CONDITIONED HYPOGLYCEMIA

CONDITIONED HYPOGLYCEMIA:

A MECHANISM FOR SACCHARIN-INDUCED SENSITIVITY TO INSULIN

By

Robert Deutsch, B.Sc., M. A.

A Thesis

Submitted to the School of Graduate Studies

in Partial Fulfilment of the Requirements

for the Degree

Doctor of Philosophy

McMaster University

November, 1971

DOCTOR OF PHILOSOPHY (1971) McMASTER UNIVERSITY

(Psychology) Hamilton, Ontario

TITLE: Conditioned Hypoglycemia: A Mechanism for Saccharin-Induced Sensitivity to Insulin.

AUTHOR: Robert Deutsch. B.Sc. (McGill University)

M. A. (McMaster University

SUPERVISOR: S. Siegel, Ph.D.

NUMBER OF PAGES: vii, 59

ABSTRACT

Evidence was presented in Experiment I which replicated previous findings, indicating that ingestion of the nonnutritive sweetener, saccharin, potentiates insulin-induced mortality in rats. Five additional experiments were conducted to determine the mechanism for this effect. The specific hypothesis examined was the view that saccharin ingestion augments the effect of insulin because the taste of saccharin elicits a conditioned hypoglycemic response. Since glycemic metabolic events have in the past been contingent on the preceding gustatory sensations associated with the ingestion of sweet nutritive substances, sweet taste alone may elicit these glycometabolic events as a conditioned preparatory response, thus leading to a reduction in blood glucose level. The present research has shown that saccharin ingestion alone results in hypoglycemia, and that this response is extinguished by longterm access to saccharin, but not by longterm access to glucose (a nutritive sweet substance). Furthermore, it was demonstrated that the hypoglycemic response can be elicited by stimuli, either gustatory or olfactory, which have been specifically paired with direct intragastric administration of glucose. These results were interpreted as supporting the conditioning account of the potentiating effect of saccharin on insulin-induced mortality. Such conditioning has implications for predicting the organism's response to drugs with glycometabolic actions, and, more generally, points out the importance of conditioned preparatory responses in the digestion and utilization of food.

ACKNOWLEDGEMENTS

I would like to thank Dr. S. Siegel for his advice, assistance, and encouragement during all phases of the research and preparation of the thesis.

I would also like to record my indebtedness to my wife, Beverley, for her patience and good sense of humour during my years as a graduate student, and for critical reading of the manuscript.

Thanks are also due to members of my thesis committee, Dr. B. G. Galef and Dr. J. M. Cleghorn, who made many helpful suggestions and criticisms.

TABLE OF CONTENTS

FIGURES

APPENDICES

INTRODUCTION

Valenstein & Weber (1965) have reported that ingestion of saccharin potentiates insulin-induced mortality. They injected rats with a dose of insulin usually sufficient to prove fatal to about 50% of the animals; one group of rats was given a saccharin solution to drink, whereas another group had water. Saccharin ingestion potentiated the effect of insulin, in that the mortality rate was higher and death latencies were shorter in the saccharin group than in the water group.

These results demonstrate that saccharin, although nonnutritive, is not physiologically inert, as has been commonly assumed (Remley & Harrel, 1969). Although the mechanism for this saccharin-induced insulin hypersensitivity is unclear, one possible explanation is suggested by Soviet research on the conditioning of digestive responses. It has been shown that a number of components of the digestive process (e.g., salivation, gastric and intestinal motility, secretion of gastric and pancreatic juice) occur in anticipation of feeding (Bykov, 1957, 1960). Thus sham-feeding (in which the animal tastes, chews, and swallows food, but the food does not enter the gastrointestinal tract) elicits the secretion of gastric and pancreatic juice, and increases gastric and intestinal motility. These anticipatory responses normally serve the function of preparing the animal to digest food. Although the sight, smell, and taste of food have a "natural" temporal contiguity with feeding, the digestive responses can be elicited by any stimulus which has been temporally associated with feeding. The associative process

which underlies the elicitation of the digestive responses by a previously-ineffective stimulus is termed classical conditioning. Such conditioning may also be responsible for saccharin-induced potentiation of the effects of insulin. That is, the exogenous insulin is more effective in inducing death in subjects who have ingested saccharin because the exogenous insulin summates with endogenous insulin released as a conditioned anticipatory response to sweet taste. In the past experience of the animal, sweet taste (e.g., lactose in milk, starch partially converted to sugar by saliva) has always been followed by a rise in blood glucose (BG). An increase in BG is a primary stimulus for insulin secretion and release (Mayhew, Wright & Ashmore, 1969). It is proposed that the association between sweet taste and the subsequent rise in BG (which elicits insulin secretion) results in the elicitation of insulin secretion by sweet taste alone. According to this view, the conditioning process would have to involve central neural integration of information from receptors in the mouth signalling sweet taste, and subsequent information from receptors in the liver (Niijima, 1969; Russek, Rodriguez-Zendeyas & Pina, 1968), intestine (Sharma & Nasset, 1962) or hypothalamus (Mayer, 1966), signalling elevation of glucose in body fluids. Thus, sweet taste, acting as a conditioned stimulus (CS), would lead to a release of insulin as a conditioned response (CR) in anticipation of the postingestional rise in BG. When the taste is of a nutritive sweet substance, the anticipatory release of insulin is

adaptive, in that it facilitates disposal of the high concentration of BG which is the result of feeding (Steffens, 1969). In the case of an artificial sweetener such as saccharin, however, there is no post-ingestional rise in BG, thus the anticipatory release of insulin is inappropriate, and results in the reduction of BG to below normal levels.

The present research was designed to evaluate the reliability of Valenstein & Weber's findings, and to test a number of predictions of the "conditioned insulin release" account of this effect.

Techniques are available for detecting changes in plasma insulin level. One approach is to assess the biological activity of the hormone, e.g., glucose uptake or glycogen deposition by the rat diaphragm (Valance-Owen & Hurlock, 1954). These techniques are generally considered to be lacking in sensitivity (Mayhew et al., 1969) and specificity (Porte & Bagdade, 1970). A second type of assay (Yalow & Berson, 1960) involves radioactive labelling of insulin, incubation of insulin and anti-insulin serum over several days, separation of "bound" and "free" components of the mixture by chromatoelectrophoresis, and measurement of the relative amounts of radioactivity in bound and free fractions of insulin. This technique is still not completely specific to insulin (Porte & Bagdade, 1970), and its use was not technologically feasible in this research project. Instead, the decision was made to look at BG levels as an indirect index of insulin levels. In the absence of other concurrent changes, an increase in circulating insulin is expected to result in a decrease in BG.

The advantage of this approach is that BG determination is relatively easy, rapid, and specific. One disadvantage is that a failure to find an effect on BG doesn't necessarily imply a lack of change in insulin level. For example, an increase in insulin may be accompanied by an increase in glucagon, which increases BG; thus, there may be no net effect on BG. Clearly, this presents a problem only in the case of negative results. Another apparent disadvantage is that a decrease in BG may not be attributable to insulin, but to some other factor, e.g., the release of an insulin-like substance of extrapancreatic origin. However, a conditioned hypoglycemic response to a gustatory stimulus is of interest whether or not insulin secretion is the mechanism whereby this response is affected, since such a conditioned hypoglycemia would be expected to potentiate the effects of exogenous insulin.

Experiment I

The purpose of Experiment I was to determine the reliability of the previously-reported results (Valenstein & Weber, 1965), indicating that saccharin ingestion potentiates the effects of insulin. The procedure was virtually identical to that used by Valenstein & Weber (1965).

Subjects and Procedure

The subjects were 60, experimentally naive, male, Sprague-Dawley-derived rats, weighing 300-350 gm., obtained from Quebec Breeding Farms, St. Eustache, Quebec. Subjects were maintained in individual cages.

For 3 consecutive days, 30 rats were given ad lib. access to 0.25% sodium saccharin solution (no water) in the home cage (Group S). An additional 30 subjects were maintained on ad lib. water (Group W). Food was available ad lib. to all subjects. On Day 4, all subjects were deprived of fluid for 24 hours. Following this deprivation period, all subjects were given an intraperitoneal injection of regular insulin ("Insulin-Toronto", Connaught Medical Research Laboratories, University of Toronto). The dose for half the subjects in each group was 28 U/Kg., with the remaining subjects receiving 24 U/Kg. Subjects were then placed in individual, clear plastic observation cages (Maryland Plastics Co., Series 50). Subjects were not given access to food, but the saccharin solution (Group S) or water (Group W) was made available for one hour

after the injection. As noted above, this fluid access schedule is identical to that used by Valenstein & Weber (1965). Subjects were monitored for 24 hr. after the injection; time of death (defined as no respiration) and fluid consumption were recorded.

Results and Discussion

The latency of the insulin-induced mortality differed in the two groups, with the Group S subjects succumbing sooner. Because of the variability of death latencies, only the first 5 subjects to expire are used in this comparison, a procedure similar to that followed by Valenstein & Weber (1965). (See Appendix A for individual death latency data for all subjects). Table 1 shows the mean latency of the first 5 deaths in each group, as well as the mortality frequencies. The effect of solution ingested on the latency of the first 5 deaths is statistically reliable ($F=5.92$, $df=1,16$, $p<.05$), as is the effect of insulin dose ($F=6.31$, $df=1,16$, $p<.025$). There was no significant difference in the frequency of deaths in each group, which is at variance with the results reported by Valenstein & Weber (1965). Another minor difference is that whereas Valenstein & Weber noted that fluid consumption after injection did not vary between the different groups, in the present experiment, subjects in Group S drank significantly more than subjects in Group W (Group S=6 ml., Group W=3 ml., $F=11.9$, $df=1, 16$, $p<.005$).

The first experiment has provided a partial replication of the results reported by Valenstein & Weber (1965): saccharin ingestion

Table 1.

Experiment I: mortality frequency and mean latency of first 5 deaths as a function of insulin dose, for Group S and Group W.

TABLE 1

	INSULIN DOSE			
	28 Units / Kg.		24 Units / Kg.	
	Mortality frequency	Mean latency of first 5 deaths, min.	Mortality frequency	Mean latency of first 5 deaths, min.
SACCHARIN GROUP	14/15	159	11/15	189
WATER GROUP	11/15	189	12/15	214

potentiates insulin-induced mortality, a finding which may be considered remarkable in view of the powerful homeostatic regulation of BG (e.g., Mayer, 1955; Mayhew et al., 1969).

