Hemoglobin A_{I_c} in the Glucose-intolerant, Streptozotocin-treated or Pancreatectomized Macaque Monkey

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SUMMARY

Hemoglobin A_{I_c} (Hb A_{I_c}), a glycosylated minor variant of Hb A, is elevated in diabetic patients. In our study, macaque monkey model with acquired glucose intolerance [both streptozotocin (STZ)-treated or pancreatectomized] was evaluated for the presence of Hb A_{I_c} and for evidence of a possible physiologic effect on oxygen-carrying capacity. Blood from 29 *Macaca* monkeys (27 rhesus), including 22 with carbohydrate intolerance (17 treated with STZ and five that were pancreatectomized), was analyzed for minor hemoglobins using Amberlite IRC-50 with cyanide-phosphate elution. Unlike in man, no Hb A_{I_c} peak was identified in any control animals. However, all five pancreatectomized animals and 10 of 17 STZ-treated animals had clearly identified peaks that eluted similarly to human A_{I_c} and had an increased glucose content after acid hydrolysis relative to the major Hb A peak. Of the more severely carbohydrate-intolerant animals (fasting blood sugar > 200 mg. per deciliter) that were receiving insulin, seven of eight monkeys had Hb A_{I_c} peaks compared with seven of 14 animals with less severe carbohydrate intolerance (fasting blood glucose < 200 mg. per deciliter) treated without insulin. During pregnancy, none of the seven STZ-treated animals had a definite peak, although one of these animals demonstrated an Hb A_{I_c} peak postpartum. In a group of five control and three STZ-treated nonpregnant animals, no differences were observed in whole blood pH, pCO_2, P50, 2,3-diphosphoglycerate, adenosine triphosphate, and adenosine diphosphate, and plasma inorganic phosphate, while plasma glucose was variably elevated. The glucose-intolerant macaque model has potential for studying the long-term effects of acquired hyperglycemia, the biochemistry of the glycohemoglobin, and the possible pathophysiologic effects of Hb A_{I_c} in pregnancy. DIABETES *27*:1182-88, December, 1978.

Hemoglobin A_{I_c} (Hb A_{I_c}), a glycosylated form of Hb A,[1] is increased in individuals with diabetes mellitus.[2] Hb A_{I_c} is the largest fraction of the minor fast hemoglobin subgroups of Hb A (Hb $A_{I_{a-c}}$)[3] and is acquired post-translationally during a slow, nonenzymatic process that continues throughout the red cell's life span.[4,5] In vitro studies suggest that glucose itself or its intermediary phosphorylated metabolites within the red blood cell may be the important reactants with the major Hb A in the formation of glycohemoglobin,[6-8] while in vivo evidence demonstrates that certain of these glycolytic intermediates

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are elevated in diabetics.[8] Furthermore, a positive clinical correlation has been made between the degree of chronic hyperglycemia in diabetics and their Hb A_{I_c} level.[9] As such, Hb A_{I_c} may serve as an indicator of the diabetic's glucose control in both nonpregnant and pregnant states.[9,10]

Physiologically, Hb A_{I_c} exhibits increased oxygen affinity relative to nonglycosylated Hb A in the presence of 2,3-diphosphoglycerate (2,3-DPG),[11,12] an important intracellular red-cell glycolytic intermediate. This phenomenon has been explained by stearic interference of 2,3-DPG by the N-terminal, β-chain glucose of Hb A_{I_c} being at a position in the hemoglobin molecule where this ligand would normally bind and exert its effect.[11] Extrapolating from these in vitro data to the in vivo situation, Ditzel suggested that elevated Hb A_{I_c} in diabetic patients may have adverse clinical effects on tissue oxygenation.[13]

Although Hb A_{I_c} has been seen in both the genetically induced and chemically induced diabetic mouse,[14] it has not been reported in any primate other than man. Our study was undertaken to determine if Hb A_{I_c} was detectable in control and in the glucose-intolerant, streptozotocin (STZ)-treated and pancreatectomized macaque. In addition, because the Hb A_{I_c} concentrations reported in pregnant diabetic patients are lower than those of nonpregnant diabetics,[16,17] the relationship to pregnancy was also explored. Finally, studies of several parameters affecting the oxygen-carrying capacity of blood from normal and glucose-intolerant monkeys were done to examine for a possible effect of Hb A_{I_c}.

