ANALYSIS OF THE POSSIBLE ROLE
OF A KETONE BODY, ACETONE,
IN THE ADJUSTMENT OF CALORIC INTAKE

by
Larry Lynn Meliza

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SIGNED: [Signature]

APPROVAL BY THESIS DIRECTOR

This thesis has been approved on the date shown below:

[Signature]  [Signature]
SIGMUND HSIAO  SIGMUND HSIAO
Associate Professor of Psychology  Date

[Signature]
[Signature]
MAY 8, 1973  Date
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ABSTRACT

Four rats were trained to barpress for food reinforcement. After the rate of daily increase in barpressing had declined to the point where the difference between days was not significant, presentation of acetone solution was found to increase the rate of barpressing significantly.

Eighteen subjects were assigned to three groups on the basis of weight and received either (a) 0.2% acetone, (b) 0.1% acetone or (c) water. Food intake on the third day was significantly altered with an increase in acetone concentration being associated with a decrease in food intake.

Twelve subjects were randomly assigned to one of three groups and received either (a) a 0.5% acetone solution, (b) an amount of acetone by injection equal to that ingested by one of the subjects in the previous group or (c) water. The difference in food intake between these three groups was not significant. When the last day of treatment was compared with the first day of food and water ad libitum, the day and treatment x day effects were significant beyond the 0.10 level.

It is proposed that a substance or substances to which acetone may be transformed may act as a hypothalamic...
satiety signal or that ketosis may function as a negative reinforcer in controlling food intake.
INTRODUCTION

Adolph (1947) has shown that rats are able to adjust their food intake when their food has been diluted by calorically inert bulk (or when they are put upon deprivation schedules) in such a manner that their caloric intake remains relatively constant. That this adjustment of caloric intake is not dependent merely upon taste or stomach distension has been demonstrated by Epstein and Teitelbaum (1962) who found that rats adjust their rate of barpressing for foods of varying caloric density (placed directly into the stomach) such that caloric intake remained relatively constant.

Walike, Jordan and Stellar (1969) studied the intake of nutritive substances in humans after preloading with either nutritive substances or water. They found that in both instances later food intake was depressed, but the greatest depression was found with the preloading of the nutritive substance. Stomach distension does play a part in the adjustment of food intake, but humans are capable of adjusting their food intake after preloading with nutritive substances to an extent that cannot be explained on the basis of stomach distension alone.

There are many hypotheses about the signal that controls calorimetric feeding. When Janowitz and Hollander
(1955) prefed dogs, they found that their subjects came to adjust their caloric intake within an average of 4.4 weeks. This adjustment was preceded by a decrease in body weight. Ehrenfreund (1959) found a high correlation between weight loss in the deprivation interval immediately proceeding feeding and later food intake. Some change in blood chemistry may result as a function of weight loss to which hypothalamic feeding centers are sensitive.

Mayer (1955) proposed that the availability of glucose to cells may be the internal stimulus which controls short-term food intake and that a hypothesis presented by Kennedy may be able to explain the long-term adjustment of food intake. Kennedy (1952) proposed that lipids or some lipid metabolites were utilized in the long-term control of caloric intake.

When individuals are given injections of insulin, which produce hypoglycemia, food intake is increased (Grossman, 1955). In this instance, insulin is present so that glucose may pass into the cells, but sufficient glucose is lacking due to the removal of glucose from the blood by insulin. In the disease diabetes mellitus, a state of hyperglycemia exists. There is, in this case, sufficient blood glucose, but insulin is not present to allow the passage of glucose into cells. Individuals with diabetus mellitus are characteristically hyperphagic. Given these facts it
appears that neither glucose nor insulin alone is acting as a satiety signal.

Panksepp, Tonge and Oatley (1972) have found that rats given mannoheptulose, which produces acute diabetes, still have the capacity to decrease their food intake after preloads of glucose and amino acids and to increase their food intake after being given the nonoxidizable glucose analogue, 2-deoxy-D-glucose.

The results of studies involving the effects of changes in blood glucose and fatty acid levels upon food intake are confounded in that many other substances vary as a function of glucose and fatty acid levels. Critical to the present study is the finding of Start and Newsholme (1968) that force-feeding rats with glucose, fructose and casein solutions led to a decrease in blood ketone body levels. It may be that ketone bodies not only vary with blood sugar levels but are also more highly correlated with food intake.

When an organism has been fasting for a long period of time, gluconeogenesis occurs. In this process, the oxaloacetate required by the Krebs cycle is utilized in the formation of glucose. In this instance, the acetyl CoA produced by the oxidation of fatty acids, glycolysis and the breakdown of some of the amino acids is not able to combine with oxaloacetate to enter the Krebs cycle. As a result, two molecules of acetyl CoA may react to form a molecule of
acetoacetyl CoA. The acetoacetyl CoA passes out of the liver into the bloodstream where it is converted to acetoacetate. Acetoacetate may be hydrogenated to form Beta-hydroxybutyric acid or it may undergo decarboxylation to form acetone (Krebs, 1966), which is the ketone body to be utilized in the present study. The details of the reactions related to the formation of acetone are given in Figure 1.