Experiment II

Experiment I confirmed the finding that saccharin ingestion potentiates insulin-induced mortality (Valenstein & Weber, 1965). The hypothesis under consideration is that this effect is attributable to a taste-elicited conditioned release of insulin or some other substance with insulin-like glycometabolic effects. If such is the case, then saccharin ingestion alone should lead to a lowering of BG.

Evidence from previous research regarding the effect of saccharin on BG is equivocal. Saccharin-induced decreases in BG have been reported by a number of investigators (Althausen & Wever, 1937; Jorgenson, 1950; Kun & Horvath, 1947; Thompson & Mayer, 1959), but others have reported increases (Nicolaidis, 1969; Pannhorst, 1935; Syllaba, 1930) or no change (Bunde & Lackey, 1948; Goldfine, Ryan & Schwartz, 1969; Remley & Harrel, 1969). Much of this research may be criticized on methodological grounds (see Steffens, 1969). Furthermore, differences in dose, deprivation conditions, and other parametric details make inter-experimental comparisons very difficult. Thus, if one is interested in the effect of saccharin ingestion on BG insofar as this is relevant to the insulin-potentiating effect of saccharin - first reported by Valenstein & Weber (1965) and substantially replicated in Experiment I - then the most appropriate approach is to look at BG following saccharin ingestion within an experimental context that is the same as the one that yields this insulin-potentiating effect. The aim of Experiment II was to determine what BG changes occur during

a procedure which is identical to that used in Experiment I except that the animals receive a placebo injection instead of insulin.

Method

Subjects, blood sampling, and BG analysis.-- The subjects were 20 experimentally naive rats of the same sex, weight, and strain as those used in Experiment I. Blood was sampled by amputating 1 mm of the tip of the rat's tail with a scalpel, and "milking" approximately 0.15 ml of blood into a heparanized, disposable, 1-ml polystyrene centrifuge tube. Blood glucose determinations were made on blood serum using the ortho-toluidine method (Dubowski, 1962), with optical density readings made on a Coleman Model 6-20 spectrophotometer at 635 nm. The reagents and glucose standard used in this procedure were obtained from Wood Scientific, Inc.

Procedure.-- The procedure was the same as in Experiment I, except for two modifications: (a) on the test day, the injection was of an equivalent volume of physiological saline rather than insulin, and (b) once a day, at the same time each day, each subject was restrained in a plastic rat restraining cage (Maryland Plastic Co., Model E-00910), and a blood sample was obtained from the tip of the animal's tail. On the day of injection, the blood sampling occurred two hours after the re-introduction of fluids. This corresponded to the approximate time of death of subjects in Experiment I.

The experiment was conducted in 2 replications, each replication using half of the subjects in each group.

Results and Discussion

BG levels of the Saccharin and Water Groups were equated at the beginning of the experiment (Group S=123 mg.%, Group W=125 mg.%). On the test day, both groups had a lower BG level than on the pretest day, a consequence of the suppression of feeding that accompanies water deprivation. The critical data for the hypothesis under consideration is the is the comparison of the magnitude of BG changes in the two groups. The mean percent change was -25.4% for Group S, and -16.1% for Group W: a highly significant difference (F=9.4, df=1, 17, p<.01). This finding is clearly in line with the prediction of the conditioning hypothesis. That is, in a situation in which it has been demonstrated that saccharin ingestion potentiates insulin-induced mortality, saccharin ingestion alone leads to relative hypoglycemia. This supports the view that, in Experiment I, the shorter death latency of Saccharin Group subjects is attributable to the fact that in that group the exogenous insulin is acting on a BG level which is already significantly lower than that of subjects in the Water Group.

One possible objection may be raised at this point. Since BG level refers to milligrams of glucose per 100 ml of plasma, the difference in BG level changes on the test day may be attributable to differences in fluid intakes, and hence plasma volume. Indeed, in the present experiment, as in Experiment I, Group S subjects consumed somewhat more fluid than Group W subjects on the test day (Group S=15 ml, Group W=11 ml), but this difference did not reach a conventional level of statistical significance (F=2.25, df=1, 1, ns).

As a further check on the "plasma dilution" interpretation, a Pearson product-moment correlation coefficient was computed between fluid intake and change in BG. The correlation, a statistically insignificant -0.15 ($t<1$), failed to support the plasma dilution hypothesis. This hypothesis also fails to account for data from experiments reported below.

If the hypoglycemic effect of saccharin is a function of its taste, then parenteral administration of saccharin should not result in hypoglycemia. In apparent contradiction to the view that the hypoglycemic effect of saccharin is a function of its taste, investigators have reported that parenteral administration of saccharin does lead to a lowering of BG (Thompson & Mayer, 1959), and also potentiates the hypoglycemic effect of insulin (Macallum, 1948; Macallum & Sivertz, 1942). However, it would be an error to assume that animals cannot taste saccharin when it is not administered orally. Carlson, Eldridge, Martin, & Foran (1923) have shown that, in dog and in man, saccharin is partially excreted through saliva. In addition, Bradley & Mistretta (1971) have provided evidence for "intravascular taste" of saccharin in the rat. Thus, the reports of the hypoglycemic effect of injected saccharin can be accounted for by the hypothesis that sweet taste acts as a conditioned stimulus for an anticipatory release of insulin (or a similar hypoglycemic substance).

Experiment III

Saccharin, according to the conditioning hypothesis, leads to hypoglycemia because of the pre-experimental contingency between sweet taste and nutritive post-ingestional events. The consequence of this contingency is that hypoglycemia occurs as a conditioned response to sweet taste. If a conditioning process does in fact underlie the response, then the response should evidence characteristics of a CR. One characteristic of a CR is that repeated presentations of the CS without accompanying presentations of the unconditioned stimulus (US) leads to a diminution and gradual elimination of the CR. This process is termed experimental extinction. Thus, if the hypoglycemic response to saccharin is a CR, then prolonged exposure to the CS (sweet taste) should lead to the extinction of this CR. Experiment III was designed to determine whether the hypoglycemic effect of saccharin ingestion is subject to experimental extinction.

Subjects and Procedure

The subjects were 14 experimentally naive rats of the same weight, sex, and strain as those used in the previous experiments. The blood sampling procedure and BG analysis were the same as those described in Experiment II.

The aim of the procedure was to give animals in the Saccharin Group exposure to the saccharin solution that would be sufficient to

extinguish the hypoglycemic response to it. Thus, 7 rats (Group S) were given continuous access to 0.25% (Wt/V) saccharin solution, but not water, in the home cage for 60 days. Another 7 subjects (Group W) were given continuous access to water, but not saccharin, in the home cage for the same period. Food was available ad lib. to all subjects. Both groups were then tested according to the procedure outlined in Experiment II, to determine the effect of saccharin on BG.

Results and Discussion

As in Experiment II, BG levels of subjects in Group S and Group W were equivalent at the start of the experiment (Group S = 113 mg.%, Group W = 115 mg.%). Similarly, both groups showed a decrease in BG from pretest to test day. However, in the present experiment there was no significane difference between BG changes shown by the two groups. In fact, the slight difference observed was opposite in direction to that found in Experiment II (Group S = -11.9%, Group W = -15.0%; $t < 1$). As was the case previously, Group S subjects did comsume more fluid than Group W subjects on the test day (Group S = 17 ml., Group W = 12 ml.; $t = 2.50$, $df = 12$, $p < .05$), which, according to the plasma dilution hypothesis, should have resulted in a greater BG decrease for that group. Thus, it would again appear that the observed glycemic effects cannot be attributed to differential fluid ingestion.

The previous experiment has shown that saccharin ingestion results in hypoglycemia. The present experiment, using the same method of testing, has shown that giving animals prolonged access to the saccharin solution abolishes this hypoglycemic response. The fact that the hypoglycemic response to saccharin appears to undergo experimental extinction lends support to the view that a conditioning process underlies the response. Furthermore, it allows us to reject the view (Kun & Horvath, 1947) that the hypoglycemic effect of saccharin is an unconditioned response (i.e., an innate reflex) to sweet taste.

Experiment IV

The extinction of the hypoglycemic response to saccharin by long-term access to saccharin (Experiment III) has provided support for the conditioning hypothesis. It is possible, however, that prolonged consumption of any sweet solution, whether nutritive of nonnutritive, would abolish the hypoglycemic response. Alternatively, it is possible that long-term access to a nutritive sweet solution would increase the magnitude of the hypoglycemic response to sweet taste, since the consumption of a nutritive sweet solution provides, in effect, additional pairings of sweet taste with nutritive post-ingestional consequences. In Experiment IV, these possibilities were evaluated by giving animals long-term access to a solution of glucose, a sweet, nutritive substance, prior to assessing the effect of saccharin on BG.

Subjects and Procedure

The subjects were 20 experimentally naive rats of the same weight, sex, and strain as those used in the previous experiments. The blood sampling procedure and BG analysis were the same as those described in Experiment II.

The procedure used in this experiment was basically similar to the procedure used in Experiment III, except that in this experiment subjects in the experimental group were given long-term access to a glucose solution rather than saccharin. Thus, the 10 subjects in the

the Glucose-Saccharin Group (Group G-S) were given continuous access to 3% (Wt/V) glucose solution [approximately equal in preference to 0.25% saccharin (Shuford, 1959; Young & Madsen, 1963)] in the home cage for 60 days. Another 10 subjects (Group W) were given water for the same period. Food was available *ad lib.* to all subjects. Subjects were then tested to determine the effect of saccharin on BG, according to the procedure outlined in Experiment II. Subjects in Group G-S continued to have access to the glucose solution for the first 3 days, but were tested with 0.25% saccharin solution on the test day. Subjects in Group W had only water to drink throughout.

