Document Delivery Pull Slip

Request Date: 12/7/2009 01:35:07 PM

Journal Title: Veterinary Clinical Pathology

Volume: 13
Issue: 2
Month/Year: 1984

Article Author: Fulmer,Loeb,Martin, Gard (et al?)
Article Title: Effects of Three Methods of Restraint on Intravenous Glucose Tolerance Testing in Rhesus and African Green Monkeys
Pages: 19-25

Location: **MSL Bound Journal Stacks**
Call Number:

* UPDATE LOANSOME DOC:

Customer Information: **yourpetsfriend@gmail.com**
Charges

Adobe Acrobat Printing Problems: If you print out a mirrored image, have missing text, or blank pages: Go to **Printing Properties** and make sure **PRINT AS IMAGE** is checked.

Scanned with Adobe Acrobat & saved to K by: KGP
Unable to pull because (initial & date)

Volume Not On Shelf_____

Title Not On Shelf _____

Not Found As Cited_____ BRING ITEM DOWN & PLACE ON PROBLEM CART

Other_____

Effects of Three Methods of Restraint on Intravenous Glucose Tolerance Testing in Rhesus and African Green Monkeys

Roger Fulmer; Walter F. Loeb, V.M.D., Ph.D.
David P. Martin, V.M.D.; Elizabeth A. Gard, D.V.M.
The Departments of Laboratory Animal Medicine and Science and the Department of Pathology
Litton Bionetics, Inc.
5516 Nicholson Lane
Kensington, Maryland 20895

Address reprint requests to Dr. Loeb.

Dr. Martin's current address is
E.I. Dupont DeNemours & Company
Biomedical Products Department
Experimental Station
Wilmington, Delaware 19898

Summary

The intravenous administration of 0.75 gm glucose per kg and the measurement of serum glucose pretest and at 10, 20, 30, 60, 90 and 120 minutes constitute a satisfactory protocol for intravenous glucose tolerance testing of Rhesus (Macaca mulatta) *and African Green* (Cercopithecus aethiops) *monkeys. No significant differences were noted between animals restrained with ketamine hydrochloride and those restrained with sodium pentobarbital, but the African Green males and females and the male Rhesus monkeys yielded significantly different results while being manually restrained.*

KEY WORDS: glucose tolerance, restraint, Rhesus monkey, African Green monkey, nonhuman primate.

Introduction

The glucose tolerance test assesses several components of the body's mechanism for regulating blood glucose. In medical practice and in lab-

This study was supported in part by NHLBI Contract NO1-HV5-3031.
The work was conducted in an AALAC accredited facility and according to the principles of animal care promulgated by the NAS-NRC (see "Guide for Laboratory Animal Facilities and Care").

oratory animals it is a valuable aid in the diagnosis of mild diabetes and prediabetic states. In the test, a standard amount of glucose is given either orally or intravenously. Blood glucose values are then monitored at intervals to follow the effect of the body's insulin and other glucose-regulating mechanisms upon blood glucose levels. This study was conducted to develop a technique for intravenous glucose tolerance test appropriate to Rhesus *(Macaca mulatta)* and African Green *(Cercopithecus aethiops)* monkeys. A major objective was to determine if the method of restraint played any role in the test results and if there were any differences between the two species or sexes.

Materials and Methods

Ten Rhesus monkeys and 10 African Green monkeys were utilized in this study. The animals were all wild-caught, mature adults, weighing between 3.5-9.5 kg, equally divided as to sex. They had been quarantined and conditioned to the labotatory for a minimum of 6 months before the study began. They were separated by species and housed in suspended, duplex, stainless steel wire cages. Water was provided *ad libitum* through automatic water valves and the diet was commercial nonhuman primate biscuits fed once daily. The ventilation in the animal quarters was approximately 15 air changes per hour. The temperature was maintained at 22-24 $\pm$ 1°C and the relative humidity was approximately 50%. Lighting was automatically controlled for 12 hours of light and 12 hours of darkness.