METHODS

Seven control rhesus, 17 STZ-treated glucose-intolerant rhesus, and five pancreatectomized female *Macaca* monkeys* from three separate colonies [University of Puerto Rico (PR); Pregnancy Research Branch, National Institutes of Health (NIH); and University of Illinois (Ill.)] were examined for the presence of Hb A_{I_c}. Streptozotocin (40 to 48 mg. per kilogram I.V.) had been administered at least 17 months before, and pancreatectomies were performed at least two months before studying the glucose tolerance of any of the monkeys. Glucose tolerance was assessed after a 16-hour overnight fast under phencyclidine anesthesia by a single intravenous bolus infusion of glucose (0.5 gm. per kilogram) within three years of these studies. The upper limit of normal glucose disappearance was 11 minutes in nonpregnant control animals.[18] Glucose disappearance rate (T½) during the intravenous glucose tolerance test ranged from 18.5 minutes to infinity in all the experimental animals but one. That monkey (20 NIH) had previously been glucose intolerant (T½ = 23 minutes) but reverted to normal (T½ = 10 minutes) when the test was repeated immediately after the third Hb A_{I_c} sampling. Eight of the 15 animals (five pancreatectomized and three STZ treated) from the Illinois colony were managed with insulin (5 to 20 U. per day) on a sliding scale in response to marked hyperglycemia (fasting blood glucose > 200 mg. per deciliter), while all others received no special therapy. The former animals were evaluated by weekly, overnight-fasting plasma glucose analyses while the latter were studied monthly. Insulin was not administered to achieve normoglycemia but, instead, only to permit survival with hyperglycemia.

Washed red blood cells were hemolyzed, and Hb A_{I_c}, along with the other fast hemoglobins, was measured by cation-exchange chromatography on an Amberlite IRC-50 column using a modification of methods described previously.[3,16,19] Hb A_{I_c} was expressed as a percentage of the hemoglobin. The glucose content of Hb A_{I_c} was measured by fluorometric analysis with stoichiometric enzyme-coupled reduction of NADP after hydrolytic release of the sugar from the hemoglobin sample for four hours in 1 N HCl.[20] The hydrolyzed samples were neutralized, and aliquots were removed for glucose analysis by the addition of hexokinase and glucose-6-phosphate dehydrogenase.

The volume of packed red cells (VPRC) and the hemoglobin concentration of whole blood were measured by the usual laboratory techniques.[21] Plasma inorganic phosphorus was measured colorimetrically after complexing with molybdate.[22] Neutralized perchloric filtrates of whole blood were assayed for 2-3-DPG by the method of Keitt[23] and for adenosine triphosphate (ATP) and adenosine diphosphate (ADP) by high-pressure liquid chromatography.[24] Plasma glucose was measured by a glucose oxidase method on the YSI glucose analyzer (Yellow Springs Instrument Co., Ohio). Plasma insulin was measured by a double-antibody radioimmunoassay technique.[25]

Free-flowing venous or arterial blood gases were measured with either a Corning blood-gas analyzer no. 165 (Corning Scientific Instruments, Medfield,

*Three *Macaca mulatta*, one *M. fascicularis*, and one *M. arctoides*.

