SUCROSE VOL II #106

K28

Monograph on Sucrose-Volume 2

#106

12/11/73

K28

MONOGRAPH

ON

SUCROSE

VOLUME II

TR-72-1552-38

Submitted Under:
Contract No. FDA 72-104

December 11, 1973

INFORMATICS INC.
6000 Executive Boulevard
Rockville, Maryland 20852

J. Lab. & Clin. Med. 36: 242-248. 1950.

Reproduced by permission of the copyright owner

ALLERGIC REACTIONS FROM THE INGESTION OR INTRAVENOUS INJECTION OF CANE SUGAR (SUCROSE)

THERON G. RANDOLPH, M.D.,* AND JOHN P. ROLLINS, M.D.†
CHICAGO, ILL.

THE possibility of cane acting as an allergen has received scant attention even though it is a member of the botanical family of cereals, the various members of which are well known for their ability to cause allergic symptoms. Cane also fulfills another requirement that should lead us to suspect its allergenicity, namely, it is ordinarily consumed several times per day. The fact that cane is most commonly eaten in the form of highly refined crystalline sugar, containing practically no protein, has apparently lead allergists to discount its allergenic possibilities. Another reason that its allergenicity may have been overlooked is the fact that it has been included in practically all basic diagnostic elimination diets employed routinely in investigating the possibilities of food sensitivity.

However, in view of the ability of highly refined corn sugar (dextrose and glucose) to produce allergic symptoms by ingestion[1] or as a result of intravenous injection[2] in at least certain highly corn-sensitive patients and the ability of granulated beet sugar (sucrose) to produce allergic responses in beet-sensitive patients,[3] the allergenicity of cane must be given more careful scrutiny.

Sensitivity to cane was first recognized by Duke[4] in 1925 (Case 23) and subsequently by Coca[5] in 1945. Sensitivity to cane sugar has been recognized by us with increasing frequency during the past two years since all undiagnosed cases of allergy possessing a wide base of food sensitivity have been subjected routinely to individual food tests with cane sugar. The technique of this test is that described by Rinkel[6] as modified slightly by Randolph and Rawling.[7]

CASE REPORTS

The following cases illustrate the clinical aspects of cane sensitivity.

A. S., a 36-year-old woman, had been subject to sick headaches since childhood; individual attacks were characterized by nervousness and tenseness, followed by extreme fatigue, distention and diarrhea, lacrimation and swelling of the affected side of the face, pulling and tautness of the posterior cervical muscles, and severe head pain. There had also been localized myalgic pains and cramps in other muscles, chronic perennial nasal allergy, and chronic fatigue and depression existing between the more severe attacks of headaches, myalgia, and gastrointestinal symptoms. The ingestion of chocolate was known to bring a severe headache but the patient suspected no other foods.

The patient had been diagnosed as having conversion hysteria, studied extensively under Sodium Amytal narcosis, and treated psychiatrically. In spite of medical efforts she remained chronically sick and had been unable to care for her six children.

There was no evidence of inhalant allergy as determined by details of the history or by means of skin testing with inhalant allergens.

Received for publication, April 22, 1950.

*Instructor in Medicine, Northwestern University Medical School.
†Research Fellow in Medicine, Northwestern University Medical School.

As a result of studying the major foods by means of individual food tests the patient was found to be highly sensitive to wheat and corn, developing a severe headache after each of these experimental tests. Lesser degrees of sensitivity were demonstrated after the ingestion of eggs, lettuce, pork, and onions. Although the specific avoidance of these incriminated foods resulted in about seventy per cent relief, she continued to complain of extreme fatigue, particularly troublesome in the morning, suggesting the diagnosis of hypoglycemia, but this could not be confirmed. She was then subjected to an individual food test with cane sugar; this was followed in twenty-five minutes by the development of belching, distention, dizziness, and scotomata, followed shortly by a severe chill and marked flushing of the face. A very sharp generalized headache began three hours after the test feeding.

With the additional avoidance of cane sugar the patient has remained free of her formerly troublesome symptoms for the past year except as she inadvertently ingested an incriminated food. On several different occasions cane sugar had been taken, either accidentally or intentionally; each time it was followed by a reaction similar to that of the original test ingestion. On one occasion she received three teaspoons of cane sugar in two cups of tea under the impression that she was taking beet sugar. Within five minutes she became logy and drowsy; this was followed by bloating and extreme fatigue and abdominal distress persisting for the remainder of the day. With the avoidance of cane sugar as well as the other incriminated food allergens, she reported that she felt mentally alert for the first time in a number of years, that she remained free of her morning fatigue and that she was able to work all day without tiring as easily as formerly. Her sleep at night was much less disturbed in that she felt more relaxed, being relatively free of her former tenseness and nervousness. For the past several months she has been able to do her own housework and care for her large family.