In an initial range-finding experiment, female Rhesus monkeys which had been fasted for 24 hours were restrained with ketamine hydrochloride and, after collection of a baseline blood sample, were given 0.5 gm of glucose per kg IV. Blood samples were collected at 30, 60 and 120 minutes post-injection and glucose values determined on all samples. Post-injection glucose values were generally below baseline, never significantly elevated. From this we concluded that the challenge dose of glucose was inadequate and that the blood samples following glucose administration were being collected too late. Based on the above experiment, the challenge dose was set at 0.75 gm/kg and the sampling times at prechallenge and 10, 20, 30, 60, 80 and 120 minutes.

Our experimental design was to subject the animals to a glucose tolerance test under three different methods of restraint. These were manual, ketamine hydrochloride (Ketaset®:Bristol Laboratories) administered intramuscularly (IM), and sodium pentobarbital (Nembutal®:Abbott) administered intravenously (IV). After collection of the prechallenge blood sample, the challenge dose of glucose (Dextrose 20%:Cutter Laboratories) was injected into the saphenous vein over a 2-minute period. Blood samples for glucose measurement were collected at the determined sampling times. A 2-week rest period was allowed after each test, so that no residual effects of the restraining drug would affect the next test. Due to the length of the testing procedure, it was necessary to perform the test in both the morning and afternoon. To minimize the effects of feeding, the animals which were tested in the morning were fasted from the previous morning with a total fasting time of 25 to 28 hours. Animals to be tested in the afternoon were fed at 4:00 p.m. on the previous day so that the fasting period was 21 to 24 hours in length. Each animal was shifted during the test period so that it was tested both in the morning and afternoon.

When manual restraint was used, the animals were captured using heavy leather gloves with gauntlets. After holding the animal for glucose injection or blood collection, it was returned to the cage until the next bleeding time when it was recaptured. The animals given ketamine hydrochloride were grasped by one leg within the cage, and 15 mg/kg was administered IM. During the course of the test, the animals were supplemented with ketamine hydrochloride (IM) to maintain an adequate degree of restraint. When sodium pentobarbital was used, the animals were restrained manually while the drug was administered IV via the saphenous vein to a light anesthetic level of effect which was approximately 30 mg/kg. No supplementation of this drug was necessary when this method of chemical restraint was utilized.

All of the blood samples were obtained by venipuncture of the femoral vein and collected in 2 ml anti-coagulant vacuum tubes containing 4 mg of potassium oxalate and 5 mg of sodium fluoride (Vacutainer®:Becton-Dickinson). Glucose measurements were made by a hexokinase method.[1]

Results

Determined glucose values are tabulated in Table 1.

An analysis of variance of the baseline fasting glucose values showed that no significant differ-

TABLE 1
Glucose Tolerance in Rhesus and African Green Monkeys

Table 1A *Macaca mulatta* Males

Animal	Restraint Method*	0′	10′	20′	30′	60′	90′	120′	K Value
419-J	M	72	397	306	272	126	94	88	2.27
422-J	M	89	332	260	226	74	57	61	3.04
431-J	M	105	443	369	356	178	68	50	1.82
598-J	M	121	323	250	176	71	64	62	3.06
599-J	M	104	503	393	395	237	110	73	1.44
419-J	N	123	332	214	120	39	61	71	4.28
422-J	N	114	335	247	141	30	35	69	5.03
431-J	N	125	377	277	194	40	60	54	4.59
598-J	N	73	334	204	150	50	55	63	3.72
599-J	N	46	236	132	64	28	43	58	4.15
419-J	K	74	338	272	164	66	52	63	3.34
422-J	K	74	367	271	192	47	48	49	4.19
431-J	K	142	396	251	160	20	66	86	6.07
598-J	K	70	321	182	91	45	62	57	3.80
599-J	K	80	343	199	98	44	55	54	4.01