Individuals with diabetes mellitus have high levels of blood ketone bodies and are characterized as having an abnormally high desire for food. In addition, kwashikior, a disease induced by diets low in proteins but relatively sufficient in carbohydrates, results in anorexia. This disease is characterized by increased fat deposition in the liver as well as an increase in glycogen storage. The result of these metabolic changes is that the individual is hypoglycemic. Under hypoglycemic conditions, increased ketone body formation would be expected except that in this instance the acetyl CoA required for their formation is being utilized in the formation of fatty acids. Kwashikior may then be characterized by higher than normal levels of ketone bodies (but levels below those found in the normal fasting animal). Ketone bodies are very sensitive to the state of food deprivation. These compounds, therefore, are likely candidates to signal states of food deprivation to the areas of the brain related to feeding.
Fig. 1. Metabolic Pathways Associated with Ketone Body Formation.
Any element in the blood which plays a role in the behavioral regulation of food intake must meet certain requirements. Primarily, this substance must be able to pass the blood-brain barrier in order to stimulate the areas related to the behavioral regulation of food intake. Ketone bodies can pass the blood-brain barrier since in fasting organisms, where the supply of glucose is limited, the brain begins oxidizing ketone bodies (Youngs and Scrimshaw, 1971).

A proposed mechanism for caloric adjustment must also account for the fact that this adjustment is not immediate. Hebb (1949) noted that food deprived rats would not increase their food intake on the first day after deprivation to an extent that would prevent further weight loss. Since with fasting there is an increase in oxaloacetate from the Krebs cycle, there would necessarily be a delay in the onset of ketosis which is related to the amount of stored glycogen.

A problem in the adjustment to deprivation schedules is the limit of adjustment. Dufort (1964) found that rats on a twenty-three hour deprivation schedule began adjusting at the end of eighteen days, while those on forty-seven and seventy-one hour deprivation schedules were unable to adjust.

A ketostatic mechanism for the adjustment of food intake may also be able to explain these results. If the increase in ketone bodies was limited to a certain level,
then adjustment to deprivation beyond that level would not be possible. The acetoacetyl CoA utilized in the formation of ketone bodies is also utilized in the formation of cholesterol and as a source of energy. As previously noted, Start and Newsholme showed that infusions of various nutritive substances led to a decrease in ketone body levels. The intake of food after a long period of fasting would be expected to decrease ketone body levels.

The purpose of this experiment was to observe the possible effects of an increase in concentration of one of the ketone bodies, acetone, upon food and water intake in rats.
METHOD

Subjects

The subjects were thirty-six Harlan Sprague-Dawley albino rats approximately seventy-five days old, with a mean body weight of 263 grams. Subjects were caged individually in a continuously lighted room. The foods used were Purina powdered lab chow and Noyes pellets. Food intake was measured to the nearest 0.10 grams. Liquids were presented in a 100 ml. graduated drinking tube mounted on each cage. Water intake was measured to the nearest ml. Two Skinner boxes were used in shaping and testing.

Procedure

Experiment I: In this experiment, barpressing was shaped in four subjects and their rate of response recorded after 23.75 hours of food deprivation each day for a period of 6 days. During the last 15 hours before testing on the sixth day, all subjects were presented with a 0.3% acetone solution in the drinking water.

Experiment II: In this experiment, 18 subjects were divided into three groups on the basis of weight. The members of each group were randomly assigned to one of the following treatments: (a) 0.2% acetone solution and food ad libitum, (b) 0.1% acetone solution and food ad libitum
or (c) water and food ad libitum. At the end of three days, the subjects' food and water intake were measured and the results analyzed using a randomized blocks design.

Experiment III: In this experiment, 12 subjects were used in a repeated measures design to test the possibility that acetone may have an effect over a period of time. Subjects were randomly divided into three groups and received treatments as follows. (a) Group I received a 0.5% acetone solution and food ad libitum. (b) Each subject in Group II received an amount of acetone by intraperitoneal injection equal to the amount taken by one of the subjects in Group I. This amount was adjusted for weight differences between the pairs with a 10% acetone solution being the material injected. (c) Group III received food and water ad libitum. Subjects were then shifted to food and water ad libitum at the end of four days. Food intake on the last day of treatment was then compared with that on the first day of ad libitum feeding.

