shape could not be determined with a zero splash height (H).

The other shape parameters, α, β, and R, did not exist for zero water depth. Because of the inability to measure the shape parameters at zero depth, Eqs. 1 through 6 can be used only for $d/D \geqslant 0.02$, which is the lower limit of the experimental independent variable.

Asymptotes for the above equations were estimated from measurements of splashes in deep water, $d = 9$ cm, by reasoning that the effects of depth on splash shape would be negligible for depths greater than the asymptotic value. Values of d/D corresponding to 99 percent of the asymptotic value of the dependent variable for Eqs. 1 through 6 are 1.3, 2.2, 3.3, 0.8, 0.6, and 0.8. Thus, depth likely has a negligible effect on splash for depths greater than three waterdrop diameters.

The parameters describing splash-shape size and time reach maxima at water depths of 0.28 D, 0.24 D, and 0.37 D for Eqs. 1, 2, and 3, respectively. Thus it can be inferred that water depth has its greatest effect on raindrop splash at depths of about one-third drop diameter.

If water depths greater than three waterdrop diameters have little effect on splash shapes, then it may be assumed that waterdrop impact has little effect on the underlying soil surface covered by such water depths. This depth is 8.7 mm for the smallest waterdrop diameter used and is not likely to occur over a significant portion of an agricultural field. However, rainfall may consist of drops much smaller than 2.9 mm. Laws and Parsons (*4*) give

$$D_{50} = 2.23\, I^{0.182} \qquad (7)$$

where I is rainfall intensity in inches per hour and where D_{50} is defined as the median raindrop diameter. The volume of drops larger than the median is 50 percent of the total volume. For a rainfall of 2 inches (5.08 cm) per hour (which is a highly erosive rainfall intensity), $D_{50} = 2.5$ mm. Most of the raindrops are smaller than the median diameters. Therefore, if Eqs. 1 through 6 may be assumed valid for drop diameters somewhat smaller than the range of waterdrops used, the required thickness of a protective water layer becomes small enough so that it could feasibly form over substantial portions of a field. This reasoning is supported by the observed effectiveness of only a small amount of mulch in reducing erosion on bare soil. Although mulch undoubtedly impedes sediment transport in runoff, it also increases the depth of surface water storage during a rainstorm. Thus, any method of maintaining a thin water layer may greatly reduce soil detachment due to raindrop impact and, hence, may reduce soil erosion.

CALVIN K. MUTCHLER
LYLE M. HANSEN
North Central Soil Conservation Research Center, Soil and Water Conservation Research Division, U.S. Agricultural Research Service, Morris, Minnesota 56267

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8 June 1970

Insulin Levels in Primates by Immunoassay

Abstract. *Only trace amounts of insulin were detected by an immunoassay system with guinea pig antibody to pork insulin in the New World primates* Cebus *and* Saimiri. *The system found insulin levels in the Old World primates rhesus and chimpanzee which were quite like those of human beings. The findings suggest important structural differences in the insulins of the two primate divisions.*

Insulin could not be detected in the plasma of two species of New World primates either before or after administration of glucose when an immunoassay system that detects the expected amount of insulin in Old World primates and human beings was used. In this immunoassay system, insulin from subprimate species is used for the production of antibodies to insulin in another species (*1*). This is usually done in a small laboratory rodent with bovine or porcine insulin. The reference standard also is typically beef or pork insulin. Since in the assay the sample and added isotopically labeled insulin compete for the antibody to insulin, the antibody must not distinguish between insulin of different species if the measurements are to be valid. The usual procedure is to use the available porcine insulin as the working standard after comparing its recovery by the system with the primary standard, human insulin. This use of nonhuman materials for assaying insulin in human plasma is possible because of the cross-reaction of many mammalian antigen systems. The structures of human, beef, and pork insulin differ in only a few amino acids (*2*). These insulins, as well as those of horse, whale, dog, cat, rabbit, hamster, and man have all been shown to be neutralized by guinea pig antibodies to beef insulin (*3*). However there are insulins derived from other species which do not react with this antibody; for example the insulin from guinea pig, coypu, and capybara are not bound by antibody to beef insulin (*4*).

In the assay method of Hales and Randle (*5*) the complex of insulin and antibody is precipitated (for counting) by still another antibody, which is made to react with gamma globulin of the species in which the antibody to insulin was produced. In some other methods, including the one used here, the insulin–antibody complex is separated by ethanol precipitation.

During studies of the long-term effects of diets on glucose tolerance in *Cebus* and in rhesus monkeys, intravenous glucose tolerance tests were done. When plasma insulin was measured during these tests, only negligible amounts could be found in the *Cebus* monkeys although the insulin content of the plasma of rhesus monkeys was quite like that of human subjects. Since the glucose tolerances were not abnormal in the *Cebus* monkeys, it was assumed that the assay was not detecting insulin in this species. Investigation of additional species suggested that Old World primates have humanlike responses of insulin level while the New World primates have no measurable response of plasma insulin to glucose. There appear to be important immunological differences in the insulins which account for these findings.