Results and Discussion

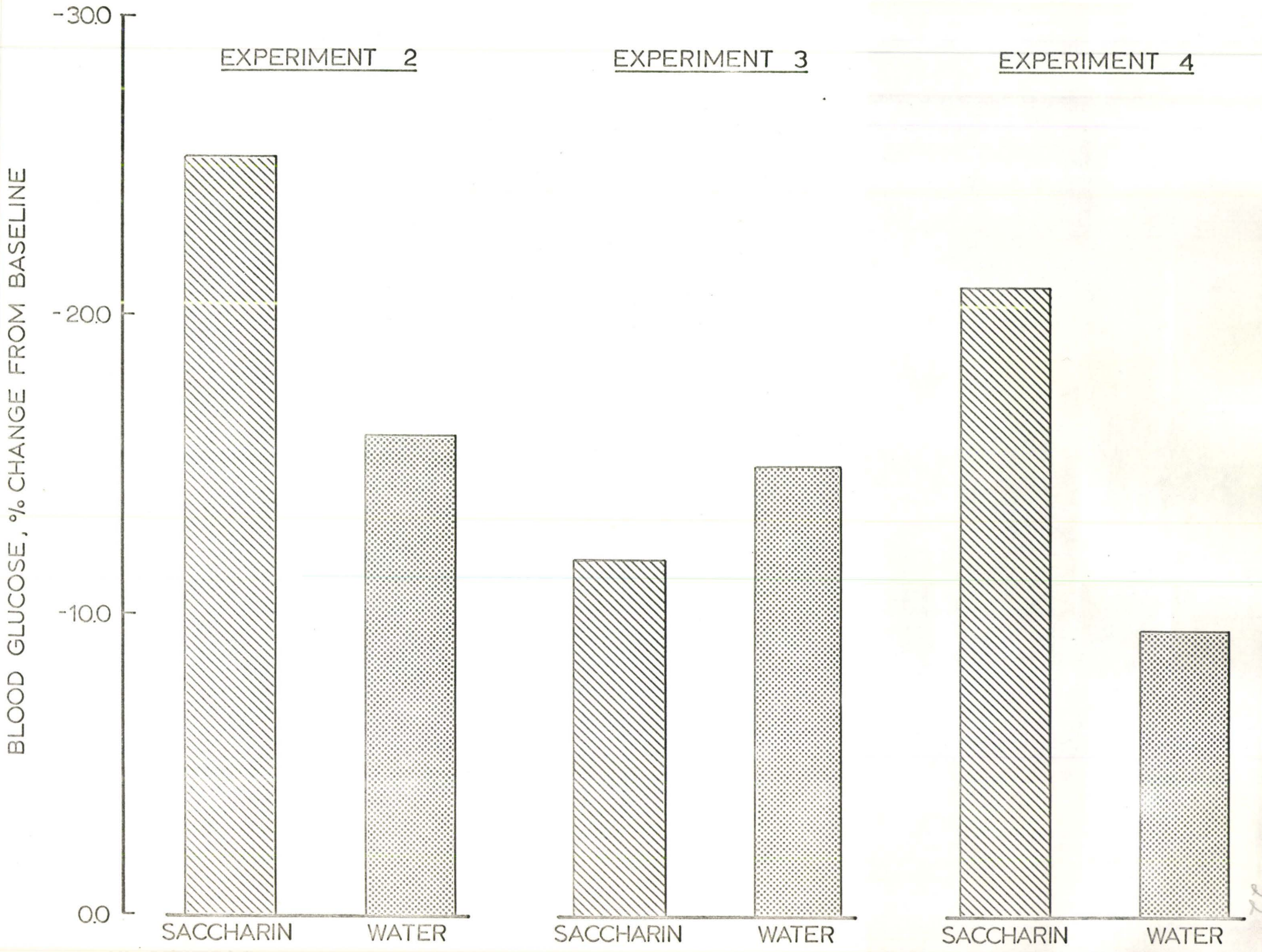
Both Group G-S and Group W had a lower BG level on the test day than on the pretest day. The subjects given saccharin on the test day (Group G-S) exhibited hypoglycemia compared to subjects in Group W (Group G-S =-20.9%, Group W = -9.6%; $t = 2.94$, $df = 18$, $p < .01$).[1] These results, as well as the results of Experiments II and III, included for comparison, are shown in Figure 1. Examination of Figure 1 indicates that the Saccharin-Water difference in the present experiment is comparable to the difference obtained in the initial demonstration of the hypoglycemic effect of saccharin (Experiment II), thus, it is clear that long-term access to a nutritive sweet solution does not diminish

---

1 All significance levels for *t* values in this report are for a two-tailed test.

Figure 1.

Experiments II, III, and IV: mean percentage change in BG for Group S and Group W.


-30.0
-20.0
-10.0
0.0
BLOOD GLUCOSE, % CHANGE FROM BASELINE
EXPERIMENT 2
EXPERIMENT 3
EXPERIMENT 4
SACCHARIN
WATER
SACCHARIN
WATER
SACCHARIN
WATER

Experiment V

According to the conditioning hypothesis, saccharin-induced hypoglycemia is an example of a "naturally-occurring conditioned response" (Bykov, 1960). The response is a "natural" one because it is a consequence of the fact that animals taste the food before it enters the gastrointestinal system, and the fact that sweet taste has been a reliable predictor of the postingestional effects of carbohydrate ingestion. This suggests that it should be possible to have any stimulus function in this manner if the contingencies are so arranged as to make it a predictor of nutritive postingestional events. This would be exactly analogous to the classic Pavlovian procedure of pairing an initially-neutral stimulus, e.g. a bell, with delivery of food in the mouth, and measuring salivation to the bell alone. In the present experiment, in initially-neutral gustatory stimulus was paired with direct intragastric administration of glucose. If the conditioning hypothesis is valid, such pairings should result in the elicitation of a hypoglycemic response by the new gustatory stimulus.

Subjects

The subjects were 19 experimentally naive rats, weighing 200-250 gm., of the same sex and strain as those used in the previous experiments. The blood sampling procedure and BG analysis were the

same as those described in Experiment II.

Procedure

Each rat participated in the experiment for 18, 60-minute sessions. Each subject was run once every other day. Subjects were deprived of water 24 hours before each session.

The experiment consisted of four successive phases: (a) adaptation to restraint (2 sessions), (b) baseline BG determinations and assessment of BG response to intubation procedure (2 sessions), (c) conditioning (10 sessions), and (d) extinction (4 sessions).

On the 2 adaptation days, each subject was placed in a plastic rat retraining cage and, two minutes after having been introduced into the restraining cage, a 1% (Wt/V) solution of instant decaffeinated coffee (Sanka), a physiologically inert substance with a distinctive taste (Revusky, 1968), was made available to the subject. The coffee solution continued to be available for the rest of the hour.

On each of the 2 "baseline" days, a blood sample was obtained from each subject immediately following insertion into the restraining cage ("pre" measure of BG). Subjects were then given access to the decaffeinated coffee solution and, after two minutes' access to it, were given 5 ml. physiological saline by acute intragastric intubation. This procedure involved removing the subject from the restraining cage,

holding the subject's mouth open with a mouth brace, inserting a feeding tube (C.R. Bard, Inc., Size 8 French) into the stomach via the mouth and the esophagus, and infusing the solution from a 5 ml. syringe which was connected to the feeding tube. After the intragastric intubation, the feeding tube was withdrawn, mouth brace removed, and the subject was returned to the restraining cage. A second blood sample was obtained 5 minutes after intubation ("post" measure of BG). Subjects were left in the restraining cage for the remainder of the hour; the decaffeinated coffee solution remained available throughout this period.

During the conditioning phase (next 10 sessions), the procedure was similar to that described above, except that 9 subjects, comprising the Experimental Group (Group E), were intubated with 5 ml., 50% (Wt/V) glucose, whereas the remaining 10 subjects, comprising the Control Group (Group C), continued to be intubated with 5 ml. physiological saline.

For the next 4 sessions (extinction phase), the same procedure was followed except that all subjects were intubated with physiological saline. Thus, the comparison of BG changes in Group E and Group C on the extinction days permits the evaluation of conditioned glycemic effects acquired by the taste of decaffeinated coffee.

Consumption of the decaffeinated coffee solution was measured (a) immediately prior to intubation, (b) 5 minutes after intubation, and (c) at the end of the session.

Results

BG levels of subjects in Group E and Group C were approximately equivalent at the beginning of the experiment (Group E = 121 mg.%, Group C = 123 mg.%), as were the glycemic responses to the intubation procedure, expressed as percentage change from "pre" to "post" level, during the baseline phase (Group E = +4.1%, Group C = +3.1%).

Figure 2 presents BG changes for Group E and Group C during the 10 conditioning days. As expected, intragastric glucose intubation resulted in a marked increase in BG as measured 5 minutes after intubation. Inspection of Figure 2 suggests that the effect of glucose intubation tended to diminish with repeated administration, but this apparent diminution of the response was not statistically significant. The control group showed a slight rise in BG during each session, presumably as a result of stress associated with the tubing procedure.

Figure 3 presents the BG change for both groups throughout the extinction phase, when all subjects were intubated with saline. On the first day of extinction, subjects in Group E showed a significant decrease in BG compared to Group C ($t = 3.32$, $df = 17$, $p < .01$). Smaller, but still significant, relative decreases were observed on the second and third extinction days (Day 2, $t = 2.47$, $df = 17$, $p < .05$; Day 3, $t = 2.83$, $df = 17$, $p < .02$). By the fourth day of extinction

Figure 2.

Experiment V: mean percentage change in BG during the baseline and conditioning phases for Group E and Group C.

