DIETARY SUGARS IN HEALTH AND DISEASE

IV. MANNITOL

August 1979

Prepared for

BUREAU OF FOODS
FOOD AND DRUG ADMINISTRATION
DÉPARTEMENT OF HEALTH, EDUCATION, AND WELFARE
WASHINGTON, D.C. 20204

under

Contract Number FDA 223-75-2090
DIETARY SUGARS IN HEALTH AND DISEASE

IV. MANNITOL

August 1979

Prepared for

BUREAU OF FOODS
FOOD AND DRUG ADMINISTRATION
DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE
WASHINGTON, D.C. 20204

under

Contract Number FDA 223-75-2090

by

Richard G. Allison, Ph.D.

LIFE SCIENCES RESEARCH OFFICE
FEDERATION OF AMERICAN SOCIETIES
FOR EXPERIMENTAL BIOLOGY
9650 Rockville Pike
Bethesda, Maryland 20014
FOREWORD

The Life Sciences Research Office (LSRO), Federation of American Societies for Experimental Biology (FASEB), provides scientific assessments of topics in the biomedical sciences. Reports are based upon comprehensive literature reviews and the scientific opinions of knowledgeable investigators engaged in work in specific areas of biology and medicine.

This technical report was prepared for the Bureau of Foods, Food and Drug Administration (FDA), by Richard G. Allison, Ph.D., Staff Scientist, LSRO, FASEB, in accordance with the provisions of Contract No. FDA 223-75-2090.

The LSRO acknowledges the contributions of the investigators who provided data and the consultants who reviewed the draft of the report. However, the listing of consultants' names in Section VII does not imply that they endorse the conclusions of the study. The LSRO accepts the responsibility for the report and the opinions expressed.

The report was reviewed and approved by the LSRO Advisory Committee (representatives of each constituent society of FASEB) under authority delegated by the Executive Committee of the Federation Board. Upon completion of these review procedures, the report was approved and transmitted to FDA by the Executive Director, FASEB.

While this is a report of the Federation of American Societies for Experimental Biology, it does not necessarily reflect the opinion of each individual member of the FASEB constituent societies.

Kenneth D. Fisher, Ph.D.
Director
Life Sciences Research Office
SUMMARY

This report on mannitol reviews technical and scientific data in relation to the health aspects of using mannitol as a food ingredient. Mannitol is a sweet-tasting hexahydric alcohol occurring naturally in brown seaweed, fungi, pumpkins, onions, and certain plant exudates, as well as in animal tissues and fluids in low concentrations. Utilization of mannitol as a nutritive sweetener, formulating aid, and for other food ingredient purposes is limited because it is about one half as sweet as sucrose and because it may cause laxation. Oral doses of 10 to 20 g of crystalline mannitol may cause laxation in adult human beings; greater amounts of mannitol can be ingested in dilute solution without laxation. In the U.S., the per capita daily consumption of mannitol as a food ingredient is about 30 mg.

The absorption and metabolism of mannitol have been studied with isolated mannitol rather than as a normal dietary component. Mannitol is absorbed by passive diffusion from the upper portion of the small intestine. Small quantities of mannitol in isotonic solution are more completely absorbed than are larger quantities presented as crystalline mannitol or in hypertonic solutions. After absorption, mannitol enters hepatic cells where it can be metabolized and contribute to the formation of glucose and glycogen. The initial step in metabolism is oxidation to fructose; however, about 20 percent of ingested mannitol passes through the liver without being metabolized and is excreted unchanged in the urine. The caloric value of mannitol is estimated to be between 2 and 2.7 kcal per g because of incomplete absorption and metabolism.

Animal feeding studies designed to test cariogenicity and measurements of acid production in human dental plaque after exposure to mannitol indicate that mannitol is less cariogenic than dextrin, glucose, or sucrose. Evidence from animal and in vitro studies indicates that mannitol is not mutagenic, teratogenic, or carcinogenic. An apparent mannitol-related occurrence of benign thymic tumors in female Wistar rats in one long-term feeding study remains unexplained. The finding was not confirmed when mannitol was retested in a long-term feeding study with female Wistar, Fischer, and Sprague-Dawley rats. The potential for changes in body fluid volume and gastrointestinal disturbances associated with chronic administration of an osmotically active substance, such as mannitol, may impose limits on the dietary level that can be tested meaningfully in experimental animals. While osmotic effects have been demonstrated experimentally, there is no evidence that they are relevant to the safety of mannitol as currently used as a food ingredient.
# TABLE OF CONTENTS

<table>
<thead>
<tr>
<th>Section</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>Foreword</td>
<td>iii</td>
</tr>
<tr>
<td>Summary</td>
<td>v</td>
</tr>
<tr>
<td>I. Introduction</td>
<td>1</td>
</tr>
<tr>
<td>A. Background</td>
<td>1</td>
</tr>
<tr>
<td>B. Scope</td>
<td>2</td>
</tr>
<tr>
<td>II. Properties, Occurrence, and Food Use</td>
<td>3</td>
</tr>
<tr>
<td>III. Absorption and Metabolism</td>
<td>5</td>
</tr>
<tr>
<td>A. Absorption</td>
<td>5</td>
</tr>
<tr>
<td>B. Metabolism</td>
<td>6</td>
</tr>
<tr>
<td>1. Endogenous mannitol</td>
<td>7</td>
</tr>
<tr>
<td>2. Exogenous mannitol</td>
<td>10</td>
</tr>
<tr>
<td>IV. Safety Evaluation</td>
<td>13</td>
</tr>
<tr>
<td>A. In Vitro and Special Tests</td>
<td>13</td>
</tr>
<tr>
<td>B. Animal Studies</td>
<td>14</td>
</tr>
<tr>
<td>1. Acute toxicity</td>
<td>14</td>
</tr>
<tr>
<td>2. Teratogenicity</td>
<td>14</td>
</tr>
<tr>
<td>3. Short-term feeding</td>
<td>14</td>
</tr>
<tr>
<td>4. Cariogenicity</td>
<td>15</td>
</tr>
<tr>
<td>5. Long-term feeding</td>
<td>16</td>
</tr>
<tr>
<td>C. Human Studies</td>
<td>17</td>
</tr>
<tr>
<td>1. Laxation</td>
<td>17</td>
</tr>
<tr>
<td>2. Cariogenicity</td>
<td>18</td>
</tr>
<tr>
<td>V. Conclusions</td>
<td>21</td>
</tr>
<tr>
<td>VI. Literature Cited</td>
<td>23</td>
</tr>
<tr>
<td>VII. Study Participants</td>
<td>31</td>
</tr>
</tbody>
</table>