TABLE 1

Characteristics of the macaque groups studied for presence of Hb A_{Ic}

	Status	Glucose (mg./dl.)	Insulin (μU./ml)	G/I	Phos. (mg./dl.)	2,3-DPG (μM/ml. RBC)	Fast Hb (%)*	Hb A_{Ic} (%)*
I. Control								
1. NIH†	Nonpreg.	70	90	0.78	1.92	4.81	9.4	NP
2. NIH	Nonpreg.	57	32	1.78	2.51	3.65	8.2	NP
3. PR	Preg.	60 (f)	90	0.67	2.39	3.96	4.3	NP
4. PR (a)	Preg.	43 (f)	32	1.34	ND	ND	11.8	NP
(b)	Nonpreg.	76 (f)	57	1.33	6.72	5.38	6.9	NP
5. PR (a)	Nonpreg.	ND	ND	ND	ND	ND	11.2	NP
(b)	Nonpreg.	ND	ND	ND	ND	ND	7.8	NP
(c)	Nonpreg.	79 (f)	27	2.93	4.04	6.13	6.5	NP
6. NIH (a)	Nonpreg.	ND	ND	ND	ND	ND	7.8	NP
(b)	Nonpreg.	ND	ND	ND	ND	ND	11.9	NP
7. NIH (a)	Preg.	ND	ND	ND	ND	ND	5.2	NP
(b)	Nonpreg.	ND	ND	ND	ND	ND	8.5	NP
II. Carbohydrate intolerant								
A. Insulin dependent (STZ treated)								
8. Ill. (a)	Nonpreg.	267 (f)	ND	ND	3.86	4.98	10.5	NP
(b)	Nonpreg.	407 (f)	ND	ND	ND	ND	16.4	5.2
9. Ill.	Nonpreg.	63 (f)	ND	ND	3.75	6.32	3.8	NP
10. Ill. (a)	Nonpreg.	72 (f)	ND	ND	2.09	7.10	4.9	NP
(b)	Nonpreg.	260 (f)	ND	ND	ND	ND	12.6	3.6
B. Insulin dependent (pancreatectomized)								
11. Ill.	Nonpreg.	285 (f)	ND	ND	ND	ND	13.2	4.4
12. Ill.	Nonpreg.	493 (f)	ND	ND	ND	ND	13.4	4.0
13. Ill.	Nonpreg.	283 (f)	ND	ND	ND	ND	3.5	1.8
14. Ill.	Nonpreg.	269 (f)	ND	ND	ND	ND	10.3	3.4
15. Ill.	Nonpreg.	240 (f)	ND	ND	ND	ND	10.3	3.1
C. Noninsulin dependent (STZ treated)								
16. NIH	Nonpreg.	342	15	22.8	3.59	5.33	15.2	8.9
17. NIH (a)	Nonpreg.	509	34	15.0	2.66	5.37	10.7	5.2
(b)	Nonpreg.	682	28	24.4	3.19	4.94	7.6	4.1
18. NIH	Nonpreg.	201	42	4.79	1.66	5.15	13.4	7.3
19. NIH (a)	Preg.	75	140	0.54	1.72	4.39	5.9	NP
(b)	Preg.	75	64	1.17	2.86	4.36	3.4	NP
(c)	Preg.	181	260	0.70	1.98	5.06	8.2	NP
20. NIH (a)	Preg.	190 (f)	ND	ND	ND	ND	7.1	NP
(b)	Nonpreg.	ND	ND	ND	ND	ND	8.2	NP
(c)	Nonpreg.	87 (f)	170	0.51	ND	ND	5.8	NP
21. NIH	Nonpreg.	ND	ND	ND	ND	ND	17.6	8.7
22. NIH	Preg.	128 (f)	ND	ND	ND	ND	5.6	NP
23. Ill. (a)	Preg.	54 (f)	160	0.34	ND	ND	ND	NP
(b)	Preg.	29 (f)	32	0.91	2.77	5.80	4.2	NP
(c)	Nonpreg.	52 (f)	ND	ND	ND	ND	3.6	NP
24. Ill. (a)	Preg.	51 (f)	65	0.78	ND	ND	9.0	NP
(b)	Preg.	38 (f)	46	0.83	4.02	4.60	5.0	NP
(c)	Nonpreg.	57 (f)	ND	ND	ND	ND	3.7	NP
25. Ill. (a)	Preg.	58 (f)	19	3.05	ND	ND	10.7	NP
(b)	Preg.	88 (f)	10	8.80	2.79	5.07	8.1	NP
(c)	Nonpreg.	184 (f)	ND	ND	ND	ND	11.3	4.3
26. Ill. (a)	Preg.	51 (f)	315	0.16	ND	ND	7.8	NP
(b)	Preg.	89 (f)	71	1.25	3.51	3.91	9.2	NP
(c)	Nonpreg.	40 (f)	ND	ND	ND	ND	4.1	NP
27. Ill.	Nonpreg.	165 (f)	ND	ND	ND	ND	13.8	8.7
28. Ill.	Nonpreg.	133 (f)	ND	ND	ND	ND	12.7	7.9
29. Ill.	Nonpreg.	80 (f)	ND	ND	ND	ND	11.1	6.7

Abbreviations: 2,3-DPG, diphosphoglycerate; f, fasting specimen; G, glucose; Hb, hemoglobin; I, insulin; ND, not done; NP, no peak; Nonpreg., nonpregnant; Phos., phosphorus; Preg., pregnant; RBC, erythrocytes; STZ, streptozotocin.