G. McL., aged 46, was first seen in 1945 at which time she gave a history of extreme fatigue associated with mental confusion and difficulty in comprehension, concentration, and memory which developed shortly after the birth of her child in 1931. Giant urticaria and intermittent episodes of abdominal cramps, diarrhea, and vomiting first occurred a year later. Leucorrhea, present to some extent at all times, was markedly accentuated in association with acute attacks of colitis and subsequently became associated with pruritus vulvae and perineal eczema. The patient also remained subject to intermittent periods of impaired hearing and frequently complained of localized or generalized muscle soreness and aching and impairment of the ability to focus her eyes. Other causes of the symptoms could not be found and it was subsequently demonstrated that her weakness and related symptoms of the fatigue syndrome,[8] mental symptoms,[9] and myalgia[10,11] as well as the more orthodox manifestations of hypersensitivity described were allergic responses reproducible at will following the manipulation of the diet in respect to specific foods.

As a result of numerous individual food tests performed during the following four-year period the patient was found to be clinically sensitive to wheat, corn, milk, eggs, coffee, beans, cane, beef, chicken, and a few additional minor foods.

The avoidance of cane prior to performing an individual food test was associated with a lessening of the general symptoms of tenseness and irritability as well as an improvement in the vaginal discharge and pruritus vulvae. Thirty minutes after the experimental ingestion of cane sugar she was observed to have a flushed face. A second feeding one hour after the first was followed immediately by intense aching and drawing sensations in her arms, progressive "dopiness", and dulled mental acuity. This persisted for the remainder of the day of the test in contrast to the absence of such symptoms the day prior to the test. The evening of the cane test the patient developed a profuse leucorrheal discharge and intense pruritus of the perineum.

Five days later she was subjected to a test with 25.0 c.c. of 5 per cent invert cane sugar intravenously, having avoided cane products in the interim and not being aware of whether she was receiving a sugar solution or saline. Seven minutes were required for the injection, the material and technique of which have previously been described.[2] Eight minutes after its completion she first noticed the onset of pain in the region of the left

elbow and an associated transient sense of chilliness. She developed flushing of the face at twenty minutes followed by itching of the skin of the face, a similar aching of the extremities, and slowness of mental reactions and somnolence. Extreme aching and drawing of various large muscles persisted for the next two days. Following the avoidance of cane she has remained relatively free of myalgia and there has been a marked improvement in the leucorrhea and mental symptoms.

Upon the performance of an identical test with saline the patient failed to develop reactive symptoms.

In the ten months elapsing since the avoidance of cane the patient developed a recurrence of myalgia and the related symptoms already described shortly after the prescription of a special desiccated thyroid tablet containing cane but free of cornstarch and other food excipients to which she was known to be sensitive. The recurrence of similar symptoms on another occasion prompted her to review the previous meal and it was determined that it had contained cane sugar by error. The experimental ingestion of cane sugar was repeated in the past month and again was associated with similar but less severe and less prolonged symptoms.

M. W., a dietitian and the wife of a physician, aged 30, has been reported in detail in the previous article of this series.[2]

Following the elimination of several major allergens from her diet, she began eating more cane sugar in an attempt to maintain her weight. Within a few weeks she noticed the onset of severe aching pains in the area of her left elbow beginning twenty to thirty minutes after meals containing cane sugar, and if an abundance of sugar was ingested, nausea and headache developed in addition. Insomnia and general tenseness and nervousness had become increasingly troublesome since she had been eating larger amounts of cane sugar. On the third and fourth nights of avoiding cane the patient was able to get to sleep more readily and slept more soundly than she had in several previous weeks. Twenty minutes after the experimental ingestion of 50 Gm. of granulated cane sugar, aching sensations in the left elbow and shoulder developed followed by the onset of an occipital headache. A second dose of cane sugar, one-half the size of the first, was followed promptly by the onset of nausea.

With the avoidance of cane in addition to other incriminated foods and the use of tolerated foods on a rotating, diversified schedule as outlined by Rinkel,[13] both the insomnia and neuralgia or myalgia symptoms were markedly improved; she slept soundly through the night instead of awakening regularly at two and again at four or five in the morning and in general felt much less tired and tense. On repeated occasions the inadvertent ingestion of cane reproduced the clinical picture as described.