Quantitative measurements of the blood ketone body concentrations were not made due to the lack of apparatus.
RESULTS

The subjects in Experiment I continued to increase their rate of barpressing after the intake of the acetone solution. Duncan's test was performed and it was found that the difference in means between days one and two was significant beyond the 0.10 level (Fig. 2). The difference between the means of the third, fourth and fifth days was not significant, but the mean of the sixth day was significantly different from the others beyond the 0.10 level. The mean intake of pure acetone by the subjects was 0.042 mls, as determined by multiplying the amount of intake by the concentration of the solution.

In Experiment II, the mean food intake for the high and low weight groups was greatest when water was presented and progressively smaller with 0.1% then 0.2% acetone. The food intake for the middle weight group was least when water was presented. The difference in mean food intake was not significant for the weight groups but was significant for the treatment and treatment x group interaction effects beyond the 0.05 level (Fig. 3).

The treatment effect, block effect and the interaction effect were all nonsignificant when mean water intake was compared for the various groups. Water intake tended to vary with food intake (Fig. 4).
Fig. 2. Mean Number of Barpresses for Four Subjects During 15 Minute Periods for 6 Consecutive Days. All Subjects were Presented with a 0.3% Acetone Solution for Drinking During the Last 15 Hours Preceding Testing on Day 6.
Fig. 3. Mean Food Intake in the Three Weight Groups after Presentation of either 0.0%, 0.1% or 0.2% Acetone in the Drinking Water.
Fig. 4. Mean Water Intake in the Three Weight Groups after Presentation of either 0.0%, 0.1% or 0.2% Acetone in the Drinking Water.
In Experiment III, all tests involving food and water intake were nonsignificant for the four days under treatment (Figs. 5 and 6). The effect of days and the interaction of days with treatment was significant beyond the 0.10 level when the last day of food intake during treatment was compared with the first day under water ad libitum (Fig. 7). Duncan's test showed that both the low and high weight groups given acetone injections were significantly different from the low weight group given water, the high weight group given water and the medium weight group given acetone solution.
Fig. 5. Mean Food Intake for Groups Receiving either Water, 0.5% Acetone Solution or Acetone Injection over a Period of Four Days.
Fig. 6. Mean Water Intake for Groups Receiving either Water, 0.5% Acetone Solution or Acetone Injection over a Period of Four Days.
Fig. 7. Mean Food Intake for Each of Three Groups Receiving Either Water, 0.5% Acetone Solution or Acetone Injection Compared with Mean Food Intake for the Same Groups Given Water and Food Ad Libitum on the Following Day.
DISCUSSION

The results of the barpressing experiment tend to support the proposal that blood acetone level is functionally related to hunger and thus to caloric intake. The response rate curve is at first positively accelerating and then changes to a negatively accelerating curve as would be expected to occur as a function of practice. The change after treatment to a positively accelerating curve would be expected with an increase in the amount of deprivation.

Those experiments involving the direct measure of food and water intake tended to show a decrease in food and water intake as a function of increasing amounts of acetone. Verplanck and Hayes (1953) have found that food intake decreases to about 60% of normal when a rat is water deprived. If the acetone solutions were less palatable than water it would be assumed that a decreased acetone solution intake would be expected, resulting in decreased food intake. Since a decrease in food intake was found with both acetone injections and acetone presented in the drinking water, this appears to rule out any palatability effects.

The apparent paradox in these two types of studies may be resolved in the satiety signal system is more complex than previously assumed. If higher than normal levels of ketone bodies always resulted in a stimulus to eating, then
it would not be to the advantage of the organism. Fats, which have the highest caloric value of the three major food substances, 9.0 kcals./gm. compared with 3.8 kcals./gm. for carbohydrates and 6.0 kcals./gm. for protein, also lead to above average ketone body levels in individuals on high fat diets.

The apparent paradox may be due to experimental error. In the barpressing experiment, the subjects were still increasing their intake daily. Subjects not adjusted to this schedule were in fact being partially deprived. Noyes pellets weigh 0.05 grams and have a caloric value of 4.34 kcals./gm., while Purina Lab Chow has a caloric value of 4.4 kcals./gm. (Valle, 1968). The maximum number of reinforcements during any session was less than 200. This means that the subjects were receiving less than 10 grams a day, which is far below their normal intake. Thus the increase in barpressing may be due to the decrease of some satiety signal.

This brings up an additional problem. As already noted, in the study by Dufort it was found that eighteen days were required for rats to begin adapting to a twenty-three hour deprivation schedule. In the present instance, rats were found to begin adapting after only eight days in the Skinner Box. Although it has already been stated that rats will increase their rate of barpressing when the hours
of food deprivation are increased this is not exactly comparable to the present situation.

