

$$e = \frac{1.3 \times 100}{5.1(1 + 6.4 \times 2.7)} = 1.4 \quad (5)$$

Since this value is clearly within the limits of experimental error, the total absorbancies of the precipitates at equivalence were taken to be proportional to antibody precipitated. If R_1 differed from R_0 by as much as 50 percent for the nonprimate albumins, e could be no greater than 5.5 percent. Changes in S due to differences in E_a introduce only minor changes in estimated errors. However, such small percentage errors occur only in systems where the value of R or S, or both, is relatively high. In assay methods such as nitrogen determination or the biuret reaction S would be low; R tends to be low in systems employing larger antigens.

3) Immunochemical correspondence is a reflection of the similarity of the surface of the cross-reacting antigen to that of the reference antigen or immunogen. In phylogenetic terms, comparison of protein homologs of living species is considered to be an analysis of the structural similarities retained from, and in common with, the homologous protein of a common ancestor (*26*). The same principle is applied to comparisons of amino acid sequences of homologous proteins (*1*). A decrease in the rate of evolutionary modification of the antigen of any species within a group, after its divergence from the reference line of descent, will result in a greater resemblance to the common ancestral molecule. This results in a higher immunochemical correspondence to the reference antigen than expected on the tentative assumption that immunochemical correspondence reflects the relative lengths of evolutionary time elapsed since divergence.

The concept of rate is employed in this context to denote net change over a period of time. It is not assumed that the modifications were introduced at a constant rate throughout that period. The longer the period of time under consideration, and the larger the units of time employed, the more regular a rate function would appear. Data obtained from immunochemical comparisons may be plotted against estimated time of divergence (*8*, *26*), or straight-line rate functions may be assumed and used to estimate probable ranges of divergence times (*9*). Margoliash has demonstrated that the latter procedure with statistical corrections is not unreasonable when applied to amino acid sequences of the cytochromes c of species whose divergences span a very long period of evolutionary time (*27*).

Table 2 summarizes the quantitative immunochemical correspondence among primate albumins. With the exceptions and reservations mentioned, there is a regular relationship between correspondence and elapsed evolutionary time.

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7 February 1966

Virus: Mixed Infection with Herpes Simplex and Simian Virus 40

Abstract. *Mixed infection, the infection of a single cell by two distinguishable viruses, has been demonstrated by electron microscopy in cultures of African green monkey kidney cells after inoculation with simian virus 40 and herpes simplex. Mixed infection occurs rarely when the two viruses are inoculated simultaneously, but if herpes is inoculated 24 hours after SV40 both viruses are found in the same nucleus in about 5 percent of intact cells.*

Mixed infection, the infection of a single cell by two distinguishable viruses, has been demonstrated by electron microscopy with African green monkey kidney (AGMK) cells infected with adenovirus 12 and simian virus 40 (SV40) (*1*). Since the two types of virus particles were intermingled within the same nucleus, the possibility of phenotypic mixing with production of virus particles with altered oncogenic properties was suggested. Subsequently, evidence of phenotypic mixing and "hybrid" formation after mixed infection of monkey kidney cells with several human adenoviruses and SV40 has been presented (*2*).

In order to determine whether mixed infection with SV40 and another type of DNA virus was possible, we have infected AGMK cultures with SV40 and herpes simplex virus (HSV). Tube cultures of the kidney cells were obtained from Microbiological Associates, Inc., Bethesda, Maryland. The strain of SV40 and methods of growth and titration have been described (*1*). The strain No. 11124 of HSV, obtained from W. K. Ashe, was grown and titrated in diploid human Wi-38 fibroblasts (*3*).

Three groups of AGMK tubes were used in each experiment. The first was inoculated with $10^{5.4}$ $TCID_{50}$ (tissue culture infective dose, 50 percent effective) of HSV; the second was infected with $10^{5.4}$ $TCID_{50}$ of HSV and $10^{6.9}$ $TCID_{50}$ of SV40 simultaneously; and the third was infected with $10^{5.4}$ $TCID_{50}$ of HSV and $10^{6.9}$ $TCID_{50}$ of SV40, but the inoculation of HSV was delayed 24, 26, or 30 hours after the initial inoculation of SV40.

Methods for infection of cultures and electron microscopy have been described (*1*). Incubation times and results are listed in Table 1.

