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19. Supported by a NSF grant. We thank J. G. Flaks for stimulating discussions during the early part of this work, and Thomas Owens Vaughan for technical assistance.

24 June 1964

Transferrin: Variations in Blood Serum of Red Howler Monkeys

Abstract. *Blood serum samples from 33 red howler monkeys* (Alouatta seniculus) *were examined. Three different phenotypes were found and denominated A, B, and C. Four serums could not be classified because their transferrin apparently did not bind iron-59, possibly owing to saturation. A difference was observed in the electrophoretic migration and pattern of the transferrins in these monkeys compared with those of other primates.*

The fact that transferrin of human blood is characterized by a genetic polymorphism (*1*) has led to the knowledge that *Macaca mulatta* (*2–4*), *M. irus* (*2, 3*), *M. radiata* (*2*), *M. nemestrina* (*2*), *Papio doguera* (*5*), and *Cebus nigrivitattus* (*3*) have a more extensive polymorphism than man. Multiple transferrins were also found in the chimpanzee (*2; 6–8*) and their genetical mechanisms have been already established (*9*). Similar information about other pongids (*2*) has been obtained, but the transferrins of other primates have not been studied, particularly those of the platyrrhines. Information about New World primates is in general very scanty, and the new trends in research, as described by Buettner-Janusch (*10*), have barely been aimed at these animals.

This report refers to a study made in 33 howler monkeys (*Alouatta seniculus*) collected in central Venezuela. Howler monkeys have peculiar vocal specializations. They belong to the infraorder Platyrrhina (*11*) and are found all over tropical America. Thirty-one of these animals were kept in the animal house of our institute, and two belonged to the local zoo (*12*). Blood was drawn by puncture of either cardiac or femoral vein under sterile conditions. For the handling of these samples we used routines which have given optimum results in our laboratory. All samples were kept at −20°C before electrophoresis. Some serums were stored for as long as 16 months, most for 4 or 5 months, and others for only 1 day before analysis.

Transferrins were studied by starch-gel electrophoresis in borate buffer by Smithies' horizontal technique (*13*) with minor modifications (*14*). Identification was made with iron-59 in sulfate form added to the serum in a proportion of 5 μc/ml, and autoradiography was performed on Ansco non-screen x-ray films (*15*). Protein fractions were stained with amido black 10B.

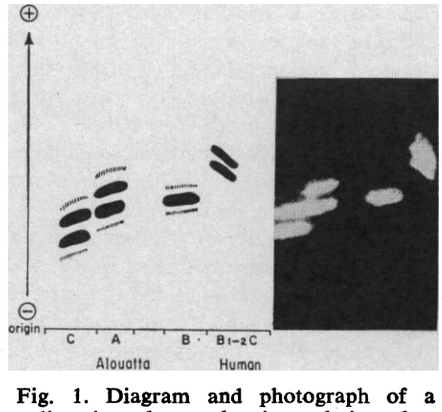

Fig. 1. Diagram and photograph of a radioactive plaque showing relative electrophoretic mobilities of transferrin bands in howler monkeys and man.

Four of the animals could not be classified because their transferrins did not bind iron-59, possibly because of saturation. Unfortunately, we did not have enough serum from these specimens to repeat the study. In those red howler monkeys where the transferrin could be made visible by autoradiography, results indicated three well-defined phenotypes, one constituted by three bands and the other two by four bands differing only in their mobility. Since there was no information about breeding and pedigree, the phenotypes were designated by letters (Fig. 1). Eight specimens were type A (27.6 percent), 20 were type B (68.9 percent), and only one was type C (4.3 percent).

The pattern of slow-moving multiple bands presented by the howler transferrins may suggest that our results are due to the presence of neuroaminidase in these serums. The splitting and slowing of transferrin bands as a consequence of contamination with agents that produce neuroaminidase has been mentioned (*16*). This possible effect was controlled with the blood of the two animals from the zoo. Their bloods, obtained and handled under checked sterile conditions, were the last studied. The serums were analyzed 30 hours after exsanguination of the animals; one of the serums was of type A and one of type B. We conclude that none of our three phenotypes are due to the action of neuroaminidase.

The phenotype patterns in this species of *Alouatta* are somewhat similar to those obtained by Boyer and Young (*6*) and Buettner-Janusch (*7*) in chimpanzees. The howler monkey transferrins move in the same general zone as those of the chimpanzee—that is, they are slower than human transferrin C.

From the meager comparative data available for transferrins in platyrrhines it might be concluded that the three howler monkey phenotypes are different from those of *Cebus* (*2, 3*), *Lagothrix* and *Ateles* (*2, 17*), and *Cacajao* (*2*). They also differ from those of all known prosimians and cercopithecoids. These serological data

agree well with those from chromosomal (*18*) and recent anatomical studies (*19*), all of which indicate that *Alouatta* is a fairly specialized creature among living New World primates. We base this statement on the known fact that it is only in *Homo* and some pongids that we find slow-moving transferrins within the Primates (*2*). As we follow the members of this order from the prosimians to the hominoids the tendency appears to be one of acquiring increasingly slower transferrins. *Alouatta* seems to have gone further in this tendency than other known platyrrhines.