After she had avoided cane sugar for a month, the patient was again subjected to an individual food test with cane, employing 50 Gm. of Domino cane sugar. The cubes were dissolved in 240 c.c. of distilled water and followed by water as desired. At ten minutes she reported the onset of the previously described pain in her left arm and shoulder; at fifteen minutes the onset of a mild frontal headache associated with discomfort in areas of the frontal sinuses and slight nausea; at twenty minutes chills associated with goose flesh started, alternating with "flashes" of excessive warmth and perspiration of the hands. These symptoms persisted and gradually became more intense during the following three hours; the patient required additional clothing for warmth during the chilling periods and became incapacitated with a throbbing generalized headache. A paroxysm of sneezing and nasal stuffiness developed at seventy-five minutes. The patient passed a very restless night, awakening several times complaining of intense pulling, drawing, and aching sensations in the posterior cervical muscles and upper back. She still had generalized aching and stiffness of all muscles the following morning and felt much more tired than usual. The joints of her hands were noticeably more stiff and motion of the fingers was painful throughout the second day although there was no evidence of edema or erythema as judged by examination.

Five days later, the patient having avoided cane products in the interim, 25.0 c.c. of 5 per cent invert cane sugar in distilled water were injected intravenously in a period of seven minutes, the patient not being aware whether she was receiving a cane solution or saline. This preparation was secured from the Baxter Laboratories, Morton Grove, Ill., and on their statement was said to be free of bacterial pyrogens; it was administered by means of pyrogen controlled technique as previously outlined.[2] The patient again became more tense and although the temperature of the room was 85° F. she voluntarily put on a heavy sweater. During the next half hour she developed marked weakness of the knees, followed by generalized aching of all extremities, tiredness, drowsiness, and irritability. With a steady increase in the intensity of her headache, in two hours she became so depressed and melancholic that she cried intermittently for the next half hour. At this point she was given codeine in an attempt to relieve the symptoms. The patient again spent a very restless night and on awakening the following morning complained of marked weakness, generalized aching of all muscles and joints and had a recurrence of the typical mouth lesions of lingua geographica.

Having avoided cane in the interim, two months later in November, 1948, the patient was subjected to an ingestion test with a solution of dextran prepared under the direction of Dr. Robert C. Hockett of the Sugar Research Foundation. This consisted of a dextran culture containing 40 per cent solids as dextran with a density of 25 Brix; this preparation had been subjected to dialysis in a standard cellophane bag against running water for twenty-four hours in an effort to remove any traces of residual sucrose which resulted in a doubling of the volume of the material in the bag. Whereas the undialyzed material was sweet to taste, the dialyzed product was tasteless; 100 c.c. of this material were ingested at a time when the patient was symptom free. Within the first five minutes she again complained of pain in the left arm and a general sensation of increased warmth. At twenty minutes she first noticed aching of the extremities; at twenty-five minutes she complained of a headache, chilliness, and increasing tenseness and "jitteriness." At forty minutes she noticed increasing nasal stuffiness and tickling sensations in her throat. At fifty minutes she complained of chilliness to the point of shivering, goose flesh, and requested a blanket for warmth. Residual effects were similar to those previously described following the ingestion or intravenous injection of cane sugar.

This patient also received 25.0 c.c. normal saline intravenously as a control, not knowing whether she was receiving saline, cane or corn sugar, and failed to develop a symptom response.

B. W., a 48-year-old man, had been subject to intermittent frontal headaches occurring in association with attacks of bloating and acute abdominal distress for many years, progressive rheumatoid arthritis, and generalized myalgia as well as perennial bronchial asthma of three years' duration. He suspected eggs, coffee, and pepper of accentuating his gastrointestinal symptoms but was unaware of intolerance to other foods.

There was no clinical or skin test evidence of sensitivity to the common inhalant allergens.

In the course of investigating his food allergy, an individual food test with granulated white cane sugar was followed by the sudden onset of dizziness and coughing at five minutes, blurring of vision at seven minutes, pulling and drawing sensations in the muscles of the arms and posterior cervical region at fifteen minutes, and, finally, marked somnolence and a severe frontal headache beginning at forty minutes. Myalgia, headache, and extreme fatigue persisted for the remainder of the day and were associated with a delayed accentuation of the bronchial asthma.

Because of the striking degree of relief of his chronic symptoms following the avoidance of sources of cane and the obvious high degree of specific sensitivity, this patient was selected for other experimental observations in respect to cane sensitivity. It is of interest that he was able to eat beet sugar with impunity and on several occasions cane sugar was substituted for beet sugar without the patient's knowledge and on each such occasion he developed a recurrence of reactive symptoms of the type described.