EXPERIMENT 5 - CONDITIONING

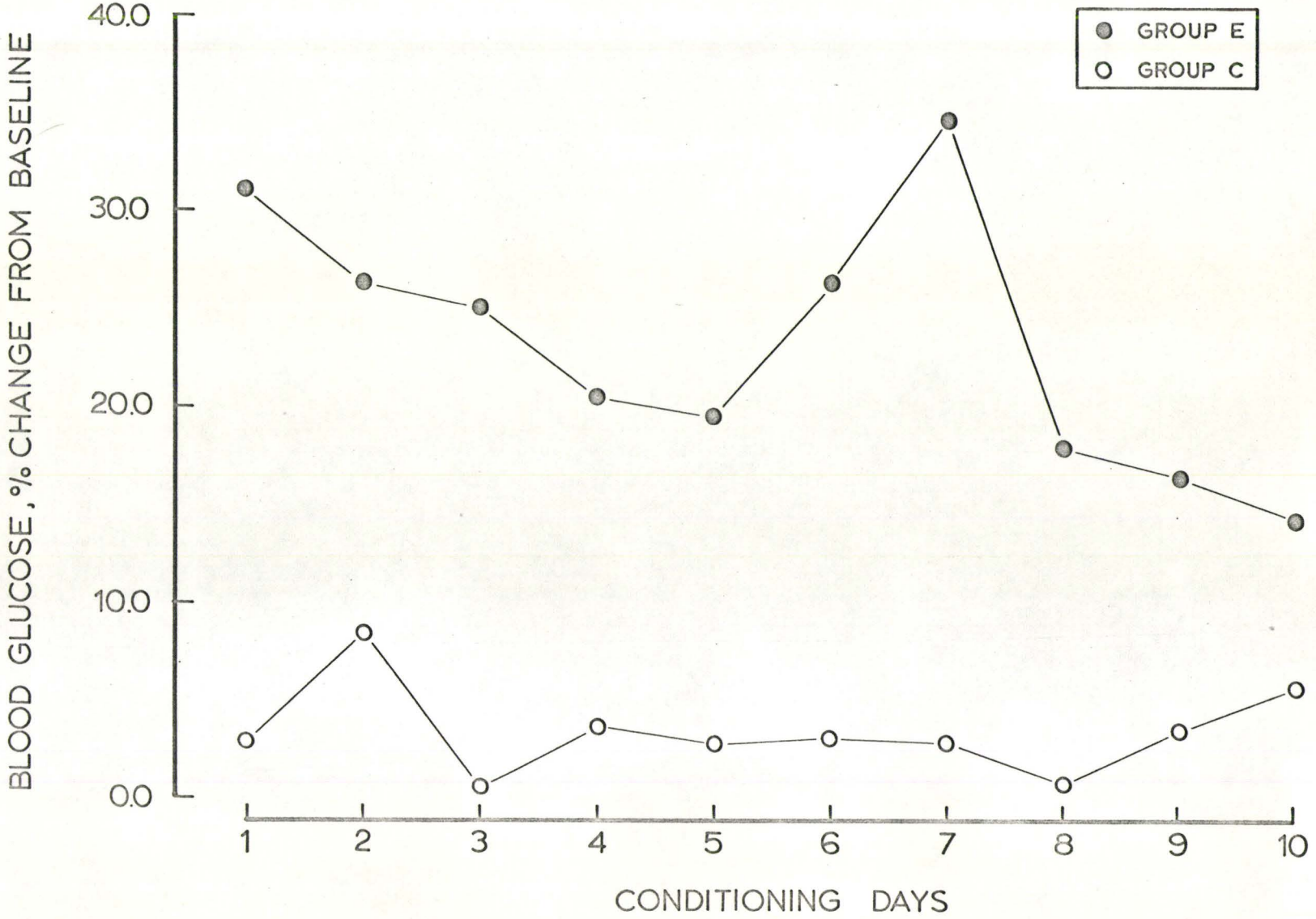
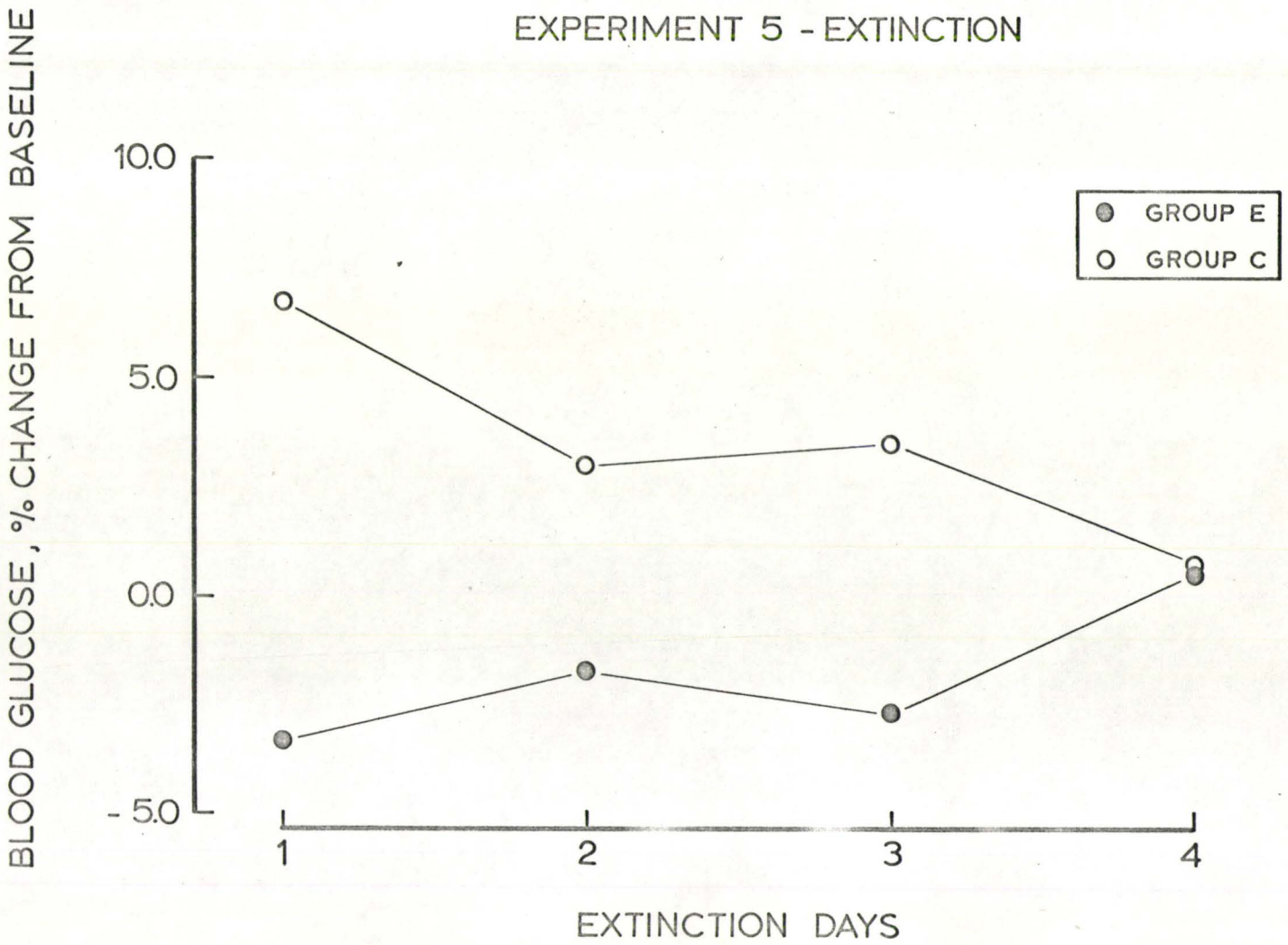


Figure 3.

Experiment V: mean percentage change in BG during the extinction phase for Group E and Group C.


EXPERIMENT 5 - EXTINCTION
BLOOD GLUCOSE, % CHANGE FROM BASELINE
10.0
5.0
0.0
- 5.0
GROUP E
GROUP C
1
2
3
4
EXTINCTION DAYS

there was no appreciable difference between changes in BG in the two groups.

Figure 4 shows the total amount of coffee solution consumed by each group 5 minutes after intubation. There was no initial difference in coffee consumption; however, subjects in Group E progressively decreased their intake as conditioning proceeded. Group E intake remained significantly depressed on the first day of extinction ($t = 2.96$, $df = 17$, $p < .01$), but gradually recovered on subsequent days. Measures of coffee intake up to the time of intubation, and coffee intake for the entire session (See Appendix B, Figures 1 and 2) show a similar pattern.

Discussion

The present experiment has shown that an initially-neutral taste cue can elicit a glycometabolic effect if it has been paired with direct intragastric glucose administration in a classical conditioning paradigm. The fact that the taste-elicited hypoglycemic response in this experiment followed an orderly course of experimental extinction is further evidence that a conditioning process is involved in producing the response.

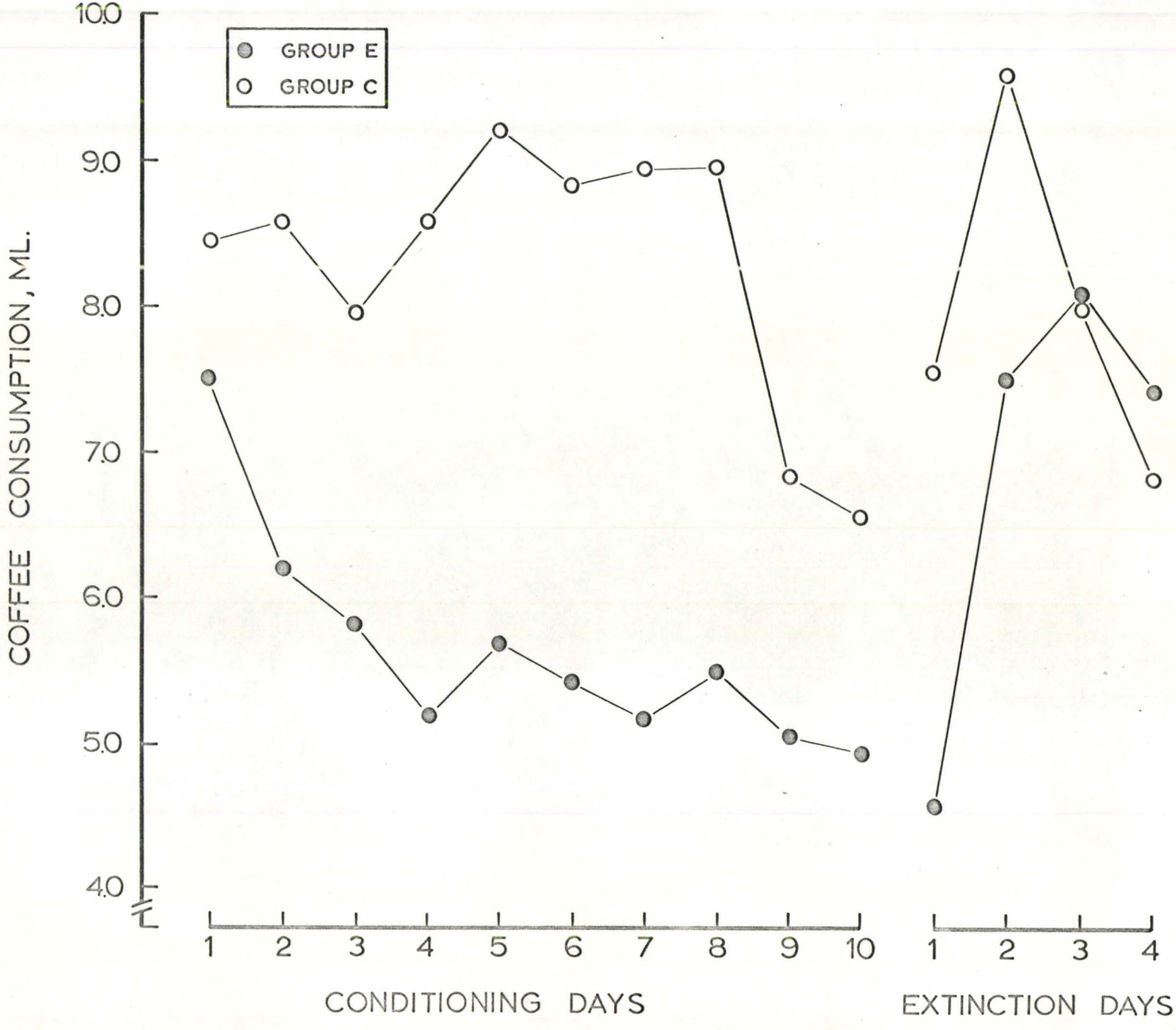
The reduced intake of the coffee solution by subjects in Group E (Figure 4) is surprising. The intubed glucose was hypertonic, thus having the effect of drawing fluid into the gastrointestional tract, which produces intracellular dehydration and hence thirst (Adolph, 1967).