The normal comparison of different levels of deprivation, such as that by Yamaguchi (1952), is one in which the rate of some response correlated with hunger is compared under two or more levels of deprivation. The cue utilized by the organism in this instance may be a change in the level of a short-term satiety signal, stomach contractions or other gastric cues. In the present experiment it was not the hours of deprivation that were being changed and thus temporal factors such as the length of time since the last meal were being held constant. The present experiment is then more comparable to the Dufort experiment, and the possibility that the level of either a long-term or short-term chemical satiety signal was changed by the increase in acetone becomes very likely.

Since the results were not as great as to be expected if a satiety signal were directly changed, it cannot be concluded here that acetone itself may be acting as a satiety signal. Another candidate which especially warrants study is the ketone body Beta-hydroxybutyrate.

In one study using obese subjects, fasting for five to six weeks led to a state in which fifty-two percent of the brain oxygen consumption was accounted for by the oxidation of Beta-hydroxybutyrate (Owen et al., 1967). In the
normal state, the oxidation by the brain of Beta-hydroxybutyrate is almost negligible. Smith, Satterthwaite and Sokoloff (1969) have found in fasting rats that within seventy-two hours the level of Beta-hydroxybutyrate increased to five times its normal amount. The enzyme Beta-hydroxybutyrate reductase, which is required for this ketone body to be oxidized also increases greatly at the same time.

Other candidates for the satiety signal are to be found in the Krebs cycle. The acetone given to the subjects, if converted to acetyl CoA, could have been utilized by the Krebs cycle. This increase in acetyl CoA could then cause the induction of the synthesis of more of the enzymes found in the Krebs cycle leading to an increase in the amount of each of the metabolites found in the cycle.

The number of possible agents acting as a satiety signal has been greatly reduced by this study since the observed changes in eating behavior can be assumed to be relatively independent of the intermediates found in glycolysis.

The theoretical model which seems best able to explain the results of the present experiment as well as those of the experiments previously discussed is as follows.

Extremely high levels of ketone bodies are found in states wherein the Krebs cycle is in some way impaired in its functioning. In this case, there is a decrease in the
metabolites of the Krebs cycle and an increase in acetyl CoA. The behavioral result is an increase in food intake as is found in diabetes mellitus, prolonged fasting and with injections or infusions of 2-deoxyglucose.

High levels of ketone bodies are found with diets high in fat and thus high in caloric value. In this situation there is an increase in acetyl CoA and an increase of the metabolites of the Krebs cycle.

The level of the metabolites in the Krebs cycle are a more adequate predictor of food intake than glucose levels, insulin levels or fatty acid levels since the level of the metabolites in the Krebs cycle tend to vary with food intake in the predicted direction. Since it is unreasonable to use the actual levels of the intermediates to predict later food intake because of their location in the cell, it is necessary to find a more practical predictor of food intake. Due to the fact that the ketone bodies are so close to the Krebs cycle and that their level is controlled by the amount of glucose, fatty acids, proteins and insulin they appear to be adequate. The use of ketone body level as a predictor of food intake would naturally require correlation studies.

The effects of infusions of ketone bodies upon other biochemicals in the blood, are dependent upon the state of the organism when the ketone bodies are infused. Infusion of ketone bodies into hyperglycemic dogs, leads
to increased hyperglycemia, while infusion of ketone bodies into hypoglycemic animals leads to increased hypoglycemia. This effect is assumed to be insulin dependent (Felts, Crofford and Park, 1964). Results from the present experiment suggest that it would be worthwhile to study the effects of infused ketone bodies in diabetic animals.

The study of the effects of high concentrations of acetone and other ketone bodies upon food intake was necessary and in time will probably be followed by additional studies of the behavioral effects of abnormal concentrations of ketone bodies. The reason for this statement lies in the previously noted relationship between ketone bodies and cholesterol. Cholesterol is a major health problem because of its role in atherosclerosis. At the present time the feedback mechanism which controls the synthesis of cholesterol is unknown. It is known that cholesterol is utilized in the formation of many steroids such as the sex hormones and the bile acids. One means of controlling cholesterol is to increase its rate of utilization in the form of bile acids (Van Belle, 1960). An increase in the level of these products may lead to undesirable situations. If the mechanism which controls the point in the metabolic pathway where it is determined whether acetoacetate or cholesterol is formed from acetoacetyl CoA can be found, then it may be possible to increase the amount of ketone bodies formed at the expense of cholesterol formation (Fig. 8).
Fig. 8. Relationship between Ketone Body Formation and Cholesterol Biosynthesis.
A second interpretation of the results of this experiment may be found in a paper by David A. Booth (1972). Booth found that the hyperphagia of rats with streptozotocin-induced diabetes eat smaller meals than control animals, but they eat more frequently so that the total daily food intake is greater than for controls. Rats with VMH lesions eat the normal number of meals, but the size of their meals is greater than for control animals. Booth has suggested that diabetic rats may learn to overeat in order to avoid some aversive situation such as ketosis.
LIST OF REFERENCES