Table 1B *Macaca mulatta* Females

Animal	Restraint Method*	0′	10′	20′	30′	60′	90′	120′	K Value
935-J	M	74	301	228	183	62	71	74	3.19
936-J	M	70	349	261	177	25	63	43	5.44
937-J	M	70	239	79	26	52	50	51	2.48
938-J	M	62	377	275	216	102	64	58	2.58
939-J	M	61	314	267	227	81	39	46	2.79
935-J	N	84	258	180	115	35	40	65	4.02
936-J	N	82	267	220	165	58	42	37	3.13
937-J	N	57	304	182	120	35	38	49	4.26
938-J	N	58	319	208	114	40	54	57	4.14
939-J	N	82	275	212	128	34	22	47	4.29
935-J	K	73	257	136	69	47	64	74	3.18
936-J	K	51	296	136	82	31	33	48	4.39
937-J	K	111	262	123	52	42	66	76	3.38
938-J	K	75	331	230	170	72	48	61	3.01
939-J	K	63	252	138	58	32	45	97	3.99

Table 1C *Cercopithecus aethiops* Males

Animal	Restraint Method*	0′	10′	20′	30′	60′	90′	120′	K Value
566-F	M	93	296	294	306	269	132	95	0.20
568-F	M	85	340	324	302	141	62	62	1.85
772-J	M	74	325	326	346	282	188	106	0.31
872-J	M	117	438	475	440	379	269	188	0.37
873-J	M	98	443	370	359	263	201	69	0.99
566-F	N	63	330	258	207	82	44	38	2.81
568-F	N	112	384	198	126	13	31	36	6.78
772-J	N	163	466	389	330	144	52	30	2.39
872-J	N	108	325	253	202	97	42	30	2.41
873-J	N	94	274	204	165	76	43	28	2.54
566-F	K	96	297	189	108	88	89	102	2.28
568-F	K	68	290	161	75	36	70	63	4.05
772-J	K	124	432	382	276	85	51	81	3.40
872-J	K	108	313	277	213	76	29	51	2.94
873-J	K	127	359	230	141	36	49	71	4.61

Table 1D *Cercopithecus aethiops* Females

Animal	Restraint Method*	0′	10′	20′	30′	60′	90′	120′	K Value
555-F	M	81	430	370	348	224	118	79	1.29
764-J	M	123	417	430	417	115	234	154	2.78
765-J	M	65	439	392	361	212	88	42	1.48
768-J	M	72	374	334	282	89	39	34	3.00
769-J	M	53	393	389	395	342	220	128	0.29
555-F	N	351	292	199	108	45	44	52	3.73
764-J	N	108	255	231	283	141	100	61	1.20
765-J	N	82	243	95	17	21	37	33	4.58
768-J	N	121	336	229	129	36	36	38	4.63
769-J	N	108	271	138	84	34	37	62	3.97
555-F	K	100	325	189	106	38	45	78	4.21
764-J	K	138	350	270	188	145	127	155	1.70
765-J	K	120	422	260	175	35	41	50	4.99
768-J	K	99	342	199	100	35	42	44	4.50
769-J	K	107	323	162	103	26	40	55	4.91

*M = Manual; N = Sodium Pentobarbital; K = Ketamine Hydrochloride
Tabulated values at time intervals are for glucose expressed in mg/dl.
Time "0" is prior to injection of glucose challenge dose.