The intravenous injection of 25.0 c.c. of invert cane sugar with pyrogen control as previously described[2] was followed by the onset of wheezing, somnolence, lacrimation, and scotomata at ten minutes and subsequently by dizziness, stiffness and drawing of the posterior

cervical muscles, and progressively severe bronchial asthma and headache persisting for the remainder of the day. The patient was unaware of the contents of the injection or the subsequent one containing normal saline; the latter was not followed by symptoms.

One week later he was fed experimentally a cube of Domino (Adant) cane sugar. He was observed to start coughing and wheezing four minutes later with the development of pulling and drawing sensations in the muscles of the extremities, back, and shoulders, with dizziness and blurring of vision at eighteen minutes. Residual increased asthma and aching and soreness of the muscles of the extremities persisted for the remainder of the day and were associated with a decreased range of motion of the elbows.

The patient was tested subsequently with 100 c.c. of a dialyzed dextran solution, as described in the previous case. This again was associated with coughing and wheezing within the first five minutes, dizziness and blurring of vision at ten minutes, followed almost immediately by progressive stiffness and soreness of the neck muscles and severe head pain. The asthma required the use of epinephrine for relief. These symptoms, entirely absent for several days prior to the test, persisted for the remainder of the day.

On another occasion the patient was fed experimentally 20.0 c.c. of rum derived exclusively from cane sugar. He again was observed to have increased wheezing and coughing beginning five minutes after ingestion, followed almost immediately by dizziness and myalgic and arthritic pains in the muscles of the arms and nuchal region. This was followed by progressive asthma, headache, lacrimation, and "dopiness." This reaction was as severe as the clinical response following the ingestion of dextran and also required the administration of epinephrine for relief of the asthma. A control experimental test with potato spirits was not associated with symptoms other than the immediate stimulating effect of the alcohol.

DISCUSSION

It is of interest that in these illustrative cases as well as in the majority of other proved instances of cane allergy specific sensitivity usually was not suspected by the patient. The most common exception to this statement is the person who is intolerant to rum; this has been noted in two patients substituting rum as an alcoholic beverage in the course of diagnostic studies for food allergy coincident with the specific avoidance of all foods and spirits derived from wheat, rye, malt, and corn. The principal reason that patients do not ordinarily suspect the existence of cane sensitivity is the fact that it is ingested so frequently that the patient does not recover from the effects of a given dose before he has received several additional feedings. These are exactly the conditions which so commonly lead to the development of a masked type of food allergy.[13]

Similarly, physicians have been loath to accept the existence of specific allergy to cane because of the nonprotein nature of granulated cane sugar. Their observations have been further hampered by the fact that practically all basic-type elimination diets include cane as one of the few permissible ingredients.

In our clinical experience, cane sensitivity should be suspected until it is ruled out in all patients who possess multiple allergy to other members of the cereal grain botanical family, and, secondly, in all individuals who possess a wide base of food involvement. As in other instances when dealing with foods ordinarily ingested several times daily, the most accurate means of diagnosing the existence or assuming the nonexistence of sensitivity is the specific avoidance of the food in question for a short period followed by performing an individual food test with it.

Another point illustrated in the cases cited as well as in many others is the nature of the clinical manifestations so commonly exhibited by cane-sensitive patients. Although any of the traditional allergic manifestations may be on the basis of specific cane sensitivity, these patients exhibit an unusually high incidence of the symptoms originally grouped by Rowe[14, 15] under the term allergic toxaemia. Many of these manifestations have been more recently described by us.[8, 9, 10, 11] Particularly striking in these individuals is the incidence of myalgia, mental confusion, depression, extreme fatigue, nervousness, tenseness, and so-called "jitteriness."

Of additional interest is the fact that two of these cane-sensitive individuals were tested with preparations derived from cane containing various proportions of the contaminating polysaccharide known as dextran. Although dextran is present in both cane and beet sugar, Neill and associates[16] believe that it is present to a slightly greater degree in cane as compared with American beet sugars. The probable origin of this polysaccharide is the brief period of fermentation of the cut cane stalk by the organism *Leuconostoc mesenteroides* during the interval after it is cut and before it reaches the mill. Traces of dextran present in the unrefined juice may not be removed as a result of the refining processes.

Of particular interest from the standpoint of possible allergic reactions is the fact that this polysaccharide is immunologically active and not dissimilar to the type-specific pneumococcus polysaccharide,[17] and Neill and associates[16] were the first to show that nearly all white sugars showed a positive precipitin reaction when tested against Type 2 pneumococcus antiserum.