*Per cent of total hemoglobin.

†The colony of origin is indicated by abbreviation—NIH, PR, or Ill (see text). Where multiple samples are indicated (a-c), they were obtained at intervals of three weeks to six months.

Mass.) or an Instrument Laboratory (IL, Lexington, Mass.) no. 213. Using a tonometer (IL model no. 237), oxygen dissociation curves were determined by plotting multiple points obtained at different oxygen concentrations but at a constant pCO_2 (40 mm.Hg). After measuring the pH and pCO_2 of a sample, the oxygen saturation was measured directly with an IL no. 182 co-oximeter. P50 actual was calculated from the P50 pH 7.4 with the Bohr correction factor.[26] The unpaired *t*-test was used for statistical analyses.

RESULTS

Data illustrating the plasma glucose and insulin concentrations at the time of study of individual female monkeys are presented in table 1. At the time of the study the glucose-insulin ratios of the experimental animals were within two standard deviations of the controls in one half of the cases measured. This feature, however, may be more dependent on whether the animals were fasted and whether they were pregnant, since there was a significant difference between plasma glucose in fasted and nonfasted ($P < 0.02$) and in pregnant and nonpregnant ($P < 0.05$) animals in which insulin was measured.

In contrast with nondiabetic man in whom an Hb A_{I_c} peak of 4 to 5 per cent of total hemoglobin is found, no peak was observed in the normal control monkeys (table 1, group I). Instead, only a single, distinct peak in the Hb $A_{I_{a+b}}$ region was evident (figure 1, top panel). This was not the case, however, for all three groups of carbohydrate-intolerant animals. All five pancreatectomized animals had Hb A_{I_c} peaks; while 10 of the STZ-treated monkeys (two of three animals treated with insulin and eight of 14 not receiving insulin) had distinct Hb A_{I_c} peaks (figure 1, middle panel). Although seven STZ-treated pregnant monkeys were examined, none had Hb A_{I_c} peaks