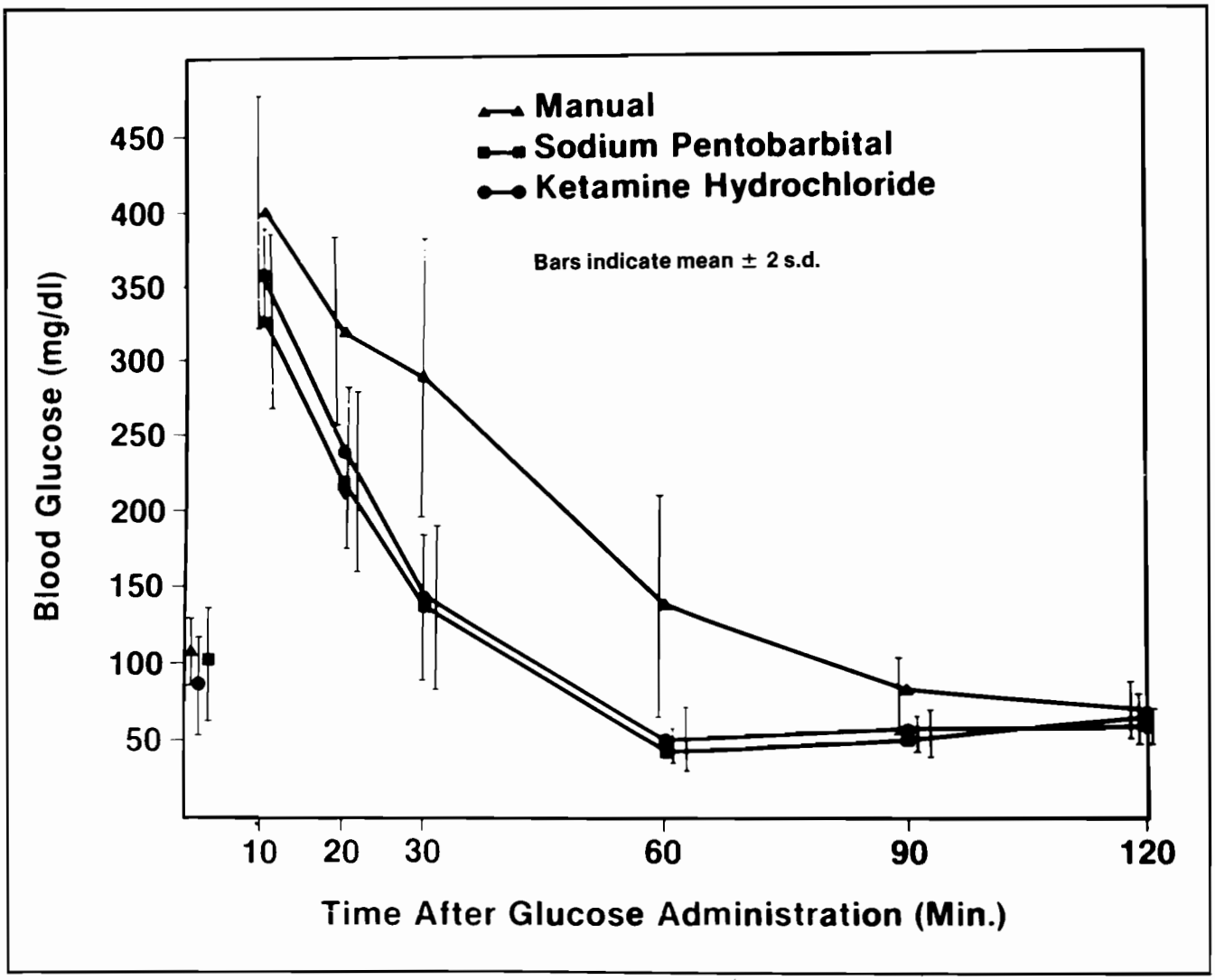

Fig. 1 — Comparison of three methods of restraint on intravenous glucose tolerance in male Rhesus monkeys.

ence was present within each group, regardless of whether the animals were tested under manual restraint, under ketamine hydrochloride, or under pentobarbital restraint.

The decrease in blood glucose post-intravenous challenge is logarithmic, an observation which we independently verified from these data, and is described by the equation:

$$C_t = C_o e^{-kt}$$

in which C_o is the glucose Time 0, C_t is the concentration at Time t, and k is the constant for the percent rate of fall of blood glucose per minute.[2] From the above formula, a working formula:

$$k = \frac{0.6931}{t\frac{1}{2}}$$

may be derived to determine k from the half time. To express k values in the conventional form, as a percent, this calculation is multiplied by 100. The k value for each tolerance test calculated from glucose values at 10 to 60 minutes is given in Table 1.