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17 August 1964

Fructose-1,6-diphosphate Requirement of Streptococcal Lactic Dehydrogenases

Abstract. *The lactic dehydrogenase of a strain of* Streptococcus bovis *specifically requires fructose-1,6-diphosphate for activity. Phosphate or fructose-1,6-diphosphate prevents inactivation of the dehydrogenase, but phosphate and other compounds cannot be substituted for the fructose-1,6-diphosphate required for activity. Lactic dehydrogenases of other species of* Streptococcus *show a similar requirement for fructose-1,6-diphosphate.*

Streptococcus bovis produces lactic acid as a major product of glucose fermentation. Examination of extracts for a pyridine nucleotide-linked lactic dehydrogenase showed occasional low rates of oxidation of reduced nicotinamide adenine dinucleotide ($NADH_2$) in the presence of pyruvate, but usually no activity was observed. After testing many possible explanations for the poor lactic dehydrogenase activity by using protective agents (thiols and chelating agents) during extract preparation, testing for nonpyridine nucleotide-linked lactate oxidation with dye acceptors, and testing other parameters without being able to explain the lack of lactic dehydrogenase activity, it was discovered that highly active lactic dehydrogenase-containing extracts could be obtained if two conditions were satisfied. These conditions were (i) the preparation of extracts in the presence of phosphate buffer and (ii) the addition of small amounts of fructose-1,6-diphosphate (F16P) to the assay system. The unusual, highly specific requirement for F16P for activity of an NAD-linked lactic dehydrogenase is described in this report, and the possible significance of the F16P requirement as a metabolic control mechanism is discussed.

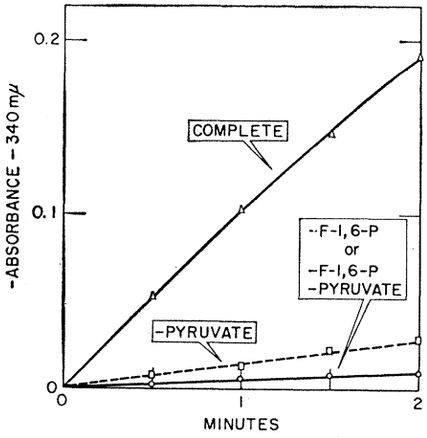

Fig. 1. Requirements for lactic dehydrogenase activity. Cuvettes (1 cm light path) contained 17m*M* tris, *p*H 7.2; 3.3m*M* neutral cysteine (*7*); 27m*M* sodium pyruvate; 20m*M* $MgCl_2$ (*7*); 0.08m*M* $NADH_2$; 1 m*M* F16P; and 10 μg of extract protein in a total volume of 3.0 ml. Extract, diluted in 0.1*M* Na_2HPO_4, *p*H 7.4, was added to start the reaction. Absorbancy changes at 340 mμ were measured with a Cary model 14 spectrophotometer.

Streptococcus bovis (*1*) was grown in 20 liters of medium as previously described (*2*). Harvested cells were washed with 0.1*M* Na_2HPO_4, *p*H 7.1, which contained 0.01 percent β-mercaptoethanol, and then suspended in the same solution. The cells were broken in a Raytheon sonic oscillator (10 kcy) under nitrogen gas and centrifuged to remove debris and residual whole cells. The supernatant solution was centrifuged at 100,000*g* for 1 hour in the model L Spinco centrifuge, and the clear supernatant solution was used in the experiments.

Figure 1 shows the absolute requirement of F16P for $NADH_2$ oxidation in the presence of pyruvate and extract. Slight $NADH_2$ oxidation was observed in the absence of pyruvate. Figure 2 shows that 10^{-4}*M* F16P was sufficient for maximum activity. No reduced nicotinamide dinucleotide phos-

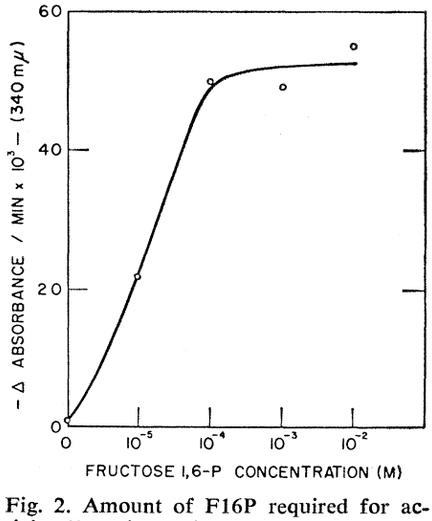

Fig. 2. Amount of F16P required for activity. Experimental procedure was as indicated for Fig. 1 except for the addition of the quantities of F16P shown in Fig. 2.