

Social Interaction and Serum Insulin Values in the Monkey (*Macaca mulatta*)

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Social interaction studies were conducted with two groups of rhesus monkeys with abnormally elevated serum insulin values. In both studies, the animals that were lowest on the dominance-submissiveness scale showed a fall in insulin levels to within the normal range for the monkey. Upon removal from the group, insulin values in these animals returned to their previous elevated level. In neither instance was the fall in insulin level accompanied by fasting hyperglycemia.

INTRODUCTION

Manipulation of parameters related to carbohydrate metabolism by stress go back at least to work by Bohm and Hoffman in 1876 quoted by Cannon et al. (1). Those investigators demonstrated a transient glycosuria associated with experimentally produced pain in the cat. Cannon and his group studied these effects and found that mere physical restraint was capable of producing the glycosuria (1). Since that time many studies have attempted, with little success, to demonstrate a causal relationship between emotional stress and prolonged glucose intolerance leading to diabetes mellitus.

The selection of appropriate subjects may be critical to studies on the psychosomatic components of diabetes.

As Danowski (2) has stated: "It still appears therefore that at present [1963] the contention that emotional stress can by itself produce permanent diabetes mellitus in a totally nondiabetic individual cannot be confirmed by clinical data, whereas considerable indirect and direct evidence can be assembled in support of the view that stress intensifies known preexistent diabetes mellitus, brings to clinical recognition previously unrecognized actual diabetes, and may convert prediabetes to actual diabetes." Thus, the appropriate subjects for such research should consist of animals (1) predisposed to the disease and (2) those in the pre-diabetic phase of the disease.

In our laboratory, a number of rhesus monkeys that attain middle-age (10-14 years) develop fasting hyperglycemia and glycosuria. These symptoms are spontaneous and show the rhesus to be an animal predisposed to the disease at least under laboratory conditions. In all animals we have observed with abnormal glucose tolerance, they first exhibited elevated fasting insulin values before the onset of hyperglycemia and glycosuria (3). We have defined this as the insulin resistant, prediabetic phase and thus

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SOCIAL INTERACTION IN THE MONKEY

have subjects believed to satisfy, in part, Danowski's criteria.

The nature of insulin resistance has not been resolved. In our animals we have demonstrated an inverse relationship between serum insulin (IRI) levels and liver cell receptor sites for insulin and no relationship between IRI and glucagon values (Lockwood, Livingston, and Hamilton, unpublished). We have not investigated the interaction between circulatory IRI and adipocytes.

The questions asked in the present work were (1) does psychological stress alter blood insulin levels in monkeys with hyperinsulinemia, and (2) can persistent glycosuria and hyperglycemia be precipitated in normoglycemic but hyperinsulinemic monkeys by such stress?

In an attempt to use a more "natural" stress situation than that provided by the usual techniques of escape from, or avoidance of, electric shock, we investigated the effects of social interaction on certain parameters related to carbohydrate metabolism. The dominance hierarchy that develops within a group of rhesus monkeys is very obvious, rigid, and not without strain on "interpersonal" relations.

Study No. 1

Method

The subjects were four mature, normoglycemic male monkeys (*Macaca mulatta*) weighing between 6 and 15 kg. All were hyperinsulinemic with fasting insulin values greater than 200 $\mu\text{U/ml}$ serum. Normal fasting values of serum insulin for monkeys in our laboratory have been $92 \pm 13 \mu\text{U/ml}$ (3). The animals were maintained ad lib. on Purina Monkey Chow (15% protein) and water. Before mixing for the social interaction portion of the work, the ani-

mals were housed individually in cages measuring 88 cm deep \times 72 cm wide. During the group studies, the animals living space was increased so that the "per capita" floor area remained constant. At first, two animals were placed together, a week later a third was introduced, and the following week the fourth animal was added to the group.

At weekly intervals, single blood samples were drawn by venipuncture from unanesthetized animals between 9 and 11 a.m. after a 15-17-hour fast. Plasma glucose was measured using the glucose oxidase method. Serum immunoreactive insulin (IRI) was determined in triplicate by a modified, double-antibody method of Hales and Randle (4) with 125I and insulin binding reagent obtained pure from Amersham Searle Co. The IRI results are expressed in micro unit equivalents of human crystalline insulin/ml serum. Plasma cholesterol and triglycerides were determined by established methods (5, 6).

Dominance ratings were based on directed aggression as defined by attack, mounting, and competition for food. Judgments of dominance-submissiveness were made by two independent observers during observation periods that varied between 1 and 2 hours/day, 6 days/week.