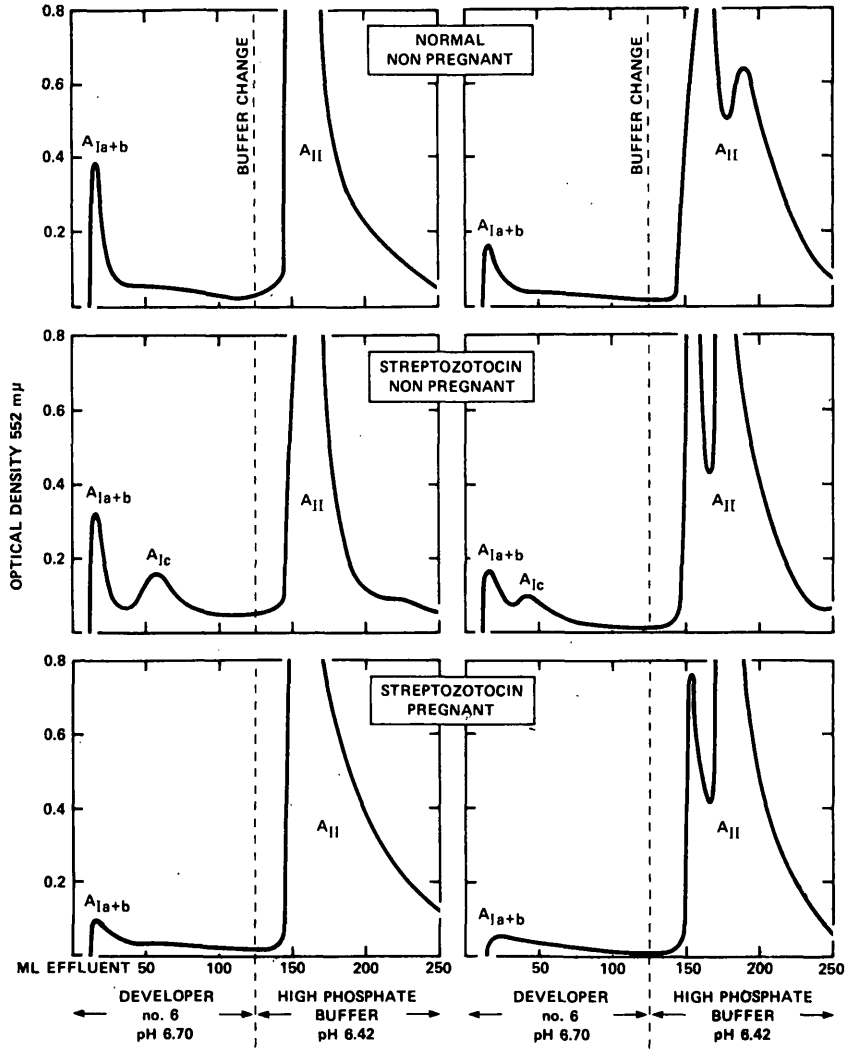

FIGURE 1

Chromatography of rhesus hemoglobin on Amberlite IRC-50. Top panel: Two normal, nonpregnant monkeys without Hb A_{I_c} peaks; (left) single major Hb A_{II} peak; (right) double major Hb A_{II} peak. Middle panel: Two nonpregnant, streptozotocin-treated animals demonstrating Hb A_{I_c} peak. Bottom panel: Two pregnant, streptozotocin-treated animals without Hb A_{I_c} peaks.

while pregnant (figure 1, bottom panel), although one manifested a peak postpartum (25 Ill.). It is noteworthy that, on chromatography, double peaks for the major hemoglobin fraction were observed in seven of the 29 macaques studied.

When the Hb A_{I_c} from an STZ-treated, nonpregnant animal was isolated chromatographically and then electrophoresed, it behaved similarly to human Hb A_{I_c} in that it migrated slightly faster towards the positive pole at pH 8.3 than did the major Hb A fraction from either man or monkey. This Hb A_{I_c}, like that isolated from man,[27] contained increased glucose after acid hydrolysis relative to the major hemoglobin peak.

In previous chromatographic analyses of human hemoglobin,[16] 95 to 100 per cent of the hemoglobin applied to the column was recovered in the separate fractions. In our study of macaque's hemoglobin, the recovery varied from 71 to 99 per cent. In one monkey (24 Ill.) the recoveries were 54 per cent in two of three determinations and may represent increased instability of that monkey's hemoglobin. When we examined the stability of the hemoglobin from four other monkeys, precipitation by 17 per cent isopropanol was observed to begin in 10 minutes in contrast with a human hemoglobin-A control of 30 minutes. These samples had been stored frozen for one month.

The VPRC and hemoglobin values for both the control and carbohydrate-intolerant, STZ-treated monkeys were not significantly different: 41 ± 5 versus 39 ± 3 per cent (Mean ± S.D.) and 13.2 ± 1.9 versus 12.1 ± 1.4 gm. per deciliter, respectively. Also, there was no significant difference between the two groups with respect to plasma inorganic phosphorus or whole blood 2,3-DPG.

Five nonpregnant control monkeys were compared with three nonpregnant, STZ-treated monkeys (table 2) with respect to several parameters of oxygen-carrying ability of blood—P50 pH 7.4, P50 in vivo, 2,3-DPG, pH, ATP, ADP, and Hb. All three STZ-treated animals manifested glycohemoglobin peaks. No significant differences were noted in this small series of observations between the two groups, although the 2,3-DPGs were all above the control mean in the three STZ-treated animals.

DISCUSSION

These are the first nonhuman primate data to show an elevation of Hb A_{I_c} in chemically induced glucose-intolerant macaque monkeys. However, when Hb A_{I_c} peaks were elevated in the nonpregnant animals, they were lower (1.8 to 8.9 per cent) than the 10 to 15 per cent Hb A_{I_c} reported for nonpregnant, insulin-dependent, adult diabetic patients. Those individuals without identifiable peaks were probably those whose glucose control was more normal. This has been suggested in diabetic patients in whom Hb A_{I_c} concentrations correlate with chronic, integrated blood glucose control over weeks or months.[9] Some of our data may be interpreted to support this suggestion. Of the glucose-intolerant animals receiving insulin (fasting blood glucose > 200 mg. per deciliter), seven of eight showed Hb A_{I_c} peaks. Of the noninsulin-requiring, glucose-intolerant group, eight of 14 animals had Hb A_{I_c} peaks. Finally, no elevations were observed in any of the noncarbohydrate-intolerant monkeys.