vii
I. INTRODUCTION

A. BACKGROUND

The Bureau of Foods, Food and Drug Administration (FDA), has a continuing interest in and responsibility for the nutritional quality of the United States dietary. The Bureau evaluates and monitors the safety of foods, establishes regulations, and provides nutrition information to consumers.

In keeping with these responsibilities, the FDA requested that the Life Sciences Research Office (LSRO), Federation of American Societies for Experimental Biology (FASEB), review current scientific information and technologic developments related to the use of mannitol in foods. This report is the fourth in a series on dietary sugars in health and disease prepared for FDA by the LSRO. Previous reports considered fructose (Kimura and Carr, 1976), xylitol (LSRO, 1978), and sorbitol (Allison, 1979) as food ingredients.

As part of a comprehensive review by FDA, a report was prepared in 1972 by the Select Committee on GRAS Substances evaluating the health aspects of mannitol as a food ingredient (Select Committee on GRAS Substances, 1972). On the basis of this report and other relevant material, the Commissioner of FDA proposed that mannitol be affirmed as generally recognized as safe (GRAS) for use as a direct human food ingredient (Office of the Federal Register, 1973). This was followed by an interim food additive order because preliminary information on a lifetime feeding study in rats raised questions about the safety of mannitol (Office of the Federal Register, 1974; Sunshine, 1973). The use of mannitol in food for human consumption is currently permitted by the Code of Federal Regulations [21 CFR 180.25] (Office of the Federal Register, 1978) on an interim basis pending additional study. The final reports of this study (Saatman, 1978) and an additional lifetime feeding study (Gongwer, 1978) in three strains of female rats have been submitted to the FDA (Sunshine, 1978).

After removal of cyclamates from the GRAS list, FDA permitted the combination of nutritive and nonnutritive sweeteners in "diet beverages" (Office of the Federal Register, 1970). That regulation [21 CFR 100.130] considers the possibility "... of injury through the inadvertent use by diabetics in the belief that the product does not contain carbohydrates ..." and requires the label of a beverage containing sorbitol, mannitol or other hexitol to bear the statement: "Contains carbohydrates, not for use by diabetics without advice of a physician." The labels of beverages containing hexitols are not permitted to bear statements such as "sugar free," "sugarless," "no sugar," or similar statements.
The Code of Federal Regulations [21 CFR 180.25] gives guidelines for the use of mannitol as a food ingredient including: the categories of foods to which it may be added; the physical or technical functional effects for which it may be added; and the levels of addition that reflect good manufacturing practice. As currently regulated, the levels are not to exceed 98 percent in pressed mints and 5 percent in all other hard candy and cough drops, 31 percent in chewing gum, 40 percent in soft candy, 8 percent in confections and frostings, and 15 percent in commercial nonstandard jams and jellies, and must be less than 2.5 percent in all other foods. The statement "excess consumption may have a laxative effect" must appear on the label of food whose reasonably foreseeable consumption may result in a daily ingestion of 20 g of mannitol (Office of the Federal Register, 1978).

B. SCOPE

This report reviews technical and scientific data on mannitol as a food ingredient and on its health effects when used in foods. The focus is on selected references and recent research on safety, metabolic, and pharmacokinetic aspects of mannitol administered orally to man and experimental animals. Sources of information include reviews of the health aspects of using mannitol in foods by the Joint FAO/WHO Expert Committee on Food Additives (1967; 1976) and by the Select Committee on GRAS Substances (1972); recent reports related to polyol and carbohydrate metabolism (Allison, 1979; Fisher et al., 1977; Kimura, 1977; LSRO, 1978; Talbot, 1978); and the computerized literature citation retrieval systems of the National Library of Medicine. Additional literature references, data, and opinions were supplied by consultants.
II. PROPERTIES, OCCURRENCE, AND FOOD USE

D-Mannitol, or mannite, C$_{6}$H$_{14}$O$_{6}$, is the chemical 1,2,3,4,5,6-hexahexehexol (CAS Registry No. 69-65-8). Crystalline mannitol occurs as odorless, white, orthorhombic needles or as a crystalline powder; a small quantity of sorbitol may be present. Mannitol is soluble in water (1 g per 5.5 ml) and ethanol (1 g per 83 ml), but insoluble in various organic solutions. Water solutions of mannitol may be very slightly levorotatory or optically inactive. Solutions of 1 to 3 percent of mannitol are about one half as sweet as similar sucrose solutions (Moskowitz, 1974). Specifications for food grade mannitol are given in the Food Chemicals Codex (National Research Council, 1972). This sweet-tasting hexahydric alcohol occurs in brown seaweed, fungi, pumpkins, onions, and certain plant exudates, as well as in animal tissues and fluids in low concentrations (Pitkänen and Servo, 1973; Robinson, 1975; Wright, 1974). From 1937 to 1948 mannitol was produced commercially by batch electrochemical reduction of glucose. Since that time it has been produced commercially by catalytic hydrogenation of glucose or invert sugar (obtained from inversion of sucrose). Wright (1974) has reviewed the chemistry of the commercial production of sorbitol and mannitol.