Single infection with HSV or simultaneous infection with the two viruses resulted in a herpes-type cytopathic effect within 24 hours. By electron microscopy, after 42, 47, and 72 hours of incubation, herpes particles were found in over 90 percent of intact cells. No cells with SV40 particles only were identified in the cultures infected with SV40 and HSV simultaneously, but a rare cell (less than 1 percent) was found containing both types of particles within the same nucleus.

When SV40 was inoculated 24 hours prior to HSV, the cytopathic effect was still of the herpes type, and, by electron microscopy, approximately 60 percent of the intact cells contained HSV only, 20 percent contained SV40 only, and 5 percent contained both types of virus particles within the same nucleus (Fig. 1). Delay of inoculation with HSV for 26 and 30 hours after SV40 produced a mixed type of cytopathic effect, and, by electron microscopy, approximately half the cells contained HSV only and half contained SV40 only. Mixed infection was seen in less than 1 percent of cells in the group with the 30-hour interval between SV40 and HSV.

Table 1. Mixed infection with herpes simplex virus (HSV) and simian virus 40 (SV40).

Subgroup	Incubation time (hr)	Cells infected (%)		
		HSV	SV40	Mixed
Group 1, HSV				
a	42	90		
b	47	90		
Group 2, HSV and SV40 simultaneously				
a	42	90		
b	47	90		
c	72	90		<1
Group 3, HSV preceded by SV40				
a*	76	60	20	5
b†	73	50	40	
c‡	72	40	50	<1

*HSV inoculated 24 hours after SV40; †HSV inoculated 26 hours after SV40; ‡HSV inoculated 30 hours after SV40.

Although it was possible to demonstrate mixed infection with SV40 and

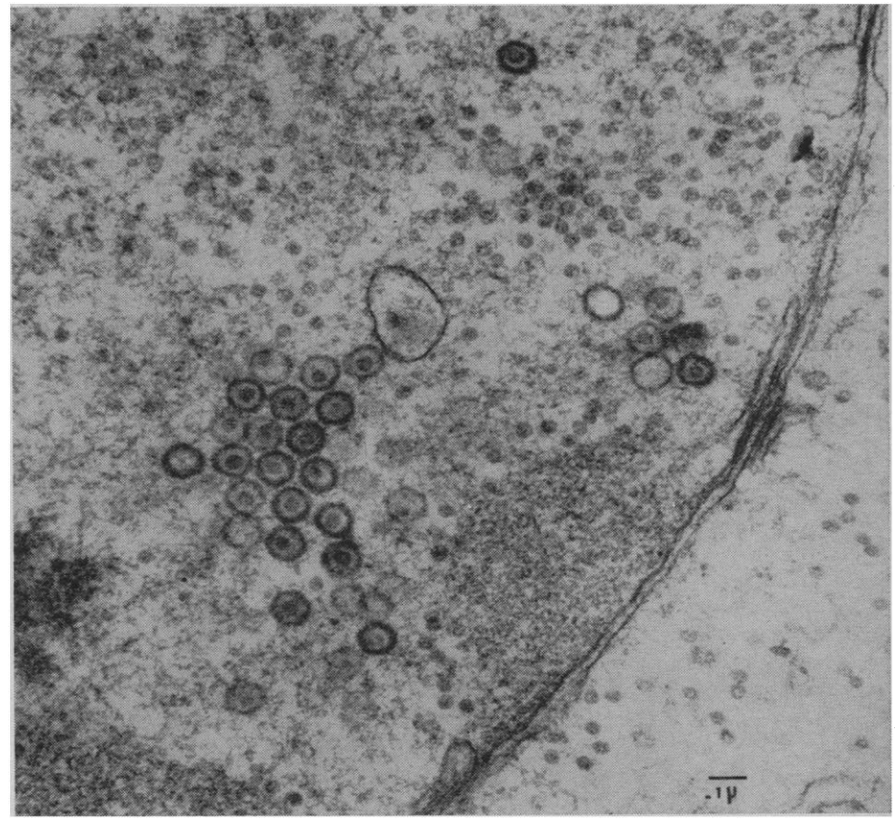

Fig. 1. African green monkey kidney cell with intranuclear clusters of herpes simplex virus (95 to 100 mμ) and SV40 (45 mμ) (× 56,500).