Table 1 shows the sequence of mixing of these animals. Animal D died on the third day following the introduction of A. It exhibited no mortal wounds and autopsy did not reveal the cause of death. This case will be discussed later since we observed a similar instance in our second study.

Results

Animal C was submissive throughout this work. Figure 1 shows that within 1 week after being placed with D and B it

TABLE 1. Sequence of "Mixing" for Monkeys in Social Interaction Study

	1st week	2nd week	3rd week	10th week	13th week
Animal	D B	D B C	D B C A	B C	C

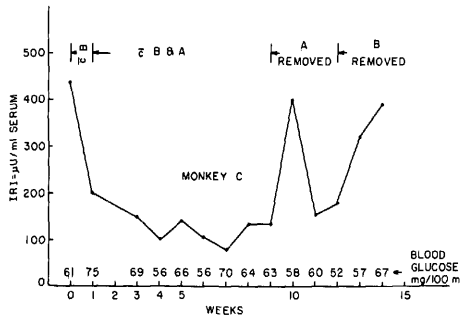


Fig. 1. Serum insulin (IRI) determinations for most submissive monkey (C) in study No. 1. Value at 0 time obtained before social interaction. Animal D not included in this figure.

showed a fall of IRI to roughly 50% of its base-line values. Among these three animals, B was dominant but there was relatively little aggressive behavior. With the introduction of A, an extremely aggressive animal, the IRI levels of C fell further and remained low until the ninth week when A was removed from the group. The following week, C competed with B for dominance with an accompanying rise in IRI values. However, B reasserted dominance over C with a resulting fall in the latter's IRI levels. After 2 weeks the two animals were separated and the IRI values of C returned to pregrouping hyperinsulinemic levels. During the social grouping, the range of IRI values ($\mu\text{U}/\text{ml}$) for animal A was 318–557 and for B 217–39.

Throughout the period of group living, none of the animals displayed evidence of glycosuria, hyperglycemia, or changes in blood cholesterol or triglycerides. Body weight variations were within the normal range for animals living alone.

Study No. 2

Method

This work was conducted using four male monkeys (*Macaca mulatta*) weighing between 11.5 and 14.5 kg. At the beginning of the study all of the subjects were considered to be hyperinsulinemic (fasting IRI range 440–520 $\mu\text{U}/\text{ml}$ serum). None of the animals had exhibited hyperglycemia or glycosuria. The procedure for this study was the same as for Study No. 1 except that all the monkeys were placed together simultaneously for the social portion.

Results

As in our first study, one of the animals (No. 82) died without apparent cause during the fifth week of the social interaction. Data are reported for the remaining animals.

Over the first 2 weeks of social grouping, animals Nos. 76 and 84 competed for the most dominant position. The situation was resolved during the third week

SOCIAL INTERACTION IN THE MONKEY

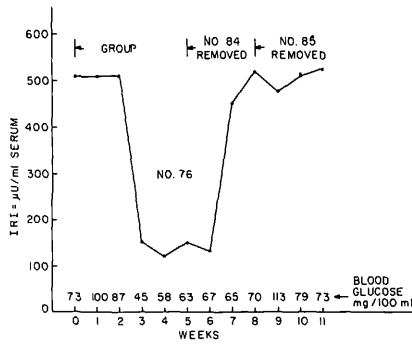


Fig. 2. Serum insulin (IRI) values for most submissive monkey (76) in study No. 2. Value at 0 time obtained before social interaction.

when No. 84 became the most dominant and No. 76 the most submissive of the group. At that time, the IRI levels of the latter animal fell to approximately 30% of its previous value (Fig. 2). No. 85 was passive throughout, initiated no aggressive behavior, and dominated No. 76 "by default." Upon removal of the most dominant monkey a week passed before No. 76 asserted dominance over No. 85, at which time its IRI returned to hyperinsulinemic, pregrouping values. Animals Nos. 84 and 85 showed no unusual change in serum IRI (Table 2), blood glucose, or lipids. There was a tendency for blood glucose levels of No. 76 to fall during his submissive experience and during group living it showed a 2.8-kg loss in body weight. However, the lowest weight was recorded on the seventh week of the study at a time when IRI values had returned to 450 μU/ml. In view of that, together with the results of the first study, we believe that weight loss did not account for the fall in IRI.

TABLE 2. Serum Insulin and Plasma Blood Glucose Values of Nonsubmissive Animals Before, During, and After Social Interaction. Study No. 2

Week	IRI (μU/ml)		BG (mg/100 ml)	
	#84	#85	#84	#85
Presocial	522	448	62	58
1	313	525	93	58
2	550	420	87	62
3	316	472	66	61
4	540	440	95	67
5 ^a	484	892	80	79
6	355	340	76	71
7	479	768	67	70
8 ^b	518	446	98	90
9	522	498	68	65

^a First data, #84 isolated from nos. 76 and 85.