Mannitol is used with boric acid in the manufacture of electrolytic condensers, in production of artificial resins and plasticizers, as a reagent for boron determinations, and as a technical formulation agent in food and drugs (Merck Index, 1976). A number of chewable medicaments and drug formulations contain mannitol as a filler.

Mannitol is added to foods for a variety of purposes, principally as a formulating aid in candies, chewing gum, and other items where its sweet taste is useful (Subcommittee on Review of the GRAS List--Phase II, 1972). However, the extensive use of mannitol as a nutritive sweetener is not considered practical because as little as 10 or 20 g of crystalline mannitol can cause diarrhea in adults (Brunzell, 1978; Ellis and Krantz, 1941; Gallagher and Pearce, 1977). The amount of mannitol used as a food ingredient by U.S. food processors in 1970 and 1975 was surveyed by the National Research Council (Committee on GRAS List Survey--Phase III, 1978). The estimated usage for 1970 was between 0.8 and 1.3 million kg. By 1975, usage had nearly doubled representing, at that time, a daily per capita consumption of approximately 20 to 30 mg of mannitol.
III. ABSORPTION AND METABOLISM

A. ABSORPTION

Mannitol is absorbed by passive diffusion from the upper portion of the small intestine (Fordtran et al., 1965; Launiala, 1968; Ruttolff and Ketz, 1961). In crystalline form or as a hypertonic solution, mannitol administered orally may cause osmotic disturbances in the gastrointestinal tract, producing laxative effects as well as decreasing its absorption. For instance, monkeys did not synthesize glycogen when given crystalline mannitol by stomach tube at a dosage of 8 g per kg body weight (Ellis and Krantz, 1941). Similarly, no glycogen was formed by fasted rats given crystalline mannitol (Carr and Krantz, 1938) or a 20-percent solution of mannitol by stomach tube (Todd et al., 1939). Field (1919) reported an increase of 10 mg per dl in the blood glucose level of men who ingested 100 g of mannitol with water (volume not identified). Doses of 10 to 20 g of crystalline mannitol produced diarrhea in the men studied by Ellis and Krantz (1941). However, Nasrallah and Iber (1969) demonstrated absorption of about 65 percent of the mannitol from 5-percent solutions containing 28 to 100 g of mannitol orally administered to 10 human subjects.

During a 30-minute period the quantity of sorbitol or mannitol absorbed from 2.5-percent solutions injected into a ligated segment of small intestine of female Wistar rats approximated the quantity of fructose absorbed under similar experimental conditions (Ruttolff and Ketz, 1961). The mean absorption rates compared to glucose (100 percent) were 41, 36, and 45 percent for fructose, sorbitol, and mannitol respectively. Although not studied experimentally, it is generally believed that the absorption rate of mannitol is less than that of fructose when each is ingested in a mixed meal rather than intubated or ingested in relatively concentrated forms under experimental conditions (Macdonald et al., 1978).

Dietary mannitol (5.1, 9.9, and 18.0 percent of diet) affected the meal patterns of adult male Wistar rats when 45 ml of a 0.2, 0.4, or 0.8 M mannitol solution was added to 30 g of diet (Bernstein and Vitiello, 1978). The rats ate about 10 g of these diets per meal. Mannitol, regardless of the dietary concentration, increased the elapsed time before the start of the next meal, i.e., postmeal interval. The postmeal interval increased from 70 minutes to 100 minutes with 0.4 M mannitol and 125 minutes with 0.8 M mannitol. The extended postmeal interval was apparently not associated with dehydration or discomfort. Compared with controls, test animals consumed more water during a 3-hour and a 24-hour period after test meals; however, water intake did not differ among the mannitol-consuming groups. An examination of the stomachs and small intestines of controls and rats consuming a meal containing added 0.8 M mannitol indicated that 75 minutes
after mannitol consumption, water and possibly food had accumulated in the upper small intestine but not in the lower intestine or stomach. The authors concluded that dietary mannitol had contributed to intestinal distension which delayed consumption of the next meal under these experimental conditions. Other investigators (Davis and Collins, 1978; Davis et al., 1975) also observed that the osmotic effects of dietary mannitol when added at levels from 0.05 to 0.2 M in a liquid diet of 0.006 M saccharin and 0.1 M glucose produced intestinal distension and altered feeding patterns over a 4-hour test period.

Fordtran et al. (1965) intubated hypertonic (550-800 mOsm per kg) mannitol solutions at a rate of 9 ml per minute into the small intestine of human subjects who had fasted for 8 hours. Samples collected 20 cm distal to the infusion site were analyzed for mannitol by the method of Corcoran and Page (1947). The absorption of mannitol was not detected from segments of jejunum (10 studies) or ileum (6 studies). The movement of water into the intestinal lumen in response to the hypertonic mannitol solutions decreased from 0.044 ml per minute per mOsm gradient near the ligament of Treitz to a value of 0.005 in the lower ileum. The investigators cautioned that the absorption of water secondary to active solute transport, particularly of glucose, can make a significant contribution to the absorption of solutes.

Launiala (1968) infused (3 ml per minute) isotonic solutions containing mannitol and glucose into the proximal jejunum of children, 8 months to 4 years old. The concentration of mannitol was varied from 50 to 150 mM and sufficient glucose was added to each infusion mixture to provide 200 millimoles of nonelectrolyte solute per litre. A 60-minute infusion period preceded the test periods of about 30 to 60 minutes. Most of the glucose was absorbed from the 50-cm test segment of the small intestine while only 9 to 18 percent of the mannitol was absorbed. Mannitol solutions of 100 mM or 150 mM caused a net secretion of water into the gut. The mean transit time decreased with increasing concentrations of mannitol from 28 minutes with no mannitol to 16.5 minutes at 150 mM mannitol.