Figure 4.

Experiment V: mean values of intake or decaffeinated coffee solution 5 minutes after intubation for Group E and Group C.

EXPERIMENT 5

COFFEE CONSUMPTION - 5 MIN. POST INTUBATION



In fact, McCleary (1953) has reported that an intragastric load of hypertonic glucose does increase water intake. However, in the present experiment, animals receiving a hypertonic glucose load drank less than animals which were loaded with physiological saline. The most likely explanation of this effect is that the intubation of 50% glucose was aversive; thus, the reduced intake of coffee represents a conditioned aversion to the taste of coffee (Revusky & Garcia, 1970). LeMagnen (1959) demonstrated a similar conditioned aversion to a distinctively-flavored diet which was associated with parenteral or intragastric glucose administration. The coffee intake data of the present experiment are especially interesting, in that the aversion to the coffee solution developed in spite of a need to take in more fluid.

The differences in intake of the decaffeinated coffee solution present a difficulty for the hypothesis under consideration: the Experimental and Control Groups differ not only with regard to the variable of interest, i.e., treatment received during the conditioning phase, but also with regard to fluid intake. Thus, it is possible to argue that the differences in BG responses in extinction were not due to differences in conditioning experience, but to differences in intake of the coffee solution.

The aim of Experiment VI was to solve this problem. In this experiment, a possible difference in fluid intakes was prevented by using an olfactory rather than a gustatory CS. Since the procedure did

not involve drinking on the part of the animal, no differences in intake could occur.

Experiment VI

Experiment VI was designed to provide a modified replication of Experiment V. Subjects in this experiment had intragastric intubation, either of glucose or saline, paired with an olfactory cue, thus, the possibility of confounding conditioning experience with differences in fluid intake was eliminated. The rationale for this study is essentially the same as for Experiment V. After repeated pairings of a CS (odor of pepperiment, in this case) with intragastric administration of glucose (US), presentation of the CS alone should elicit insulin-like glycometabolic responses, resulting in a decrease in BG. Further presentations of the CS without the US should result in the extinction of this conditioned glycemic response.

The present experiment differs in some methodological aspects from Experiment V. In Experiment V, the gastric tube was inserted and withdrawn through the mouth; thus, it is possible that the subjects may have tasted some glucose when the tube was withdrawn. In Experiment VI, this possibility was eliminated by fitting subjects with chronic nasopharyngeal gastric tubes. This also permitted more precise control of temporal parameters of intubation. Furthermore, no "baseline" trials were given in this experiment, in order to prevent a possible retardation of acquisition, a frequently-reported consequence of CS preexposure (e.g., Siegel, 1971).

Subjects and Procedure

The subjects were 10 experimentally naive rats, weighing 260-320 g., of the same sex and strain as those used in the previous

experiments. Conditions of housing, method of obtaining blood samples, and the technique of BG analysis were the same as in Experiment II. Five subjects were used in the Experimental Group (Group E) and 5 subjects in the Control Group (Group C).

All subjects were fitted with chronic nasopharyngeal gastric tubes, using a modification of the technique described by Epstein (1960). At least one week was allowed to pass between the day of the surgery and the day that the experiment proper was started.

Each rat participated in the experiment for 16, daily, 60-minute sessions. Subjects were deprived of food for 14 hours before each session.

The experiment consisted of three successive phases: (a) adaptation to the restraint (2 sessions), (b) conditioning (10 sessions), and (c) extinction (4 sessions).

On the 2 adaptation days, each subject was simply placed in a plastic restraining cage for 60 minutes.

On each of the 10 conditioning days, a blood sample was obtained from each subject immediately following insertion into the restraining cage ("pre" measure of BG). Then, a cotton ball saturated with 1 ml. oil of peppermint (B.P.) was placed in a perforated compartment of the restraining cage about 4 cm. from the rat's nose. Immediately after the introduction of the olfactory cue, Group E subjects were intragastrically intubated with 5 ml., 50% (W/V) glucose solution and Group C subjects were intubated with 5 ml. physiological saline, via the nasopharyngeal gastric tube. The rate of intragastric infusion,

controlled by a syringe pump (Harvard Apparatus Co., Inc., Model 941), was 3 ml. per minute. A second blood sample was obtained 5 minutes after intubation ("post" measure of BG).

During the next 4 sessions ("extinction"), the procedure was the same as for the conditioning phase, except that all subjects were intubated with physiological saline.

Results and Discussion

Figure 5 presents BG changes, expressed as percentage change from "pre" to "post" level, during the 10 conditioning days. As indicated in Figure 5, infusion of glucose into the stomach led to a marked BG increase by 5 minutes after the infusion. Control subjects, which received an equivalent volume of physiological saline, showed a small BG increase.

BG changes for the 4 extinction days are shown in Figure 6. On the first day of extinction, the amount of BG change was significantly lower for Group E than for Group C ($t = 3.92$, $df = 8$, $p < .01$). The difference was smaller, but still approached significance on Day 2 of extinction ($t = 2.12$, $df = 8$, $p < .07$). By days 3 and 4, there were no significant differences between the two groups.

The pattern of results observed in this sixth experiment confirms the findings obtained in Experiment V. In both experiments, a CS was paired with intragastric administration of a glucose solution (US). In both experiments, after 10 pairings of the CS and the US, presentation of the CS alone resulted in hypoglycemia compared to the

Figure 5.

Experiment VI: mean percentage change in BG during the conditioning phase for Group E and Group C.