Analysis of variances performed on the k values and sub-tested by least significant difference showed that no significant difference existed among the three methods of restraint for female Rhesus monkeys. For African Green monkeys and for male Rhesus monkeys, no difference existed between animals restrained by ketamine or by pentobarbital, but animals manually restrained yielded significantly different (lower) k values at the 0.05 level of significance.

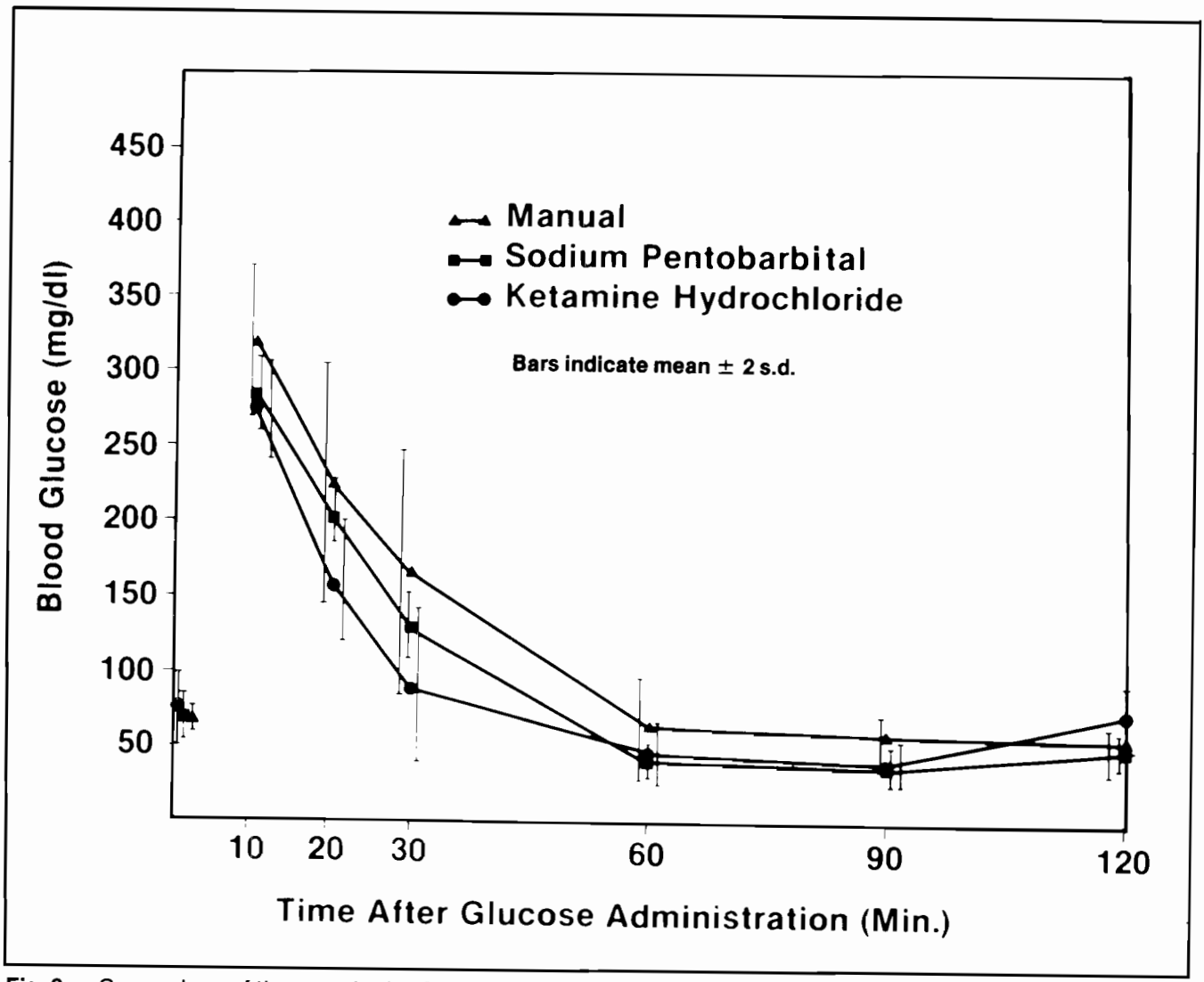

Fig. 2 — Comparison of three methods of restraint on intravenous glucose tolerance in female Rhesus monkeys.