B. METABOLISM

The available data support the generally held concept that the liver is the primary site of mannitol metabolism; however, the enzymes and metabolic pathways are not well characterized for animals and humans. Early attempts to demonstrate gluconeogenesis from mannitol in rat kidney slices were unsuccessful as were trials with extracts of rat liver tissue containing sorbitol dehydrogenase (Blakley, 1951; Krebs and Lund, 1966). Sorbitol dehydrogenase, which has been isolated from many different animal tissues, has been shown to have slight activity towards mannitol (Dills and Meyer, 1976; Rehg and Torack, 1977; Touster and Shaw, 1962). In addition, at least two laboratories
report the existence of enzymes, one in liver and one in red blood cells, that will oxidize mannitol to fructose in human beings. An NAD-linked polyol oxidoreductase capable of oxidizing D-mannitol to a ketosugar, probably D-fructose, was detected in blood serum after the liver was damaged in a case of acute hepatitis (Pitkänen and Servo, 1971). In the other report (Wang and van Eys, 1970), an NAD-linked enzymic activity was found in red blood cells of patients with essential pentosuria (a defect in NADP-linked xylitol dehydrogenase) as well as in normal controls.

The metabolism of mannitol in the liver possibly reflects the fact that liver cells are freely permeable to mannitol (Cahill et al., 1958) while other cells such as erythrocytes are impermeable (LeFeure and Davies, 1951). Consequently, polyol dehydrogenases in tissues other than the liver probably metabolize endogenous rather than exogenous mannitol. This represents a situation parallel to that for sorbitol; only hepatic sorbitol dehydrogenase is believed to make a significant contribution to the metabolism of exogenous polyol (Allison, 1979). These reports and the data reviewed below suggest that in animals an hepatic enzyme with the characteristics of mannitol dehydrogenase (E.C. 1.1.1.67), an enzyme which has been well characterized in bacterial systems, may catalyze the oxidation of dietary mannitol to fructose (Figure 1). However, the evidence as to the identity of the polyol dehydrogenase principally responsible for the hepatic metabolism of mannitol is inconclusive.

1. **Endogenous mannitol.**

Mannitol is synthesized in vivo by humans. Mean levels of mannitol in cerebrospinal fluid (CSF) and urine of normal individuals have been measured as 4.8 ± 2.6 (SD) and 65.1 ± 22.9 μmol per litre, respectively (Pitkänen and Servo, 1973). In diabetic patients, these levels tended to be higher, but only urinary mannitol reached a level, 138.7 ± 81.5 μmol per litre, statistically different from the normal mean of 65.1 ± 22.9 μmol per litre. The investigators did not speculate on the metabolic source of the urinary mannitol. However, the work of Soummer-Dauphant et al. (1976) suggests that urinary mannitol, detected in patients receiving sorbitol as part of a parenteral nutrition regimen, may result from a secondary catabolism of fructose, which was produced in excessive quantities by the metabolism of sorbitol.

Mannitol concentration in CSF of 27 healthy individuals ranged from 3 to 9 μmol per litre, about one fourth of the sorbitol concentrations of 14 to 38 mol per litre in the same subjects (Servo et al., 1977). Mannitol levels up to 20 or 25 μmol per litre of CSF were measured in patients with meningitis or other neurological disorders. Although no clinical significance was attached to these higher levels of mannitol in CSF, the investigators noted that the total polyol concentration may have clinical significance. It was proposed that the seven polyols
FIGURE 1. Schematic diagram of hepatic metabolism of mannitol, sorbitol, and related carbohydrates.

Reaction number, enzyme nomenclature, and Enzyme Commission* number: 1, aldose reductase, (1.1.1.21); 2, glucokinase, (2.7.1.2); 2', glucose-6-phosphatase, (3.1.3.9); 3, glucosephosphate isomerase, (5.3.1.9); 4, 6-phosphofructokinase, (2.7.1.11); 4', fructose-bisphosphatase, (3.1.3.11); 5, fructose-bisphosphate aldolase, (4.1.2.13); 6, triosephosphate isomerase, (5.3.1.1); 7, L-iditol dehydrogenase (sorbitol dehydrogenase), (1.1.1.14); 8, ketoheoxokinase, (2.7.1.3); 9, fructose-bisphosphate aldolase, (4.1.2.13); 10, triokinase, (2.7.1.28); 11, aldehyde dehydrogenase (1.2.1.3); 12, glycerate kinase, (2.7.1.31); 13, glycerol dehydrogenase (NADP), (1.1.1.72), or alcohol dehydrogenase (NAD), (1.1.1.1); 14, lactate dehydrogenase, (1.1.1.27); 15, mannitol dehydrogenase, (1.1.1.67).

Abbreviations: NAD = nicotinamide adenine dinucleotide; NADH = reduced NAD; NADP = NAD phosphate; NADPH = reduced NADP; ATP = adenosine triphosphate; ADP = adenosine diphosphate;

— — — ➔ indicates multistep pathway.

identified in CSF (anhydroglucitol, arabinitol, myoinositol, erythritol, sorbitol, mannitol, and possibly ribitol) originated from the brain and spinal cord rather than plasma. Only traces (<10 μmol per litre) of mannitol and sorbitol were seen occasionally in plasma samples from any of the 74 individuals examined.