Hockett[18] has theorized that the presence of dextran in sucrose may be responsible for cases of so-called sugar allergy. The third patient in this series, clinically sensitive to both beet and cane, reacted with clear-cut symptoms following the ingestion of a dialyzed culture of dextran. The fourth patient, highly sensitive to cane but not to beet sugar, not only reacted with sharp allergic symptoms following the ingestion of the dextran solution but also had a definite clinical reaction of the general type associated with the ingestion of cane sugar after the ingestion of a cube of Domino Adant sugar selected because of its relatively low content of polysaccharide or dextran.

This datum plus the fact that beet sensitivity exists in the absence of specific sensitivity to cane, as will be pointed out in a forthcoming presentation,[8] is suggestive evidence that factors other than the contaminating dextran may be responsible for the allergenicity of cane and beet sugars. This answer awaits the more detailed immunologic study of specifically diagnosed cases of sugar sensitivity than our facilities permit.

Finally, there is the fact that invert sugar of cane origin will cause unmistakable allergic reactions when it is injected in certain specifically cane-sensitive persons. In this respect, our experience in cane sensitivity is identical with that previously found to exist in respect to the intravenous injection of dextrose in patients found to be specifically sensitive to the ingestion of corn sugar.

SUMMARY

Cane sensitivity exists as a cause of chronic allergic symptoms in certain patients found to have a wide base of food allergy reactions; it should be suspected in all uncontrolled allergic persons sensitive to other members of the cereal grain botanical family and should be ruled out by the performance of an individual food test with cane.

Sensitivity to cane may be the cause of any type of clinical allergic response but is commonly responsible for a group of symptoms referred to as the fatigue syndrome of allergic origin.

Whether cane sensitivity is related to the presence in cane sugar of immunologically specific polysaccharides requires further study.

The intravenous injection of invert sugar of cane origin causes allergic responses in many cane-sensitive patients.

REFERENCES

1. Randolph, T. G., and Yeager, L. B.: Corn Sugar as an Allergen, Ann. Allergy 7: 651, 1949.
2. Randolph, T. G., Rollins, J. P., and Walter, C. K.: Allergic Reactions Following the Intravenous Injection of Corn Sugar (Dextrose or Glucose), Arch. Surg. In press.
3. Randolph, T. G., and Rollins, J. P.: Beet Sensitivity: Allergic Reactions From the Ingestion of Beet Sugar (Sucrose) and Monosodium Glutamate of Beet Origin, J. Lab. & Clin. Med. To be published.
4. Duke, W. W.: Asthma, Hay Fever, Urticaria and Allied Manifestations of Allergy, ed. 2, St. Louis, 1920, C. V. Mosby Company.
5. Coca, A. F.: Familial Nonreagenic Food Allergy, ed. 2, Springfield, Ill., 1945, Charles C Thomas.
6. Rinkel, H. J.: Food Allergy. II. The Technique and Clinical Application of the Individual Food Test, Ann. Allergy 2: 504, 1944.
7. Randolph, T. G., and Rawling, F. F. A.: Blood Studies in Allergy. V. Variations in Total Leucocytes Following the Test Feeding of Foods, An Appraisal of the Individual Food Test, Ann. Allergy 4: 163, 1946.
8. Randolph, T. G.: Fatigue and Weakness of Allergic Origin; To Be Differentiated From "Nervous Fatigue" or Neurasthenia. Ann. Allergy 3: 418, 1946.
9. Randolph, T. G.: Mental Symptoms of Allergic Origin. To be published.
10. Randolph, T. G.: Allergic Myalgia. To be published.
11. Randolph, T. G.: Allergy as a Cause of Nuchal Myalgia and Associated Headache, Arch. Otolaryng. 50: 745, 1949.
12. Rinkel, H. J.: The Function and Clinical Application of the Rotary Diversified Diet, J. Pediat. 32: 266, 1948.
13. Rinkel, H. J., Randolph, T. G., and Zeller, M.: Food Allergy, Springfield, Ill., Charles C Thomas. In press.
14. Rowe, A. H.: Food Allergy, Its Manifestations, Diagnosis and Treatment, J.A.M.A. 91: 1623, 1928.
15. Rowe, A. H.: Allergic Toxaemia and Migraine Due to Food Allergy, California & West. Med. 33: 785, 1930.
16. Neill, J. M., Sugg, J. Y., Hehre, E. J., and Jaffe, E.: Serological Studies on Sugar. II. Reactions of the Antiserums of Type 2 Pneumococcus and of Leuconostoc mesenteroides With Cane and Beet Sugars and With Cane Juice, Am. J. Hyg. 34: 65, 1941.
17. Neill, J. M., Hehre, E. J., Sugg, J. Y., and Jaffe, E.: Serological Studies on Sugar. I. Reactions Between Solutions of Reagent Sucrose and Type II Antipneumococcus Serum, J. Exper. Med. 70: 427, 1939.
18. Hockett, R. C.: Scientific Director, Sugar Research Foundation. Personal Communication.