None of the pregnant, STZ-treated monkeys had a definite Hb A_{I_c} peak. It has been reported previously that pregnant, diabetic patients have lower Hb A_{I_c} concentrations than those who are not pregnant.[16,17] This finding might be due to either lower mean blood glucose values during pregnancy, or an intrinsic hormonal effect of pregnancy itself, or decreased red blood cell survival, or an increased population of

TABLE 2

Parameters of oxygen-carrying ability in control and glucose-intolerant rhesus monkeys

	VPRC* (%)	pH actual	P50 pH 7.40 (mm.Hg)	P50 in vivo (mm.Hg)	2,3-DPG (μM/ml. RBC)	ATP (μM/ml. RBC)	ADP (μM/ml. RBC)	ATP/ADP
I. Control (n = 5)	41	7.37	31.1	33.4	4.69	1.27	0.183	9.36
Mean ± S.D.	2	0.09	0.8	2.0	0.97	0.26	0.119	4.53
II. Glucose intolerant								
16 NIH	38	7.24	30.6	36.3	5.33	3.59	1.38	4.6
17 NIH	36	7.35	30.5	30.5	5.37	2.66	1.50	5.9
18 NIH	37	7.41	30.5	30.5	5.15	1.66	1.51	6.2

*VPRC, volume of packed red cells. For other abbreviations, see footnote to table 1.

young erythrocytes[4] in the diabetic pregnancy. These possibilities will require further investigation in both man and monkey.

The presence of two major hemoglobins in *Macaca nemestrina* and *Macaca speciosa* is due to duplication of the alpha chain loci.[28-30] However, the significance of the two major peaks we observed in seven of 29 macaques is unclear. Recent studies[31] using isoelectric focusing in polyacrylamide gel found only a single major hemoglobin in *Macaca mulatta* and no evidence of polymorphism. This is in contrast with an earlier report[32] of double peaks in 16 of 39 samples analyzed by the same isoelectric-focusing technique. It has been suggested that the colonies differ because of their geographic location;[31] the instability of the major hemoglobin may be another explanation. We have found the major hemoglobin from the *Macaca mulatta* to be less stable than human hemoglobin A when measured by an isopropanol test.[32] This instability may variably affect the characterization of the hemoglobin and the per cent recovery when analyzed by different physical chemical methods. The nature of this instability is not understood. Since Hb A_{I_c} is glycosylated at the amino terminal of the beta chain, one would have expected two Hb A_{I_c} peaks if the alpha chain were duplicated; yet, we observed only one Hb A_{I_c} peak.

Given the close homology of the amino acid sequences of both the alpha and beta chains of human and macaque hemoglobin (four and eight amino acid differences, respectively),[33] the finding of an Hb A_{I_c} fraction with an identifiable carbohydrate moiety in monkeys is not surprising. The Hb A_{I_c} isolated from the monkey had electrophoretic properties and glucose content similar to those of human Hb A_{I_c}.

Since none of the amino acid substitutions in the rhesus hemoglobin molecules are at sites known to alter oxygen binding,[10] rhesus Hb A_{I_c}, like that in man, might be expected to have increased oxygen affinity. Ditzel[13] measured whole blood oxygen affinity (P50), 2,3-DPG, and hemoglobin concentration in a large number of nondiabetic and diabetic patients. He found an increased hemoglobin concentration and whole blood 2,3-DPG level in diabetics in the presence of a normal P50. He interpreted these data to indicate that diabetics have an increased tissue oxygen requirement. Although he had not measured Hb A_{I_c}, Ditzel further suggested that the higher 2,3-DPG concentrations in the face of normal P50s in diabetics were due to an abnormal hemoglobin with increased oxygen affinity (i.e. Hb A_{I_c}). In the macaque model the 2,3-DPG levels were slightly, but not significantly, higher in the glucose-intolerant animals. However, this may be due to either less severe glucose intolerance in the macaque or the small sample size.

Our finding of an Hb A_{I_c} peak in the monkey offers a model for studying this minor hemoglobin subgroup in a nonhuman primate. Possible areas for further investigation with this model are the biosynthesis of Hb A_{I_c} (as well as other minor hemoglobin variants of Hb A), the relationship of Hb A_{I_c} to control of glucose and to long-term diabetic sequelae, and the potential pathophysiologic consequences in vivo of adversely altered oxygen transport in the already vulnerable diabetic individual.[13] This model might also provide a means by which to explore the role of adverse maternal-fetal oxygen transport in both the nonketoacidotic and ketoacidotic states.

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