Under conditions of chronic renal failure and in the final stages of renal insufficiency, the concentration of mannitol in plasma may reach relatively high levels (Pitkänen et al., 1976). In 10 uremic patients on conservative treatment and not receiving mannitol-containing drugs, the plasma mannitol concentration was elevated [105 ± 120 (SEM) μmol per litre] while plasma sorbitol remained low or undetectable. One chronic uremic patient with glomerulonephritis exhibited a plasma mannitol concentration of 1270 μmol per litre. Mannitol content of red cells and CSF increased in a parallel manner in these patients. There is no evidence that dietary mannitol would pass from plasma to CSF or enter red blood cells of healthy individuals.

2. Exogenous mannitol.

Suggestions in the scientific literature that mannitol is neither absorbed from the gastrointestinal tract nor metabolized by humans and animals have been attributed by Ginn (1974) to difficulties of mannitol analysis using the method of Corcoran and Page (1947). Studies by Nasrallah and Iber (1969) with humans and by Wick et al. (1954) with rats indicate that in both species ingested [14C]mannitol is absorbed and oxidized to varying degrees depending on the amount and method of administration. Intestinal microflora may convert mannitol to more readily absorbed and utilized compounds. Early studies (Carr and Krantz, 1938; Carr et al., 1933; Field, 1919; Todd et al., 1939) demonstrated qualitatively the formation of blood glucose or hepatic glycogen from mannitol ingested by experimental animals and human beings. Feeding studies in rats also suggested that mannitol was inferior to glucose or starch as an energy source (Ariyama and Takahashi, 1929; Ellis and Krantz, 1941).

Nasrallah and Iber (1969) administered 28 to 100 g of [U-14C]mannitol orally as a 5-percent aqueous solution to 10 patients who had fasted overnight. Within this dose range, about 20 percent of the mannitol ingested was excreted unchanged in the urine, indicating appreciable absorption. The level of radioactivity in the blood rose for the first 2 hours and remained at a plateau for 2 to 4 hours; the radioactive compounds present in blood were not identified and data on blood glucose levels were not reported. The specific activity of expired CO2 increased for 8 hours after mannitol ingestion; however, intravenously administered [U-14C]mannitol produced very little radioactive CO2. Oral doses of 40 g or more generally caused frequent bowel movements, diarrhea, and excretion in the stool of a higher percentage of the dose. Only traces of radioactivity occurred in the urine and stools after 48 hours. Nasrallah and Iber (1969) concluded
that within an oral dose range of 40 to 100 g, approximately 65 percent of ingested mannitol was absorbed; and about one third of the absorbed mannitol was excreted in the urine, the remainder being metabolized presumably in the liver. Based on this study, the caloric value of dietary mannitol is considered to be about 2 kcal per g (Dwivedi, 1977).

Wick et al. (1954) provided evidence for the hepatic metabolism of mannitol by administering mannitol to rats orally, intravenously, and intrasplenically (into portal venous system). Oral administration and intrasplenic administration of mannitol resulted in 50- and 70-percent metabolism to CO₂, respectively. In contrast, after intravenous administration, only 2 or 3 percent of mannitol was recovered as CO₂ with most being excreted in the urine. Mannitol is not reabsorbed by the kidneys but excreted at the full glomerular filtration rate (Ginn, 1974; Smith et al., 1940; Wick et al., 1954). Thus mannitol has not been considered as a source of metabolizable carbohydrate for intravenous hyperalimentation. Therapeutic applications that utilize the osmotic effects of parenterally administered mannitol are beyond the scope of this report.

A distinguishing feature between orally administered doses of mannitol and sorbitol is the extent of their ultimate conversion under experimental conditions to other metabolic products by the liver. Virtually all absorbed sorbitol is removed from the circulatory system by the liver, while about one third to one half of absorbed mannitol bypasses liver metabolism and can therefore be excreted in the urine (Macdonald et al., 1978; Nasrallah and Iber, 1969; Ruttlloff and Ketz, 1961; Wick et al., 1954).

In summary, neither the absorption nor the subsequent metabolism of mannitol as part of a normal mixed diet has been studied. However, it is reasonable to expect the rate and extent of absorption of small quantities of mannitol from a mixed diet to differ from those observed from hypertonic test solutions studied clinically or in experimental animals.
IV. SAFETY EVALUATION

A 1972 review of available information on the health aspects of mannitol as a food ingredient led the Select Committee on GRAS Substances (1972) to conclude that there was no evidence of possible hazard to the public. Similarly, in an earlier review of the safety of mannitol as a food ingredient, the Joint FAO/WHO Expert Committee on Food Additives (1967) established an ADI (acceptable daily intake) for mannitol of 0 to 50 mg per kg as "unconditional" and 50 to 150 mg per kg as "conditional." However, after preliminary results of a long-term feeding study (Sunshine, 1973) with Wistar rats indicated the possibility of adverse effects related to mannitol ingestion by female rats, the ADI was revised to a "temporary" level of 0 to 50 mg per kg (Joint FAO/WHO Expert Committee on Food Additives, 1974, 1976). Several long-term feeding studies, cariogenicity trials, and in vitro tests are presented in more detail in the following sections because they became available after the reviews by the Select Committee on GRAS Substances (1972) and the Joint FAO/WHO Expert Committee on Food Additives (1976). The concerns raised of possible adverse effects (Sunshine, 1973) are addressed by the final reports of the long-term feeding study (Saatman, 1978) describing an apparent mannitol-related incidence of benign thymic tumors in female Wistar rats and a subsequent long-term study (Gongwer, 1978) with female rats of the Wistar, Sprague-Dawley, and Fischer strains in which no adverse effects on the thymus were observed.