J. Lab. & Clin. Med. 36: 407-415. 1950.

Reproduced by permission of the copyright owner

BEET SENSITIVITY: ALLERGIC REACTIONS FROM THE INGESTION OF BEET SUGAR (SUCROSE) AND MONOSODIUM GLUTAMATE OF BEET ORIGIN

THERON G. RANDOLPH, M.D.,* AND JOHN P. ROLLINS, M.D.†
CHICAGO, ILL.

DUKE[1] was the first to describe sensitivity to beet sugar although sensitivity to beets as a vegetable has long been recognized. In more recent years numerous cases of beet sensitivity in which the ingestion of beet sugar has resulted in allergic symptoms have been noted also by Rinkel[2] and Zindler.[3]

The fact that other crystalline sugars[4, 5, 6] are known to produce allergic symptoms warrants an investigation of the probable allergenicity of beet sugar. Another factor favoring such a study is the relative frequency with which beet sugar is used, for it is generally known that the incidence of specific sensitivity to foods is closely related to the incidence of such foods in the dietary.[7]

Beet sugar is the principal source of granulated sugar used in certain wide geographic regions of this country, particularly in the areas in which beet sugar mills are located and in regions relatively far removed from cane refineries or otherwise inaccessible to water transportation therefrom. In general this would include the entire western half of the United States and to some extent the north central states, particularly Michigan, Minnesota, and Wisconsin.

In recent years beet sugar has been favored by manufacturers of processed foods not only in the regions mentioned but elsewhere in view of the favorable price differential as compared with cane sugar. The current widespread use of beet sugar in food processing makes it probable that most individuals in this country are receiving some beet sugar daily, frequently several times daily.

Another source of exposure to beet is the rapidly increasing use of monosodium glutamate,[8] a so-called chemical condiment derived either from beets, wheat, corn or soy bean, incorporated into many processed foods as well as being added directly to various foodstuffs in their preparation in public eating places as well as in the home. It is most commonly employed to accentuate meat flavors in soups, chowders, and bouillons, sauces, gravies, canapés, salads, creamed dishes, and cheese dishes and is added directly to vegetables, meats, poultry, game, and fish.

The detection of the presence of a specific monosodium glutamate in a prepared food is exceedingly difficult because of the reluctance of cooks to

Received for publication, May 23, 1950.
*Instructor in Medicine, Northwestern University Medical School.
†Research Fellow in Medicine, Northwestern University Medical School.

admit that they have used such an agent and the difficulty to ascertain whether the product used might have been derived from beets or one of the other sources from which this material is made. The latter is particularly true in the usual absence of specific food sources on the labels of these commercial products.

CASE REPORTS

The following are examples of beet sensitivity.

F. A., a college mathematics professor, aged 43, had been subject to perennial nasal allergy since adolescence. During the past fifteen years he had also complained of chronic fatigue and intermittent bilateral frontal headaches. The fatigue syndrome in his case was characterized by mental confusion, impairment of memory, and a certain degree of aphasia which materially interfered with his teaching in the mid- and late afternoons. In addition he had also been subject to troublesome backaches for the past decade which usually awakened him between two and four in the morning. Superimposed on chronic symptoms of this type were acute episodes of very severe cramping pains in the leg muscles to the point that it was exceedingly difficult ot walk. In 1945 he was diagnosed as having a ruptured intervertebral disc for which a spinal fusion was performed. Although the back symptoms were improved following this operation, he reported accentuation of his residual chronic back and leg symptoms coincident with each recurrence of soreness, scratchiness, and irritation of his throat. Beginning a few months prior to the operation he also reported drawing, pulling pains across the upper back and shoulders and bouts of acute torticollis on a few occasions. He commonly reported feeling sore and stiff in all muscles on awakening in the morning.

He was found to be dust sensitive on the basis of intradermal tests, but dust therapy failed to alter the course of the symptoms. There was no other evidence of inhalant sensitivity.

During the third day of wheat avoidance in preparation for an individual food test with wheat he reported an improvement in the nasal symptoms and fatigue; the experimental feeding of wheat was followed by the development of a frontal headache associated with pulling, drawing, and tautness of the posterior cervical muscles and the delayed development of an acute lower backache. Tests with corn and eggs were followed by similar but less severe symptoms. Test ingestion of lettuce was followed by the development of a headache three hours after the meal. Potatoes were associated with the precipitation of a severe headache, abdominal pains, and excessive belching. Reactions of lesser degree followed the individual test ingestion of oats, rice, pork, orange, and tomatoes. Tests with several other major foods were compatible. At this point the chronic symptoms were materially relieved but the patient still complained of localized and generalized muscle symptoms, some fatigue, and mental symptoms.