EXPERIMENT 6 - CONDITIONING

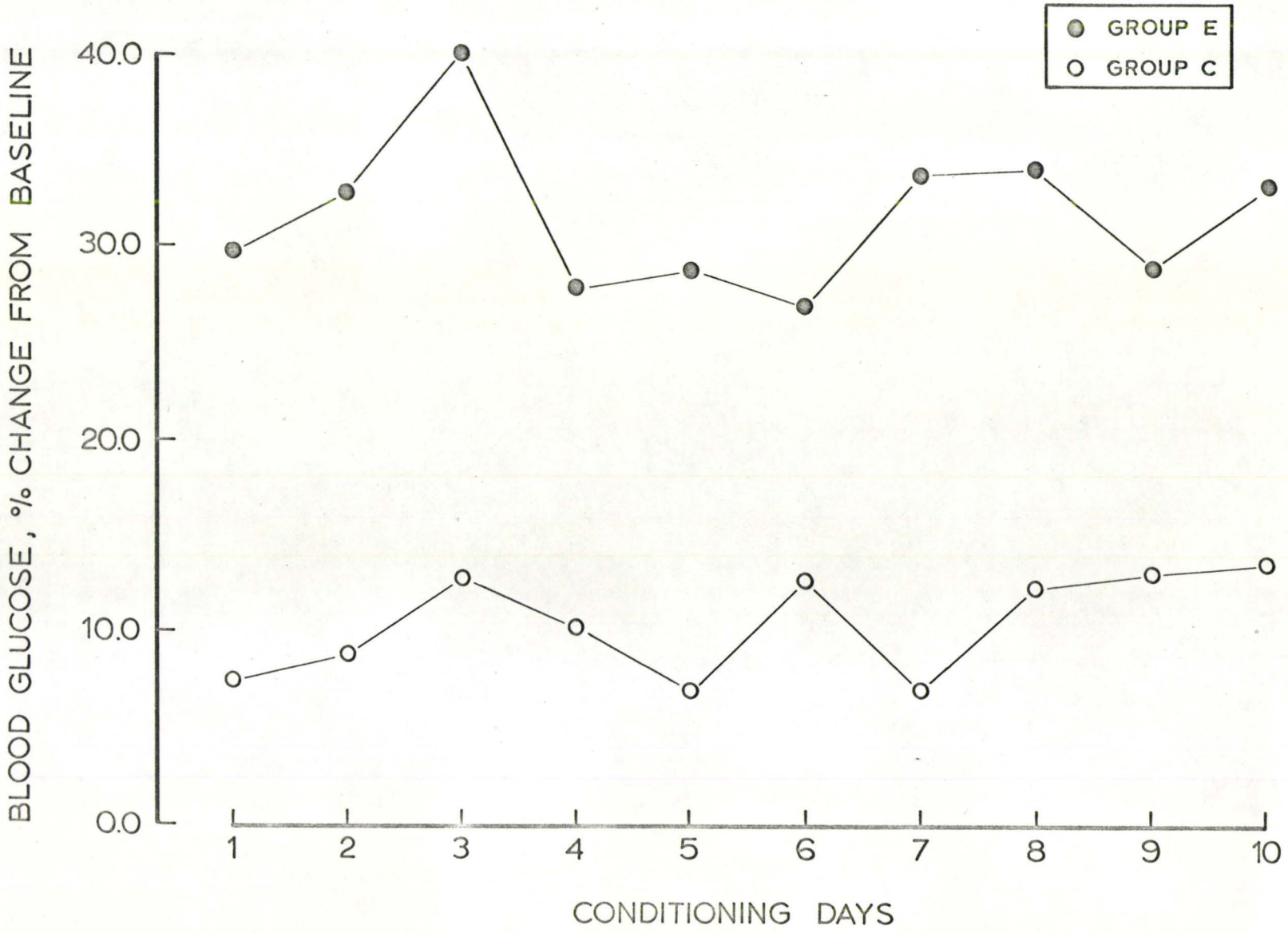
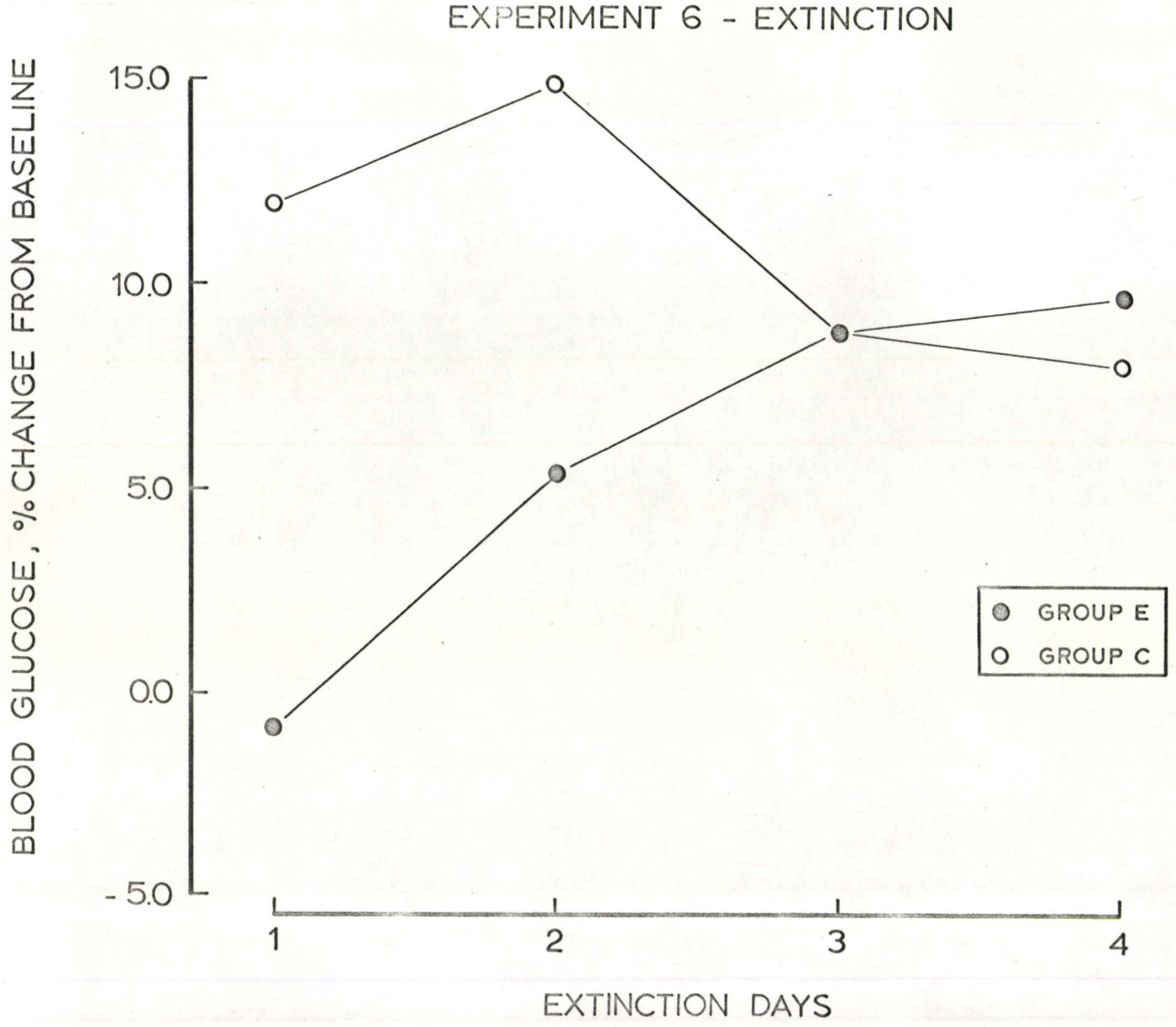


Figure 6.

Experiment VI: mean percentage change in BG during the extinction phase for Group E and Group C.


EXPERIMENT 6 - EXTINCTION
BLOOD GLUCOSE, % CHANGE FROM BASELINE
15.0
10.0
5.0
0.0
- 5.0
1
2
3
4
EXTINCTION DAYS
GROUP E
GROUP C

control group. The hypoglycemic response followed a similar course of extinction in both experiments. Experiment VI extended these results,in that the CS was in the olfactory rather than the gustatory modality, thus eliminating possible confounding between conditioning and intake of the fluid which serves as the CS.

General Discussion

The experiments reported here were designed to evaluate the hypothesis that the potentiation of insulin-induced mortality by saccharin ingestion can be accounted for by a conditioning mechanism. Evidence presented included the demonstration that saccharin ingestion alone results in hypoglycemia, and that this hypoglycemic response is extinguished by longterm access to saccharin, a non-nutritive sweet-tasting substance, but not by longterm access to glucose, a nutritive sweet-tasting substance. In an explicit test of the conditioning hypothesis, it was shown that the hypoglycemic response is also elicited by stimuli, either gustatory or olfactory, which have been specifically paired with intragastric glucose administration. Taken as a whole, these results lend strong support to the conditioning account of the potentiating effect of saccharin on insulin-induced mortality.

A more general picture that emerges from these data is a view of hypoglycemia as a conditioned response preparatory to feeding. Thus, it is seen as similar in kind to "classic" conditioned responses, such as salivation, gastric and intestinal motility, and secretion of gastric and pancreatic juice in anticipation of food (Bykov, 1957). The experiments reported here provide support for the view that hypoglycemia can occur as a learned preparatory response, and results reported by other investigators are consistent with it. For example, Mityushov (1954) has reported that, in dogs and in humans, after 8 to 10 intravenous injections of glucose, the injection of a physiological saline placebo

results in hypoglycemia. Thus, the injection procedure, which in the past has reliably predicted the metabolic consequences of systemic glucose, becomes capable of anticipatorily eliciting these responses. Steffens (1969) has shown that the removal of food just as the rat is about to eat is followed by a decline in BG level. These results may be interpreted as demonstrating that stimuli associated with feeding, i.e., sight and smell of food, result in hypoglycemia as a consequence of the animal's natural conditioning history, which is characterized by pairing of these stimuli and nutritive postingestional events. In addition, Booth & Miller (1969) examined BG levels following presentation of a visual stimulus which functioned as a discriminative stimulus for food-reinforced barpressing in previous training. Blood glucose showed an "oscillatory" response: an initial rise followed by distinct hypoglycemia, followed by a second peak. The fact that a stimulus signalling shock was also followed by a rise in BG suggests that only the hypoglycemic phase of the response is uniquely characteristic of the food-signalling function of the stimulus. The conditioning experiments reported here differ in numerous respects from Booth & Miller's; perhaps the most important differences are that no operant response was required of the animals, and that rather than visual, the present experiments used gustatory and olfactory cues, which might be particularly effective with visceral conditioning (Garcia & Ervin, 1968; Seligman, 1970).

The research reported here used BG level as a dependent variable. A particularly interesting question concerns the nature of

the mechanism underlying the observed conditioned hypoglycemic responses. The results of these experiments are as predicted by a hypothesis that insulin release occurs as a conditioned response; however, since insulin levels were not measured in these experiments, no unequivocal conclusion can be drawn regarding the involvement of insulin in the observed hypoglycemic response. Alternative possibilities include a conditioned suppression of neoglucogenesis, and a conditioned release of a hypoglycemic substance of extrapancreatic origin (Alvarez-Buylla, Segura & Alvarez-Buylla, 1961). The first of these is highly unlikely since there is no evidence that neoglucogenesis can be selectively altered by the CNS without also affecting glucose utilization. Furthermore, Mityushov (1954) has reported the blood from dogs exhibiting a conditioned hypoglycemic response has increased insulin-like activity as determined by a bioassay procedure. The possibility of conditioned release of an insulin-like substance of extrapancreatic origin is difficult to evaluate, since only limited and circumstantial evidence is available concerning its existence (Alvarez-Buylla et al., 1961). It appears, then, that conditioned insulin release is the most likely mechanism for the observed hypoglycemic responses.

The hypothesis that the secretion of insulin is under the control of a conditioned mechanism, and hence the central nervous system, runs counter to the traditional view of the control of insulin secretion, which holds that insulin secretion is regulated almost entirely by the concentration of glucose in arterial blood reaching the pancreas (Keele & Neil, 1965). However, there is evidence which suggests that central

neural control of insulin secretion is present, and that it may play an important role in normal regulation of insulin level and carbohydrate metabolism. Pancreatic islets are richly innervated, and direct neural control of insulin secretion has been suspected since the 1920's (Britton, 1925). The development of the radioimmunoassay for insulin (Yalow & Berson, 1960) made it possible to demonstrate unequivocally that vagal stimulation results in an immediate increase in insulin in venous blood, and that this increase is not mediated by BG (Frohman, Ezdinli & Javid, 1966; Daniel & Henderson, 1967; Keneto, Kosaka & Nakao, 1967). In addition to direct vagal control, it is likely that insulin secretion is also controlled by neuroendocrine mechanisms, since many of the hormones which stimulate insulin secretion, e.g., secretin, gastrin, and pancreozymin, are themselves under neural control (Chisolm, Young & Lazarus, 1969).

The second major line of evidence which implicates the CNS in the control of insulin secretion concerns the effects of direct interference with CNS function on insulin levels. Thus, it has been shown that ventromedial hypothalamic lesions result in an elevation of plasma insulin level (Frohman, Bernardis, Schnatz & Burek, 1969) and enlargement of pancreatic islet size (Han, Yu & Chow, 1970) even when food intake is controlled. Also, Kuzuya (1962) has shown that hypothalamic stimulation causes an increase in insulin secretion in dogs. These results suggest that the hypothalamus exerts a tonic inhibitory as well as an excitatory control over insulin secretion.