A. IN VITRO AND SPECIAL TESTS

Mannitol was not mutagenic as evaluated by the host-mediated assay, cytogenetic studies, or a dominant lethal assay (Litton Bionetics, 1974). In the host-mediated assay in mice, mannitol caused no significant increases in frequency of mutations with Salmonella typhimurium TA-1530 and G-46, nor did mannitol increase recombinant frequencies of Saccharomyces cerevisiae D3. Tests with these microorganisms in vitro were also negative. No aberration of the bone marrow metaphase chromosomes of rats occurred after oral administration of up to 5 g of mannitol per kg body weight daily for 5 days. Similarly, in vitro studies of anaphase preparations of human embryonic lung culture cells showed no aberrations after exposure to mannitol. The results of a dominant lethal assay using random bred rats indicated that mannitol did not induce dominant lethal mutations under the conditions of testing (Litton Bionetics, 1974).

Additional in vitro assays using either tryptophan (Escherichia coli WP2, uvrA) or histidine auxotrophs (S. typhi-
murium TA1535, TA1537, TA1538, TA98, and TA100), with and without a rat liver microsome activation system, showed that mannitol did not induce mutations in these microbial systems (Simon and Eckford, 1978).
B. ANIMAL STUDIES

1. Acute toxicity.

The acute oral toxicity (LD₅₀) of mannitol in the mouse is reported to be 22 g per kg (Gongwer, 1960) and, in the rat, between 9.5 g per kg (Litton Bionetics, 1974) and 17.3 g per kg (Gongwer, 1960). Carr and Krantz (1938) reported the minimum lethal dose of mannitol for the rat to be in excess of 13 g per kg body weight. Mice that died after receiving large doses of mannitol died with signs of central nervous system depression; however, both species showed gastrointestinal tract mucosal damage which was judged the predominant cause of death in the rat (Gongwer, 1960, 1961). Infusion for a period of 1 hour with a 10-percent mannitol solution caused reversible injury to the mucosa of rat jejunal loops; injured mucosa demonstrated a reduced absorptive capacity for glucose and neutral fat (Nasrallah et al., 1970).

2. Teratogenicity.

Mannitol was tested for teratogenic effects in mice, rats, and hamsters (Food and Drug Research Laboratories, 1972). Pregnant mice and rats given oral doses of mannitol up to 1.6 g per kg for 10 consecutive days and hamsters up to 1.2 g per kg for 5 consecutive days showed no effects on maternal or fetal survival. Mannitol was not teratogenic under the test conditions. No teratogenic effects were observed when the developing chicken embryo was exposed to mannitol at levels up to 200 mg per kg of egg (Mississippi State University, 1974).


No toxic effects occurred in male rats fed a diet containing 5-percent mannitol for 3 months or in monkeys given 3 g of mannitol daily for a similar period (Ellis and Krantz, 1941). The growth rate of male rats fed mannitol was slightly less than that of controls fed glucose. It is generally agreed that the limited absorption and metabolism of mannitol as well as its contribution to osmotic disturbances in the gastrointestinal tract can contribute to reduced weight gain in experimental animals. On the basis of growth rate of rats fed 3- to 24-percent mannitol in their diet, Staub (1978) estimated from preliminary studies that the availability of energy from mannitol was about 2.7 kcal per g. This value is somewhat higher than the 2 kcal per g calculated on the basis of the study of Nasrallah and Iber (1969) in which mannitol was given as a 5-percent solution rather than as a component of a meal.

Several investigators have attempted to quantitate the cariogenicity of mannitol relative to other nutritive sweeteners used in chewing gum and various types of candies. In short-term feeding studies with the rat as the animal model, various polyols and sugars have been mixed individually with basal diets, some considered "normal" and others "cariogenic". Results have not been definitive and data appear to conflict. For example, preliminary results reported by Navia et al. (1974) indicated that the cariogenicity of mannitol was not significantly less than that of sucrose when measured in a 20-day study of weanling Sprague-Dawley (CRL) rats fed a cornstarch diet containing sucrose or mannitol at a level of 5 percent. However, on the basis of longer term studies, other investigators consistently reported mannitol to be less cariogenic than carbohydrates such as dextrin, glucose, and sucrose (Grunberg et al., 1973; Shaw, 1976; Shaw and Griffiths, 1960).

Shaw and Griffiths (1960) fed cariogenic diets, based on dextrin or sucrose, to a strain of "caries-susceptible" rats for periods of 13 to 20 weeks. Substitution of mannitol or sorbitol for dextrin or sucrose at a level of 20 to 67 percent of the diet consistently reduced the number of carious lesions in the surviving animals. Moderate to severe diarrhea and poor survival occurred in animals fed 20 percent or more mannitol. The age of the rats at the beginning of the feeding trial and the rate at which mannitol levels were increased in the diets affected these parameters. The small numbers of animals used in the study precluded statistical comparisons; the investigators concluded that mannitol had not supported the initiation and progression of carious lesions at a rate comparable to sucrose or dextrin.

The incidence of caries in the lower molars of Wistar rats fed either a cariogenic diet (66 percent rice, 30 percent whole milk powder, 3 percent alfalfa leaf meal, and 1 percent sodium chloride) or this cariogenic diet supplemented with 10 percent glucose, sucrose, mannitol, sorbitol, or xylitol was evaluated in a 21-week trial (Grunberg et al., 1973). Only about one half of the rats consuming these diets survived the trial period and rats fed the polyol-containing diets weighed about 20 percent less than members of the other groups at the end of the trial period. Groups consuming the cariogenic diet alone or with either added glucose or sucrose had the highest incidence of caries while those fed xylitol exhibited the lowest. The incidence of dental caries with mannitol or sorbitol in the diet was appreciably less than with glucose, sucrose, or the cariogenic diet. For mannitol, the incidence of caries in the first, second, and third molars was from 9 to 27 percent less than with sucrose, from 28 to 50 percent less than with glucose, and from 23 to 54 percent less than with the unsupplemented cariogenic diet.
Shaw (1976) found in a 60-day trial that the addition of 5 or 10 percent of sorbitol or mannitol to a cornstarch-based diet of "relatively low caries potential" did not change the caries activity in HCS (Harvard caries susceptible) rats from the level supported by a 67-percent starch diet. However, in another experiment, caries activity increased significantly when 10-percent sucrose was added to the cornstarch diet. Thus, the ability of mannitol to support the initiation of carious lesions was not detected under these experimental conditions.