^b First data, #85 isolated from no. 76.

Both animals that died from unknown cause during the social interaction studies exhibited similar behavior; neither battled for dominance nor did they flee from attack. In general they

crouched in the cage corner and although snipped at on occasion, were not maltreated grossly. They appeared helpless in the situation without displaying "fight or flight" syndrome. By coincidence, the last blood sample drawn on both animals was obtained 3 days before death. Assays revealed that serum IRI rose for D from 410 to 502 $\mu\text{U}/\text{ml}$ and for No. 82 from 505 to 982 $\mu\text{U}/\text{ml}$. Changes in blood glucose and lipids were undistinguished. Weight loss was 0.5 kg for D and 4.0 kg for No. 82. Thus, for the latter animal there was a large drop in body weight in the presence of a rise in IRI values (cf. No. 76). Autopsy revealed no gross abnormalities in these animals.

Although we do not know if the death of these animals was "sudden" in nature, since they were discovered the following morning, they may be related to the long literature of investigations of sudden death. Cannon (7), writing about sudden death accompanying psychological trauma, maintained that it resulted from overstimulation of the sympathetic nervous system. Later, Richter (8) studied the phenomenon in wild rats placed in a water tank with no means of escape. Under such circumstances he considered their condition "helpless." Richter observed that just before death the animals exhibited a slowing of heart rate and respiration and postulated that the sudden death was the result of overstimulation of the vagal system. This was substantiated by the demonstration that atropine reduced the mortality rate. Thus, there were two theories attempting to explain the phenomenon. Although our work was not designed to explore these possibilities, our data lend support to the Richter hypothesis in that both the "helpless" animals showed a rise in IRI before death, and there is evidence that vagal

stimulation is followed by increased insulin release (9).

Discussion

Although the effects of social interaction on IRI levels were dramatic for the submissive animals in our studies, in no instance did we precipitate persistent glycosuria or hyperglycemia. The large fall in IRI values for these animals lies far outside the range of determinations for hyperinsulinemic monkeys in our colony (mean $594 \pm 70 \mu\text{U}/\text{ml}$ serum) (3). These results with hyperinsulinemic monkeys are similar to the data of Mason et al. (10) with normoinsulinemic monkeys exposed to shock avoidance procedures. In that work, monkeys were trained to avoid electric shock to the feet. During the test sessions, there was a significant fall in IRI levels that could not be explained by changes in blood values of glucose or epinephrine.

On the assumption that hyperinsulinemia in the presence of normoglycemia in our monkeys is the result of "insulin resistance" (11), we are pressed to explain the results of social stress on the insulin system without accompanying elevations of blood glucose. Findings from other laboratories present at least one explanation. Mason et al. (12) reported that along with a fall in insulin levels in their stressed monkeys, there was also a reduction in blood levels of testosterone. In addition, Rose et al. (13) demonstrated a significant fall in testosterone levels in submissive monkeys during social interaction studies. These findings suggest that the fall in insulin values in our submissive animals was secondary to reductions in circulating testosterone. This hypothesis requires

SOCIAL INTERACTION IN THE MONKEY

evidence that testosterone is involved in some manner with glucose utilization. A suggestion is provided by the work of Craighead (14) who showed that in mice injected with the M variant of EMC virus, the incidence of diabetes was greater for males. In follow-up studies using EMC virus and castrated mice of both sexes, the incidence of diabetes was enhanced by the administration of testosterone (14). It remains to be seen whether this is merely an instance where testosterone has its primary effect on the size of the population of the virus and not directly on carbohydrate metabolism.

The problem of the effects of psychological stress on carbohydrate metabolism appears to be a fruitful area for investigation. Certainly, in the future, one must make determinations of a multiplicity of hormones and attempt to decipher relevance by experimental intervention. For example, one might ask if testosterone replacement in submissive monkeys is followed by a rise in insulin values. Such experiments would serve as a starting point for the consideration of

the psychosomatic components of certain metabolic diseases.

Summary

The effects of social interaction on serum insulin levels were studied in two groups of hyperinsulinemic but normoglycemic monkeys. In both studies, the most submissive animals showed a fall of serum insulin to within the normal range for the laboratory monkey, without impairment of blood glucose levels. The findings are discussed in terms of hormonal interaction and in particular to the possible effects of the interaction of insulin and testosterone on carbohydrate metabolism.

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C. L. HAMILTON AND T. CHADDOCK

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