The third approach to the study of central control of insulin secretion is to investigate the role of "psychological" variables in the modulation of insulin levels. For example, Goldfine, Abraira, Gruenwald & Goldstein (1970) studied the effect of "imaginary food ingestion" on plasma insulin levels. These investigators hypnotized human subjects and told them to imagine that they're having their favourite foods in a restaurant. In 3 out of 7 subjects this led to a prompt rise in immunoreactive insulin. These results support the view that the hypoglycemic responses observed in the present series of experiments indeed represent anticipatory release of insulin. The data reported by Goldfine et al., combined with the results of research reported here, suggest that "psychic secretion" of insulin is possible, and that it - like "psychic secretion" of gastric acid (Pavlov, 1897) - can be accounted for by a conditioning mechanism.

Several areas of further investigation are suggested by the present findings. One major question concerns the exact nature of the effector system involved in the conditioned hypoglycemic response. The first step here is to assess the role of insulin by carrying out radioimmunoassay procedures. Another approach would be to attempt to conditioned depancreatized animals or animals made diabetic by alloxan or streptozotocin injections. If a pancreas with functioning beta cells proves to be necessary for conditioning, then the possible role of intermediary hormones becomes important. The question here is whether conditioned release of insulin is effected through direct neural control or through a neuroendocrine mechanism. One approach to this problem would

be to attempt to condition animals with a denervated pancreas. Another major question concerns the central neural mechanism underlying the response. Conditioning animals with specific lesions of the CNS is the method of choice here. For example, if the lateral hypothalamus is necessary for the "glucostatic" regulation of feeding, as the findings of Epstein & Teitelbaum (1967) suggest, then one would expect that an intact lateral hypothalamus is also necessary for the development of conditioned hypoglycemia of the type reported here.

According to the conditioning hypothesis, the hypoglycemic effect of sweet taste is the function of the natural conditioning history of the organism. This suggest investigation of the role of early experience in the development of the hypoglycemic response to sweet taste. Bykov (1960) has reported that dogs which are not allowed to taste meat until adulthood do not show the usual salivary response to the smell and sight of meat when first exposed to these stimuli. Analogously, it may be proposed that animals raised without the opportunity to taste nutritive sweet substances should not manifest the hypoglycemic response to sweet taste.

The present research has demonstrated that hypoglycemia can occur as a conditioned response in preparation for, or in anticipation of, the entry of glucose into the stomach. It has been demonstrated here that such glycemic conditioning has implications for predicting an organism's response to drugs with glycometabolic actions.

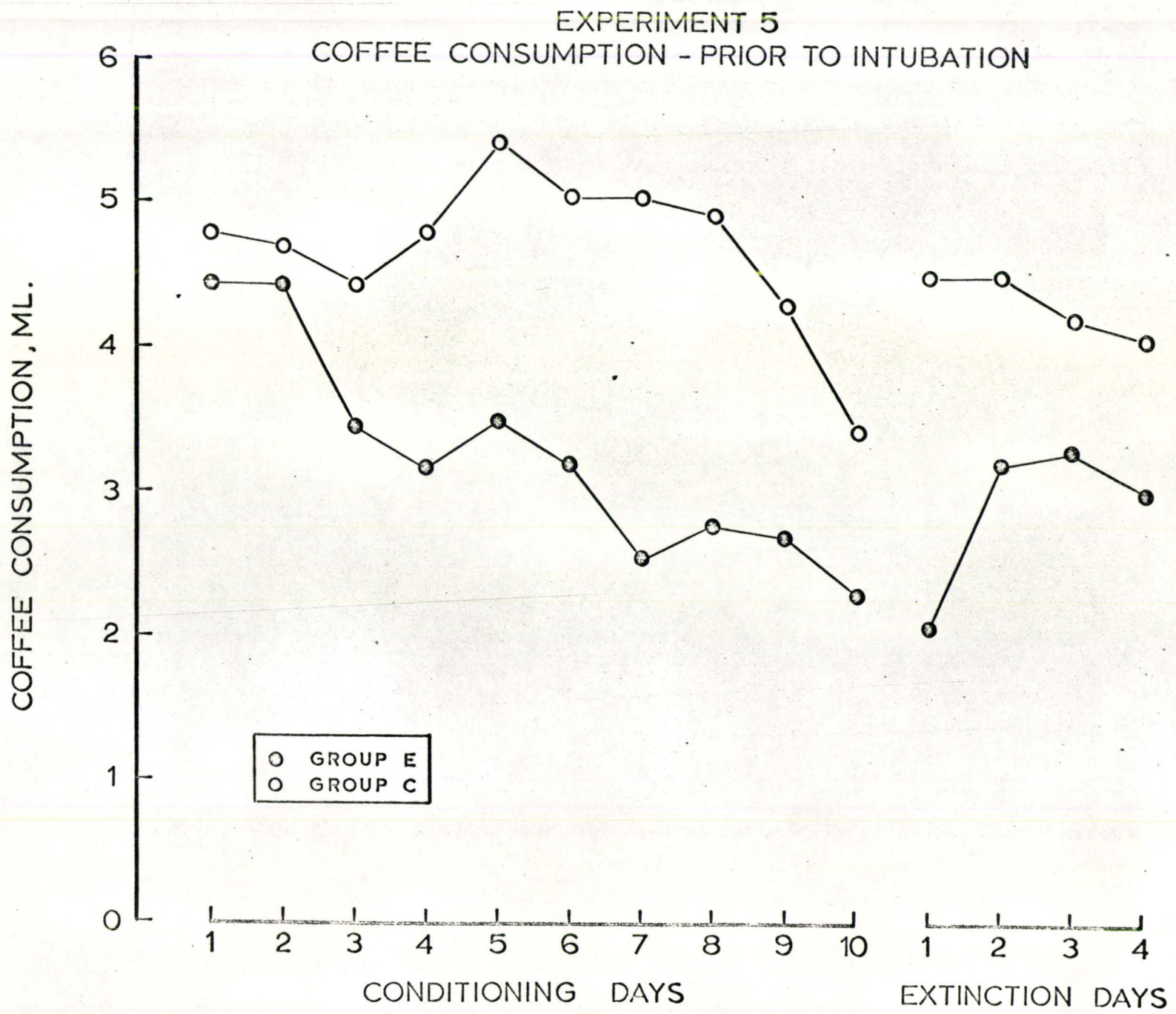
This work, like the research on the conditioning of secretion of saliva, gastric juice, pancreatic juice, and bile, points out the importance of the contribution of conditioned responses to the digestion and utilization of food. The view that conditioning processes play an important role in the physiology of the organism, although generally accepted by Soviet (e.g., Bykov, 1960) and Eastern European (e.g., Ádám, 1967) investigators, is only beginning to be acknowledged by Western researchers in the fields of physiology and medicine (e.g., Tepperman, 1970).

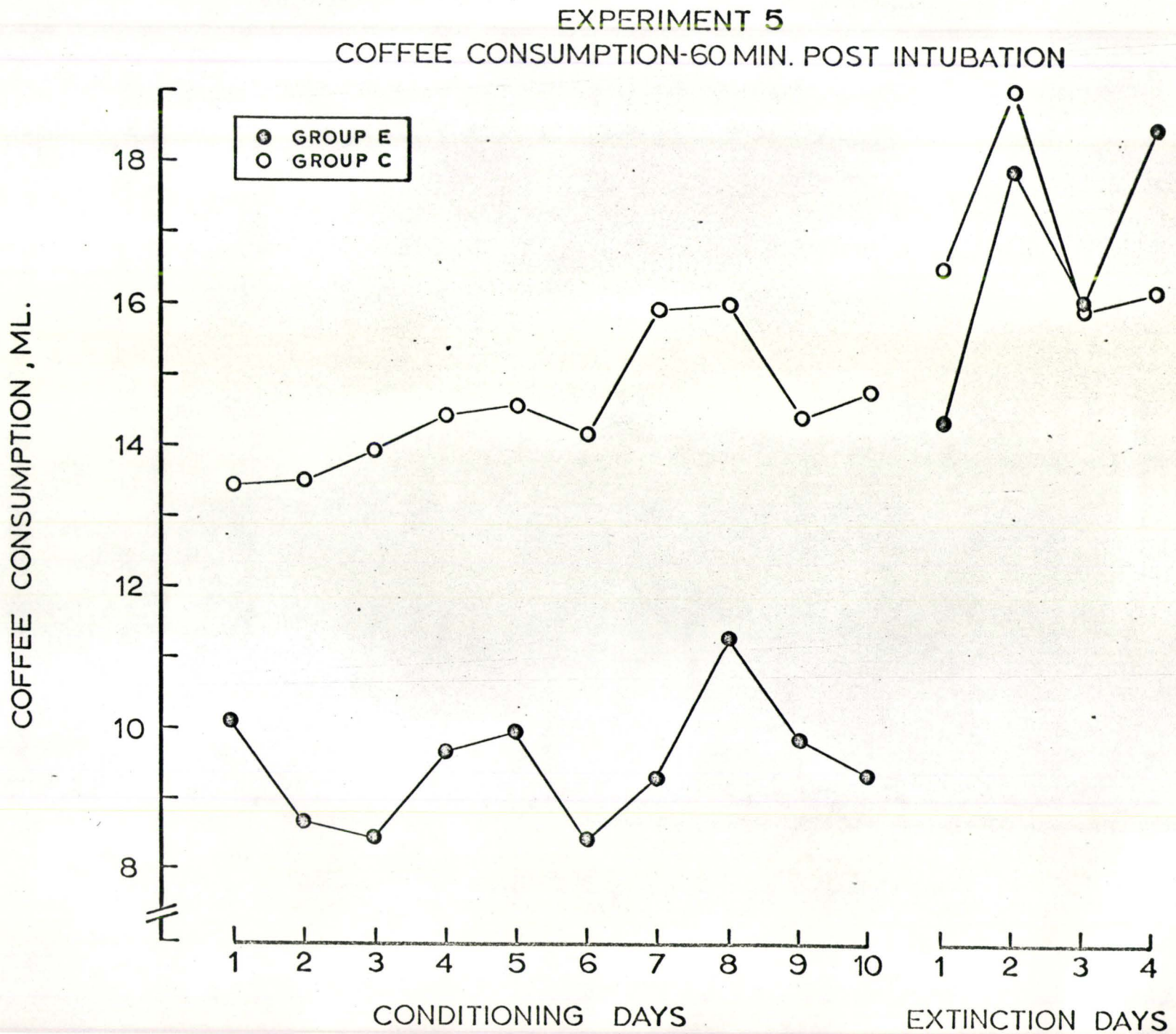
APPENDIX A

Experiment I - Death Latencies

	INSULIN DOSE	
	28 Units / Kg.	24 Units / Kg.
	Death Latency, min.	Death Latency, min.
SACCHARIN GROUP	132, 155, 160, 168, 180, 190, 198, 209, 213, 215, 266, 267, 277, 281.	162, 191, 194, 197, 205, 213, 223, 263, 277, 334, 594.
WATER GROUP	161, 165, 186, 214, 219, 223, 248, 261, 267, 278, 381.	154, 218, 229, 234, 236, 265, 265, 267, 282, 329, 378, 440.