Not only are the cariogenicity studies in rats difficult to compare because of differences in experimental design, the absolute cariogenicity of mannitol remains undefined. This is particularly true in terms of mannitol's ability to support the initiation of carious lesions. However, most authorities express the cariogenic potential of a dietary substance in terms of acid production and plaque formation by oral microflora (Kimura and Carr, 1976; LSRO, 1978). Streptococcus mutans, a bacterial species important in plaque formation, and a wide variety of other bacteria can ferment mannitol (Gallagher and Pearce, 1977; Guggenheim, 1968). While not specifically measured in animal feeding studies, the extent and rate of acid production, plaque formation, and adaptation of oral microflora -- as indicated by the incidence of dental caries -- appear to contribute less to the carious process in experimental animals ingesting mannitol than in animals ingesting an equivalent amount of glucose, dextrin, or sucrose.

5. Long-term feeding.

Results of two long-term feeding studies have become available recently (Gongwer, 1978; Saatman, 1978). The study of Saatman (1978), a chronic toxicity and lifetime tumorigenicity trial in male and female Wistar rats, was terminated after 22 months (94 weeks) because of high mortality in the male control group. Rats in the treatment groups (40 male and 40 female rats per group) received mannitol at a level of 1, 5, or 10 percent of the diet. In the 5- and 10-percent mannitol groups, body weights of males were 5 to 7 percent less than controls. Female rats fed mannitol exhibited slightly increased water consumption and urine volume. Necropsy revealed an apparent mannitol-related incidence of benign thymic tumors in female rats. There were 2 benign thymic tumors in female controls, 6 in each of the 1- and 5-percent mannitol groups, and 10 in the 10-percent mannitol group. The occurrence of thymic tumors in males (0 in controls, 3 at 1 percent, 1 at 5 percent, and 0 at 10 percent of mannitol in the diet) did not suggest a relationship to mannitol treatment.

Subsequently, a lifetime feeding study of mannitol at 1, 5, and 10 percent of the diet was undertaken with female rats of three strains, Sprague-Dawley, Fischer, and Wistar (Gongwer, 1978). The Wistar rats used in the previous study (Saatman, 1978) were obtained from a different supplier than the Wistar rats employed
in this study. Mannitol did not affect mortality, general health and behavior, food consumption, urinary magnesium and volume, terminal organ and body weight, or the occurrence of subcutaneous tissue masses (in live animals). An increased incidence of certain tissue masses in the mannitol-treated groups was judged by the author to be of slight or no biological importance and generally within the expected spontaneous incidence for these strains. Specifically, the incidence of tissue masses increased in the anogenital area, cervix, and uterus in Fischer rats given the 10-percent mannitol diet, and in the cervix and/or uterus of Wistar rats. Histopathological evaluations of the thymus of females of all three strains revealed no adverse effects of mannitol in this study.

The adrenal glands of control rats and rats fed the mannitol-supplemented diet showed several different types of lesions which were considered generally consistent with those spontaneously occurring in aged rats of the three strains (Gongwer, 1978). In Fischer rats receiving the 10-percent mannitol-supplemented diet, the combined incidence of focal medullary hyperplasia (unilateral plus bilateral) and medullary pheochromocytoma (unilateral plus bilateral) was 47 of 96 animals examined, and was statistically greater than the control incidence, 33 of 99 animals (P<0.05, two-tailed Fisher's exact test). Viewed separately, neither the incidence of focal medullary hyperplasia (21 of 96) nor the incidence of pheochromocytoma (26 of 96) was found by the author to differ significantly from respective control values, 18 of 99 and 15 of 99. In either case the incidence in controls, which appears unusually high for this strain, exceeded those observed in the 1- and 5-percent mannitol-supplemented groups. Because there was no clear dosage response of focal medullary hyperplasia and pheochromocytoma in the Fischer rats and no treatment-related effect on the adrenal glands of the Sprague-Dawley and Wistar rats, the investigating pathologists concluded that the increased incidence of adrenal lesions in the Fischer rats was probably a chance occurrence unrelated to dietary mannitol (Gongwer, 1978).

C. HUMAN STUDIES

1. Laxation.

Ingestion of 10 to 20 g of crystalline mannitol is known to produce laxative effects in man (Ellis and Krantz, 1941). When given as a 5-percent solution, doses above 40 g of mannitol were likely to produce diarrhea and excretion of a higher percentage of the dose in the stool (Nasrallah and Iber, 1969). Apparently, no studies designed to examine the effects of mannitol as part of a normal mixed diet have been done. It is probable that mannitol, as observed for sorbitol, may reduce small bowel transit time when included in the diet at a level below that resulting in laxation (Duane, 1978; Launiala, 1968).
2. Cariogenicity.

Six subjects dissolved 94-percent mannitol tablets (4 g) in their mouths three times daily after meals for 4 weeks (Clark et al., 1961). Morning saliva was collected daily and acid production was measured in a Warburg manometer with mannitol or glucose as substrate. The amount of acid produced from mannitol was 5.5 percent of the amount produced from glucose. The rate of acid production did not change, indicating that no adaptation of the oral microflora occurred during the 4-week trial. However, Brown and Bowles (1977) have cautioned recently that the widespread use of mannitol may provide S. mutans with a competitive advantage in the oral environment.