The comparison of the three methods of restraint on intravenous glucose tolerance in the four groups of monkeys is presented in graphic form in Figures 1-4.

Discussion

Carbohydrate tolerance testing has been utilized as a diagnostic tool in the nonhuman primate by many investigators. Restraint methods have been both manual and chemical and the carbohydrate has been administered both orally and intravenously.[3-8] We found only one report which compared methods of restraint.[7] The oral route of glucose administration was utilized in that study and the results suggested that ketamine might have an adverse effect on the absorption of glucose from the gastrointestinal tract. In another study, however, ketamine did not have an effect when the glucose values of ketamine-sedated animals were compared to the values of animals restrained by a squeeze cage.[8]

We conducted the study described here to fulfill a requirement to annually test the glucose tolerance level on several species of nonhuman primates. We chose the IV route of administration because of its ease of administration, safety and uniform delivery of the glucose to the blood stream.

In man, when the intravenous glucose tolerance test is applied to the diagnosis of diabetes mellitus, k values over 1.2 are regarded as normal,

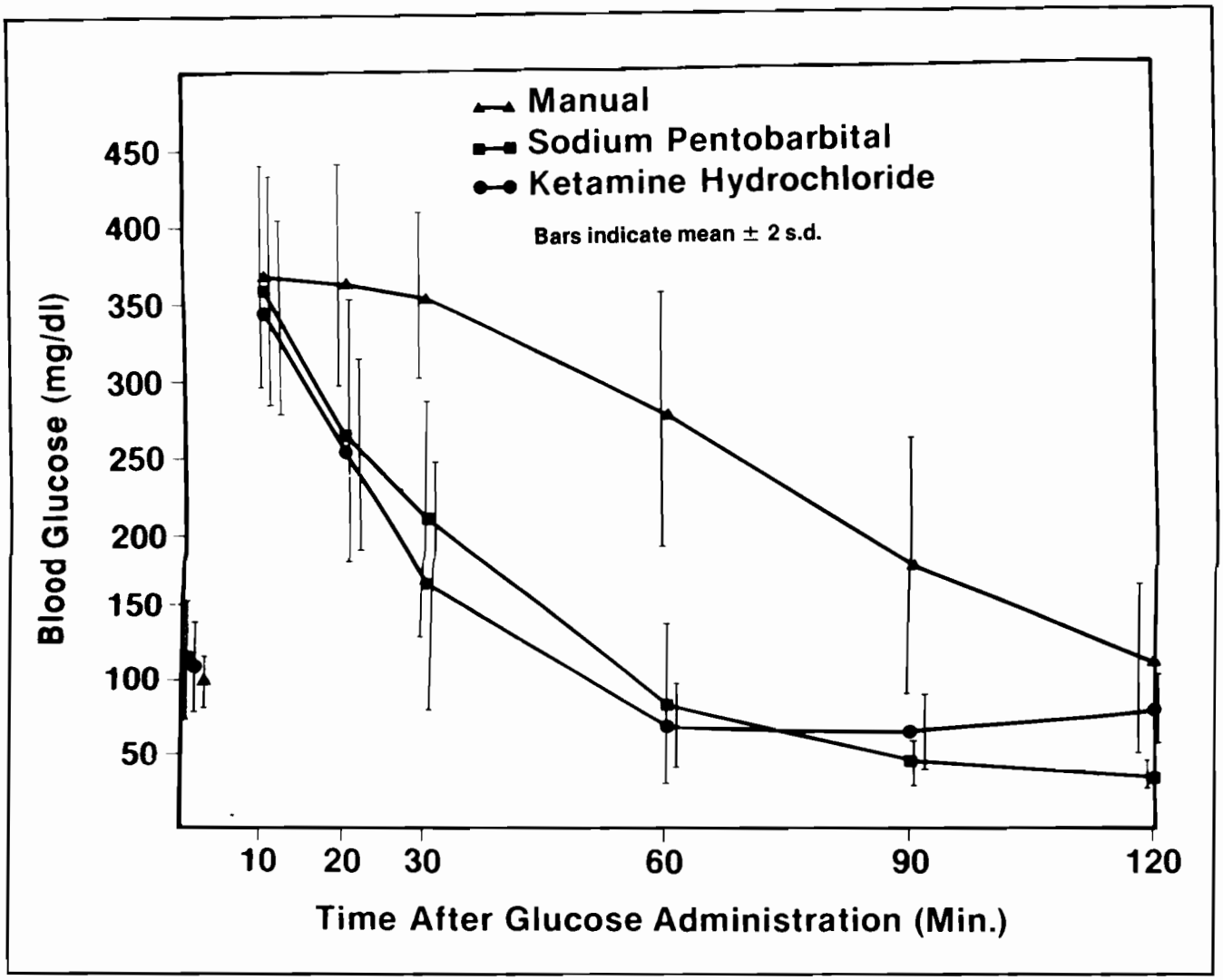

Fig. 3 — Comparison of three methods of restraint on intravenous glucose tolerance in male African Green monkeys.

values of 1 to 1.2 as borderline, and values below 1 as diagnostic of diabetes mellitus.[2] As none of the animals in this study was diabetic, this study does not enable us to draw corresponding conclusions for this test protocol nor for the species studied. However, all k values for chemically restrained animals ranged from 1.20 to 6.78. No k values between 1.2 and 1 were observed in this study. Five values fell below 1, from 0.20 to 0.99. These were all in manually restrained African Green monkeys, four males and one female. These values, in part, produce the significant difference in k values between manually and chemically restrained African Green monkeys. We have generally observed that African Green monkeys are more resistant to restraint than Rhesus, and that in both species females tend to be more tractable and docile than males. We speculate that biochemical effects related to restraint and struggling, in particular epinephrine release, invalidate the serum glucose decreases, an effect which is most marked in male African Green monkeys and so slight in female Rhesus as not to be statistically significant.

The results of the study described here indicate that either ketamine hydrochloride or sodium pentobarbital is a satisfactory restraining agent for both sexes of the species tested. The use of either one allows for direct comparison of the results among the animals.