Appendix B - Figure 1




EXPERIMENT 5
COFFEE CONSUMPTION-60 MIN. POST INTUBATION
GROUP E
GROUP C
COFFEE CONSUMPTION, ML.
18
16
14
12
10
8
1 2 3 4 5 6 7 8 9 10
CONDITIONING DAYS
1 2 3 4
EXTINCTION DAYS

REFERENCES

Adolph, E. F. Regulation of water intake in relation to body water content. In Handbook of Physiology, Section 6, Alimentary Canal, Volume 1. Washington, D. C.: American Physiological Society, 1967.

Althausen, T. L., & Wever, G. K. The effect of saccharin and galactose on the blood-sugar. Proceedings of the Society for Experimental Biology and Medicine, 1937, 35, 517-519.

Alvarez-Buylla, R., Segura, E. T., & Alvarez-Buylla, E. R. Participation of the hypophysis in the conditioned reflex which reproduces the hypoglycemic effect of insulin. Acta Physiologica Latinoamericana, 1961, 11, 113-119.

Booth, D. A. & Miller, N. E. Lateral hypothalamus mediated effects of a food signal on blood glucose concentration. Physiology and Behavior, 1969, 4, 1003-1009.

Bradley, R. M. & Mistretta, C. M. Intravascular taste in rats as demonstrated by conditioned aversion to sodium saccharin. Journal of Comparative and Physiological Psychology, 1971, 75, 186-189.

Britton, S. W. Studies on the conditions of activity in endocrine glands: XVII. The nervous control of insulin secretion. American Journal of Physiology, 1925, 74, 291-308.

Bykov, K. M. Cerebral Cortex and Internal Organs. (W. H. Gantt, Tr.) New York: Chemical Publishing Co., 1957.

Bykov, K. M. Textbook of Physiology. Moscow: Foreign Languages Publishing House, 1960.

Carlson, A. J., Eldridge, C. J., Martin, H. P., & Foran, F. L. Studies on the physiological action of saccharin. Journal of Metabolic Research, 1923, 451-477.

Chisholm, D. J., Young, J. D., & Lazarus, L. The gastrointestinal stimulus to insulin release. I. Secretin. Journal of Clinical Investigation, 1969, 48, 1453-1460.

Daniel, P. M. & Henderson, J. R. The effect of vagal stimulation on plasma insulin and glucose levels in the baboon. Journal of Physiology, 1967, 192, 317-327.

Dubowski, K. M. An o-toluidine method for body fluid glucose determination. Clinical Chemistry, 1962, 8, 215-235.

Epstein, A. N. Water intake without the act of drinking. Science, 1960, 131, 497-498.

Epstein, A. N. & Teitelbaum, P. Specific loss of hypoglycemic control of feeding in recovered lateral rats. American Journal of Physiology, 1967, 213, 1159-1167.

Frohman, L. A., Bernardis, L. L., Schnatz, J. D., & Burek, L. Plasma insulin and trigliceride levels after hypothalamic lesions in weanling rats. American Journal of Physiology, 1969, 216, 1496.

Frohman, L. A., Ezdinli, E. Z., Javid, R. Effect of vagal stimulation on insulin secretion. Diabetes, 1966, 15, 522.

Garcia, J. & Ervin, F. R. Gustatory-visceral and telereceptor-cutaneous conditioning -- adaptation in internal and external milieus. Communications in Behavioral Biology, 1968, 1, 389-415.

Goldfine, I. D., Abraira, C., Gruenwald, D., & Goldstein, M. S. Plasma insulin levels during imaginary food ingestion under hypnosis. Proceedings of the Society for Experimental Biology and Medicine, 1970, 133, 274-276.

Goldfine, I. D., Ryan, W. G., & Schwartz, T. B. The effect of glucola, diet cola and water ingestion on blood glucose and plasma insulin. Proceedings of the Society for Experimental Biology and Medicine, 1969, 131, 329-330.

Jorgensen, H. The influence of saccharin on the blood-sugar. Acta Physiologica Scandinavia, 1950, 20, 33, 37.

Kaneto, A., Kosaka, K. & Nakao, K. Effects of stimulation of the vagus nerve on insulin secretion. Endocrinology, 1967, 80, 530-536.

Keele, C. A., & Neil, E. Samson Wright's Applied Physiology. London: Oxford University Press, 1965.

Kun, E., & Horvath, I. The influence of oral saccharin on blood sugar. Proceedings of the Society for Experimental Biology and Medicine, 1947, 66, 175-177.

Kuzuya, T. Regulation of insulin secretion by the central nervous system. II The role of the hypothalamus and the pituitary gland upon insulin secretion. Journal of the Japanese Society for Internal Medicine, 1962, 51, 65-74.

Le Magnen, J. Effets des administrations post-prandiales de glucose sur l'établissement des appetits. Compte rendu des séances du la societé de biologie, Paris, 1959, 153, 212-215.

Macallum, A. B. The potentiation of insulin by sulphones. Canadian Journal of Research, 1948, 26, 232-238.

Macallum & Sivertz, C. The potentiation of insulin by sulphones. Canadian Chemistry and Process Industries, 1942, 26, 569.

Mayer, J. Regulation of energy intake and the body weight: the glucostatic theory and the lipostatic hypothesis. Annals of the New York Academy of Sciences, 1955, 63, 15-43.

Mayer, J. Some aspects of the problems of regulation of food intake and obesity. New England Journal of Medicine, 1966, 274, 662-673.

Mayhew, D. A., Wright, P. H., & Ashmore, J. Regulation of insulin secretion. Pharmacological Reviews, 1969, 21, 183-212.

McCleary, R. A. Taste and post-ingestion factors in specific-hunger behavior. Journal of Comparative and Physiological Psychology, 1953, 46, 411-421.

Mityushov, M. I. The conditional-reflex incretion of insulin. Pavlovian Journal of Higher Nervous Activity, 1954, 4, 206-212. (Unofficial translation by G. F. Chorley, Department of Russian, McMaster University.)

Nicolaidis, S. Early systemic responses to orogastric stimulation in the regulation of food and water balance: functional and electrophysiological data. Annals of the New York Academy of Sciences, 1969, 157, 1176-1203.

Niijima, A. Afferent impulse discharges from glucoreceptors in the liver of the guinea pig. Annals of the New York Academy of Sciences, 1969, 157, 690-700.

Pavlov, I. P. The Work of the Digestive Glands. (W. H. Thomson, Tr.) London: Griffin, 1902.

Porte, D., Jr., & Bagdade, J. D. Human insulin secretion: an integrated approach. Annual Review of Medicine, 1970, 21, 219-240.

Remley, N. R. & Harrell, E. H. Biological effects of saccharin ingestion in rats: A preliminary report. Psychonomic Science, 1969, 17, 295-297.

Revusky, S. Effects of thirst level during consumption of flavored water on subsequent preference. Journal of Comparative and Physiological Psychology, 1968, 66, 777-779.

Revusky, S., & Garcia, J. Learned associations over long delays. In G. H. Bower & J. T. Spence (Eds.) Psychology of Learning and Motivation. New York: Academic Press, 1970.

Russek, M., Rodriguez-Zendeyas, A. M., & Pina, S. Hypothetical liver receptors and the anorexia caused by adrenaline and glucose. Physiology and Behavior, 1968, 3, 249-257.

Seligman, M. E. P. On the generality of the laws of learning. Psychological Review, 1970, 77, 406-418.

Scow, R. O., & Cornfield, J. Quantitative relations between oral and intravenous glucose tolerance curves. American Journal of Physiology, 1954, 179, 435-438.

Sharma, K. N., & Nassett, E. Electrical activity in mesenteric nerves after perfusion of gut lumen. American Journal of Physiology, 1962, 202, 725-730.

Shuford, E. H., Jr. Palatibility and osmotic pressure of glucose and sucrose solutions as determinants of intake. Journal of Comparative and Physiological Psychology, 1959, 52, 150-153.

Siegel, S. Latent inhibition and eyelid conditioning. In A. H. Black & W. F. Prokasy (Eds.), Classical Conditioning II. New York: Appleton-Century-Crofts, 1971.

Steffens, A. B. Rapid absorption of glucose in the intestinal tract of the rat after ingestion of a meal. Physiology and Behavior, 1969, 4, 829-832.

Syllaba, G. The influence of saccharin on the respiratory exchange and on the blood-sugar level - An example of the conditioned reflex Guy's Hospital Report, 1939, 80, 230-232.

Tepperman, J. Some aspects of the control of carbohydrate and fat metabolism. Paper presented at Pennsylvania State University Symposium, 1970.

Thompson, M. M. & Mayer, J. Hypoglycemic effects of saccharin in experimental animals. American Journal of Clinical Nutrition, 1959, 7, 80-85.

Valenstein, E. S., & Weber, M. L. Potentiation of insulin coma by saccharin. Journal of Comparative and Physiological Psychology, 1965, 60, 443-446.

Vallance-Owen, J., & Hurlock, B. Estimation of plasma-insulin by the rat diaphragm method. Lancet, 1954, 1, 68-70.

Yalow, R. S., & Berson, S. A. Immunoassay of endogenous plasma insulin in man. Journal of Clinical Investigation, 1960, 39, 1157-1175.

Young, P. T., & Madsen, C. H. Individual isohedons sucrose-sodium chloride and sucrose-saccharin gustatory areas. Journal of Comparative and Physiological Psychology, 1963, 56, 903-909.