The *Cebus* monkeys were jungle-born *Cebus apella* that had been fed purified diets for 8 to 10 years for studies of sterol metabolism and atherogenesis (*6*). These diets were formulated to contain marginal amounts of the essential, sulfur-containing amino acids, and supplementary cholesterol was added. The ani-

mals grew normally and were healthy although they developed hypercholesteremia. The squirrel monkeys (*Saimiri sciurea*), the rhesus monkeys (*Macaca mulatta*), and the chimpanzees (*Pan troglodytes*) were obtained from dealers. The chimpanzees had been obtained in infancy and fed purified diets like those used for the *Cebus*. They were 7 to 10 years of age. The rhesus monkeys were young adult animals fed Purina monkey chow freely. None of these animals had received insulin injections.

Glucose tolerance testing was done by administering a small dose of barbiturates (*7*) intravenously to induce somnolence followed by 500 mg of glucose per kilogram of body weight in a 50 percent solution given intravenously in 1 minute. Blood samples were drawn from the opposite arm at 10-minute intervals for 40 minutes in a syringe wet with heparin (10 mg/ml). The bloods were deproteinized with Somogyi's reagent (*8*), and glucose was measured in the supernatant with a glucose oxidase method (*9*). The K_{gl} (*10*) was calculated according to Lundbaek (*11*). Additional plasma was stored at −20°C for measurement of insulin by the Heding (*12*) procedure. In this method pork insulin labeled with ^{125}I, guinea pig antibody to pork insulin, and porcine reference insulin are used. The antibody to insulin was obtained (*13*) from 15 guinea pigs and pooled. The insulin-antibody complex was separated from free insulin by precipitation with 80 percent ethanol, and the radioactivity in the supernatant was determined by scintillation counting. The method was adjusted to optimize the precision in the range between 10 and 100 micro units of insulin per milliliter of plasma.

The insulin data are shown for the four species, along with the values of K_{gl} in Table 1. Only trace amounts of insulin were found in 13 individuals representing two New World primate species. Seven individuals from two Old World species showed a range of insulin levels like those seen in human beings. Porcine insulin was added to the plasma of both *Cebus* and squirrel monkeys and was completely recovered, ruling out an interference.

The explanation for this difference in insulin response between New and Old World primates is not known. While the New World primates appear to have little or no plasma insulin, it is more likely that the antibody used in this immunoassay system does not react with the insulin of the New World primates, especially since the K_{gl} values were quite as expected in those animals. This interpretation suggests that there are antigenic differences in the insulin of these New and Old World primate species. The findings suggest a diverse evolutionary development of these branches of the primate family (*14*).

Other workers have also found the insulin content of rhesus plasma to be quite like that of human beings when an immunoreactive method with pork insulin and a guinea pig–generated antibody was used (*15*). Certain other New World mammals including the guinea pig, the coypu, and the copybara have insulin which is not reactive with the antibody to beef insulin (*4*). Thus at least five New World species are known to have plasma insulin which is nonreactive to either beef or pork insulin antibodies. Insulin from a variety of other mammals including man, several Old World primates, beef, sheep, pig, horse, whale, dog, cat, rabbit, rat, muskrat, chinchilla, hamster, and mouse are reactive (*4*).

The nonreactivity of guinea pig and coypu insulin as compared to beef insulin may be attributed to differences in amino acid sequence. A sequence analysis of these New World primate insulins has not been reported and would be of great interest. The possibility of a biologically active insulin which is nonantigenic to the recipient might be of practical importance in the management of diabetes.

Table 1. Glucose tolerance and plasma insulin levels in four primate species. C, commercial diet; P, purified diet.

No.	Age (yr)	Sex	Diet	K_{gl}	Insulin (μunit/ml plasma)				
					Before glucose	Minutes after glucose			
						10	20	30	40
New World primates									
Saimiri sciurea									
60	3	♀	C	1.71	0	0	0	0	0
70	4	♂	C	1.81	0	0	4	0	0
73	3	♂	C	1.65	0	0	0	0	0
Cebus apella									
10	8	♀	P	3.25	0	1	3	1	0
11	8	♀	P	2.66	0	0	0	0	1
12	10	♂	P	2.61	0	0	2	0	0
14	9	♂	P	2.04	0	0	0	1	0
15	9	♂	P	2.47	0	0.5	0	0	0
47	3	♀	P	3.75	0	0	0	0	0
32	7	♂	P	0.96	0	0	0	2	3
33	7	♂	P	1.59	0	0	0	0	1
43	4	♀	P	1.55	0	0	0	0	1
49	3	♀	P	0.56	0	3	7	7	2
Old World primates									
Macaca mulatta									
A	3 ±	♀	C	1.69	4	37	27	22	21
B	3 ±	♂	C	2.43	13	156	27	10	0
C	3 ±	♂	C	2.57	10	11	41	59	19
252	8	♀	P	1.74	39	183	149	102	
Pan troglodytes									
1	10	♀	P	3.89	30	172	152	58	17
2	9	♀	P	5.77	10	112	47	21	11
3	7	♂	P	2.47	18	177	197	194	151

GEORGE V. MANN
Department of Biochemistry, Vanderbilt University School of Medicine, Nashville, Tennessee
OSCAR B. CROFFORD
Department of Medicine, Vanderbilt University School of Medicine

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16. Supported by PHS grants HE-3986 and HE-08195. G.V.M. is a career investigator of the National Heart Institute 5-K6-HE-8288-07; O.B.C. is an Investigator of the Howard Hughes Medical Institute.

8 June 1970; revised 22 July 1970