In checking his food diary it was learned that he had awakened two or three times nightly with backache whenever he had eaten beets; this agreed with the childhood history of vomiting after spinach and severe headaches following the ingestion of Swiss chard, members of the same botanical family.

He was prepared for an individual food test with beet sugar by the avoidance of all beets and beet sugar for a period of four days prior to the experimental ingestion of 75 Gm. of beet sugar. Fifty minutes later he reported the gradual onset of pressure in the frontal area of the head, tautness of the posterior cervical muscles, and heartburn. As the headache became accentuated he developed severe abdominal cramps and flushing of the face. He remained unusually fatigued for the following two days. With the avoidance of beets and commercially sweetened foods containing beet sugar he reported marked and sustained improvement in the remainder of the chronic symptoms. The patient slept more soundly at night and was able to sleep on his abdomen for the first time since the spinal fusion. He noted that he was much less restless than formerly, being able to sit in his chair for long periods of time without discomfort or the necessity of frequently changing his position, that he had what

he considered a normal amount of energy, and that he was much more alert mentally, which reflected itself in greater degrees of lucidity and ease of expression than he had enjoyed in several years. At the time of his last visit, a year after the diagnosis of beet sensitivity, he was actively engaged in a research project in addition to his full-time teaching position.

The patient remained highly sensitive to beets, either in the forms of beets as such or beet sugar. On several occasions the accidental ingestion of beet sugar was followed by a precipitation of fatigue, headache, myalgia, and mental confusion.

At this time he volunteered for testing the effect of monosodium glutamate, a chemical condiment prepared from Steffen's waste, a beet residue following the extraction of the sugar. After the ingestion of one teaspoon of the powder dissolved in a glass of water, he developed a frontal headache which became progressively more severe for two hours. The following morning he awakened with marked stiffness of the neck, shoulders, and lower back and remained lame, stiff, and tired for the rest of that day. He noticed residual fatigue and mental confusion the second day and then remained symptom free. A control test with monosodium glutamate prepared by the same company* but derived from wheat was not associated with reactive symptoms. At the time of this test, wheat had been excluded from the diet for a year and the patient was known to have a tolerance for an occasional feeding of wheat. The patient was unaware whether he was receiving the wheat or the beet preparation at the time of the tests.

R. C., a housewife, aged 42, had been subject to intermittent hives for the past ten years prior to her initial visit in March, 1948. Although she had had attacks of urticaria at any time of the year, her symptoms were much more troublesome from the fourth of July to Labor Day. In the last year, however, this seasonal incidence had changed and she had continued to have daily chronic urticaria throughout the winter.

She had known that the ingestion of clams was followed by pruritus and swelling of the buccal mucous membranes, nasal stuffiness, and lacrimation. Other foods were not suspected.

There were no abnormalities of the physical examination except for the typical urticarial plaque lesions. Skin tests with inhalants were negative except for a borderline reaction to house dust, but specific therapy with dust failed to alter the course of the symptoms.

The patient was studied for specific food allergy by means of individual food tests. The experimental ingestion of wheat, corn, milk, egg, and tomato was not associated with reactive symptoms. A similar test with potato was followed by the development of a severe headache beginning ten minutes after the initial feeding and became associated with marked fatigue and pulling and drawing sensations of the posterior cervical muscles. One hour after the test ingestion of coffee the patient developed tenseness and nausea, followed shortly by the onset of severe generalized urticaria which persisted for four days, whereas for a few days prior to this test she had been having only mild, intermittent hives.

Although the avoidance of potato and coffee were helpful, this plan did not relieve the chronic urticaria and neither did various elimination diets tried successively at this juncture.

The additional history then became available that the hives had been present only since the patient began spending the summers in Michigan, and until the past year the hives had subsided upon her return to Chicago. This immediately aroused the suspicion of beet sensitivity inasmuch as this appears to be more common in Michigan than in Illinois because of the wider use of beet sugar in Michigan. Then the additional pertinent fact came to light that during the war she had bought a large supply of Michigan beet sugar and had used it exclusively each summer. Furthermore, in returning to Chicago in the fall of 1947 she had brought the remainder of this sugar with her and had continued using from it for several months. Upon the exhaustion of this source of supply she had purchased additional beet sugar in the local market.