Hassell (1971) has demonstrated that the pH of plaque in vivo, which has been established by a normal diet, changes from a value of 7 to a value of about 6 within 10 minutes after exposure to a 10-percent solution of mannitol. This result agrees with the pH decrease of 0.8 units in a similar study by Mühlemann and De Boever (1970). Others report no acid production from mannitol. During 30-minute trials, Ahlden and Frostell (1975) found no increase in the hydrogen ion concentration of dental plaque isolated from subjects immediately after they rinsed their mouths with a saturated solution of mannitol. Birkhed (1978) was also unable to demonstrate acid production from mannitol by samples of plaque isolated from volunteers consuming their normal diets. A pH value of 5.5 or lower is generally considered necessary before decalcification begins (Gallagher and Pearce, 1977).

Koulourides et al. (1976) employed an intraoral cariogenicity test to evaluate the relative cariogenicity of a series of sugars and sugar alcohols, including mannitol. This test measured changes in the microhardness of samples of bovine enamel, which were exposed periodically to 3-percent solutions of sucrose or another test sugar. The samples of bovine enamel were worn in a special denture by human volunteers who consumed their normal diets. Mannitol, sorbitol, lactose, and melibiose were judged significantly less cariogenic than sucrose on the basis of changes in the microhardness of the bovine enamel samples.

Several studies conducted with children who chewed polyol-sweetened gums were designed primarily to examine the effect on caries development of ingredients such as dicalcium phosphate (Finn and Jamison, 1967; Richardson et al., 1972) and sodium trimetaphosphate (Finn et al., 1978). The studies were not designed to examine the cariogenicity of the polyols per se and the exact compositions of the gums were not given. Children who chewed a polyol-sweetened gum (containing 50 to 70 percent sorbitol and mannitol) or those who did not chew gum had greater caries increments over a 30-month period than children who chewed polyol-sweetened gum to which sodium trimetaphosphate had been added (Finn et al., 1978). Imfeld and Mühlemann (1977) observed that chewing of certain polyol-containing gums, which do not con-
tain sucrose, increased the pH of dental plaque over fasting values and suggested that the failure of plaque pH to reach values below pH 5.5 after exposure to polyol-containing chewing gum and candies represents an important contribution to preventive cariology. None of these studies were designed specifically to study mannitol's effects on caries development. However, the results are consistent with the cariogenicity trials in experimental animals indicating that mannitol, in the absence of adaptation of the oral microflora, is less cariogenic than sucrose.
V. CONCLUSIONS

- Animal studies and clinical investigations indicate that 50 to 70 percent of mannitol ingested in aqueous solutions may be absorbed by diffusion from the gastrointestinal tract. In human subjects approximately 50 percent of an oral dose containing up to 100 g of mannitol is metabolized; absorbed mannitol which is not metabolized is excreted unchanged in the urine. The extent of absorption and metabolism of mannitol included as a component of a normal mixed diet has not been determined.

- Orally administered mannitol contributes to the formation of blood glucose and hepatic glycogen in experimental animals and in human beings. Mannitol is apparently metabolized in the liver but this process is poorly characterized.

- While oral doses of 10 to 20 g of crystalline mannitol may cause laxation in adult human beings, the influence of diet composition on laxative effects has not been studied in animals or human beings.

- The perceived sweetness of mannitol is about one half that of sucrose. This plus the possibility of gastrointestinal disturbances may limit the usefulness of mannitol as a sweetener.

- Most animal feeding studies designed to test the cariogenicity of mannitol and measurements of acid production in human dental plaque after exposure to mannitol indicate that mannitol is less cariogenic than dextrin, glucose, or sucrose.

- Evidence from animal and in vitro studies indicates that mannitol is not mutagenic, teratogenic, or carcinogenic. The unexplained benign thymic tumors that occurred in one feeding study with female Wistar rats did not occur in females of that strain or two other strains of rats when mannitol was retested with a similar long-term feeding protocol.
VI. LITERATURE CITED


[Mississippi State University. 1974]. Investigation of the toxic and teratogenic effects of GRAS substances to the developing chicken embryo: mannitol. State College, MS. [7p.]


VII. STUDY PARTICIPANTS

A. CONSULTANTS

Dills, William L., Jr., Ph.D.
Assistant Professor of
Nutritional Biochemistry
Division of Nutritional Sciences
Savage Hall
Cornell University
Ithaca, New York 14853

Schachtele, Charles F., Ph.D.
Professor of Dentistry and
Microbiology
School of Dentistry
University of Minnesota
515 Delaware Street, S.E.
Minneapolis, Minnesota 55455

Kashgarian, Michael, M.D.
Department of Pathology
Yale University School of
Medicine
New Haven, Connecticut 06510

Touster, Oscar, Ph.D.
Professor and Chairman,
Department of Molecular
Biology
Vanderbilt University
Nashville, Tennessee 37235

B. SPECIAL CONSULTANT

Huber, Tyron E., M.D.
6002 Roosevelt Street
Bethesda, Maryland 20014
C. LIFE SCIENCES RESEARCH OFFICE

Kenneth D. Fisher, Ph.D.
Director
Life Sciences Research Office
Federation of American Societies
for Experimental Biology
Bethesda, Maryland 20014

Richard G. Allison, Ph.D.
Staff Scientist

Herman I. Chinn, Ph.D.
Senior Staff Scientist

Frederic R. Senti, Ph.D.
Associate Director

John M. Talbot, M.D.
Senior Medical Consultant

OTHER CONTRIBUTING LIFE SCIENCES RESEARCH OFFICE STAFF

Elizabeth M. DeWitt
Administrative Aide

C. Grace Gurtowski
Bibliographer

Beverly Keder
Literature Retrieval/
Technical Report Specialist