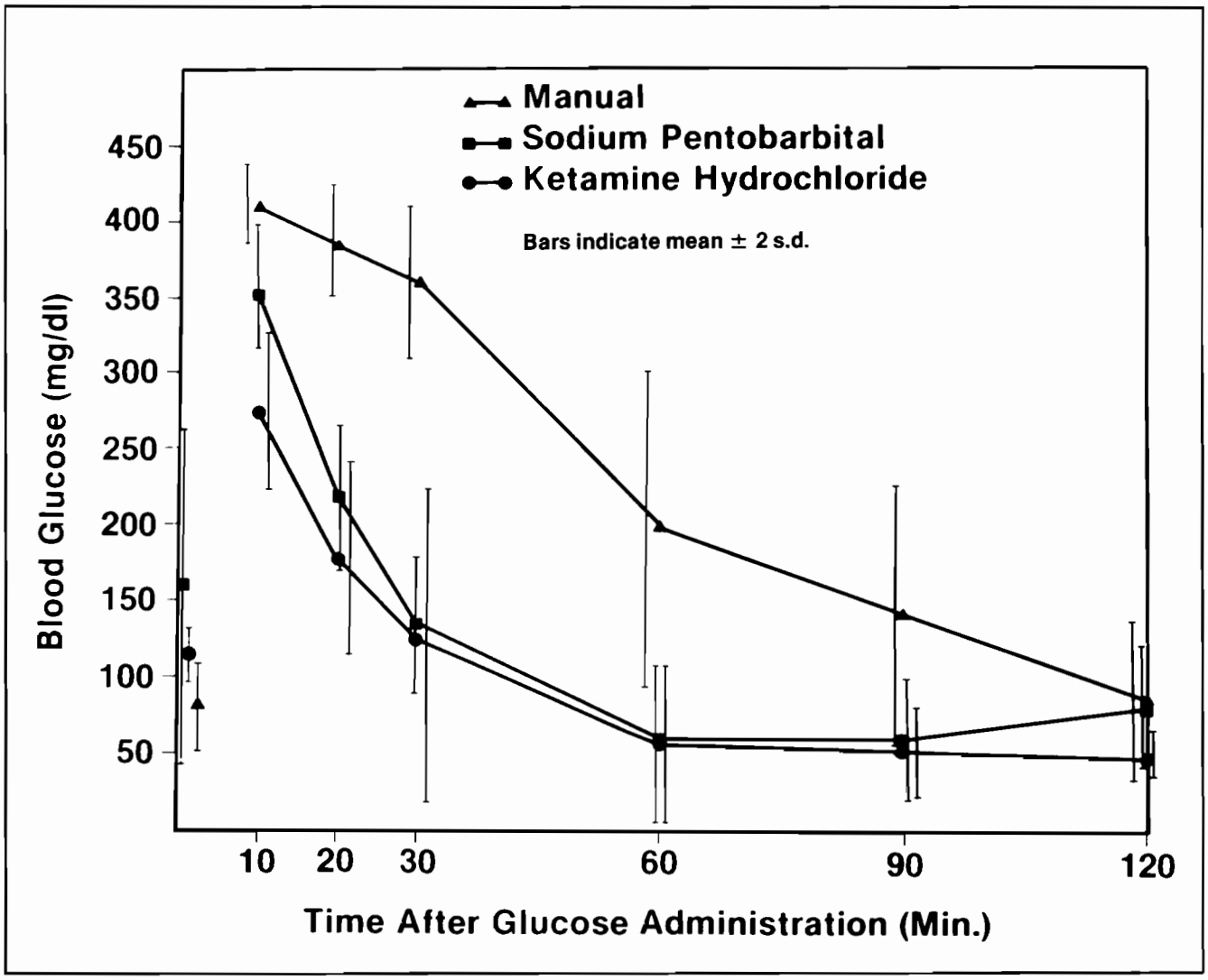

Fig. 4 — Comparison of three methods of restraint on intravenous glucose tolerance in female African Green monkeys.

REFERENCES

1. Leon LP, Chu DK, Stasin RO, Snyder LR: Advances in Automated Analysis. Technicon International Congress 1:152, 1976.
2. Park D Jr, Halter JB: The Endocrine Pancreas and Diabetes Mellitus. In: Textbook of Endocrinology, Chapter 15, ed by RH Williams. Philadelphia, WB Saunders Co, 1981.
3. Honjo S, Kondo Y, Cho F: Oral Glucose Tolerance Test in *Macaca fascicularis*. Lab Anim Sci 26:771-776, 1976.
4. Kirk JH, Casey HW, Harwell JF Jr: Diabetes Mellitus in Two Rhesus Monkeys. Lab Anim Sci 22:245-248, 1972.
5. Howard CF Jr, Palotay JL: Spontaneous Diabetes Mellitus in *Macaca cyclopis* and *Mandrillus leucophaeus*: Case Reports. Lab Anim Sci 25:191-196, 1975.
6. Streett JW, Jonas AM: Lactose Intolerance in the Stump-tail Macaque *(Macaca arctoides)*: A Case Report. Lab Anim Sci 30:80-84, 1980.
7. Streett JW, Jonas AM: Differential Effects of Chemical and Physical Restraint on Carbohydrate Tolerance Testing in Nonhuman Primates. Lab Anim Sci 32:362-366, 1982.
8. Kemnitz JW, Kraemer GW: Assessment of Glucoregulation in Rhesus Monkeys Sedated with Ketamine. Amer J Prim 3:201-210, 1982.

ACKNOWLEDGEMENT

The authors express their sincere appreciation to Robin Pomerantz for mathematical and statistical consultations and analyses.