The patient was tested with beets and beet sugar after four days of avoidance and developed a sharp recurrence of urticaria one-half hour following the second feeding which persisted for the following two days.

*Samples were furnished through the courtesy of International Minerals and Chemical Corporation, Chicago, Ill.

She then remained free of urticaria and other allergic symptoms except for an occasional recurrence following meals away from her own home. However, continual urticaria again developed in the spring of 1949 coincident with the onset of the golf season. She played golf on the average of four or five days a week and always drank an "old fashioned" after her game. It then occurred to her to investigate the type of sugar used in the bar. Finding that beet sugar had been used exclusively, henceforth she had her drinks made with cane sugar and the urticaria ceased at the end of three days. She also learned from experience that she was able to tolerate the bakery products from one neighborhood bakery and not from another; inquiry led to the fact that cane sugar was used routinely in the former and beet sugar in the latter.

Upon another occasion she was fed experimentally one-half teaspoonful of monosodium glutamate of beet origin in a half glass of water (at the time of the test she was unaware whether she was receiving monosodium glutamate of beet, wheat, or corn origin). Ten minutes later she noticed a sense of pressure in her temples, followed shortly by extreme weakness and faintness which persisted for an hour. Intense erythema and pruritus of the skin about the eyes, anterior neck, and upper back developed at eighty minutes; scratching was followed by urticarial lesions. At the end of two hours the patient was observed to have generalized urticaria of the entire upper part of the body; this persisted and spread to involve the entire body during the night of the test. Three days were required for this attack to subside. A similar amount of monosodium glutamate of corn* and of wheat origin was not followed by urticaria or other symptoms.

L. T., a housewife, aged 32, had been subject to intermittent eczema and ragweed hay fever since childhood; the latter had been complicated by seasonal bronchial asthma in recent years. She also complained of intermittent stiffness and aching of the posterior neck muscles, frequently followed by dizziness and generalized headaches. There had also been intermittent bouts of burning and smarting on urination, swelling and soreness of the knees and finger joints, and a chronic degree of fatigue, all of which had failed to respond to various types of treatment

On examination the patient had an erythematous scaly eruption of several fingers, a fusiform enlargement of the mid-finger of her right hand with edema and discoloration of the skin of the infraorbital areas.

She was found highly sensitive to corn, wheat, and beets on the basis of individual food tests; the corn reaction consisted of excessive gas and belching with pronounced clearing of the throat beginning at forty minutes, followed by flushing of the face, somnolence, and the development of a severe headache. The wheat test was followed by the development of transitory tightness of the chest, somnolence, and marked flushing of the face beginning twenty minutes after the initial feeding. Difficulty in focusing her eyes, extreme fatigue, and nervousness persisted for the remainder of the day. Beet sensitivity was suspected on the basis of an acute reaction following the ingestion of beets as determined from evidence from her food diary and the fact that she had been using beet sugar exclusively for the past two years. An individual food test with beets was followed by somnolence beginning at twenty minutes and a severe frontal and occipital headache at sixty minutes which persisted for the remainder of the day. The following morning she passed several diarrheic stools and there was a definite flare of the dermatitis of her hands, they being more pruritic and erythematous than any time in several recent days. The patient also developed a port wine colored urine the morning after eating beets.

She was then fed 50 Gm. of beet sugar after not having eaten any type of beet product for several days; this was followed by sleepiness at five minutes which interfered with her ability to read comprehendingly. She complained of feeling nervous and tense and particularly of restlessness of her legs. Associated with the leg symptoms was a sensation of tightness and stiffness of the knee joints and the desire alternately to flex and extend the knees.

*Furnished through the courtesy of A. E. Staley Mfg. Co., Decatur, Ill.

With the complete avoidance of beets the patient noticed relief of her formerly troublesome arthritis in that she remained free of the stiffness and soreness of her knees and finger joints and was able to move them painlessly through their full range of motion.

The dermatitis of her hands flared from contact with cornstarch in ironing or with wheat flour in the course of baking. It is interesting, however, that she was able to iron without an accentuation of the dermatitis if she used arrowroot starch in place of cornstarch or if she only inhaled the fumes of baking wheat-containing products and avoided direct contact of her hands with uncooked flour.

Because of the exceedingly high degree of specific sensitivity to corn and beets in this patient and the fact that the ingestion of either corn sugar (glucose or dextrose) or beet sugar (sucrose) caused an immediate accentuation of her symptoms, she was fed experimentally 50.0 Gm. of alpha D glucose* prepared from beet sucrose with the understanding that the source material was cane sugar. As she had tolerated the ingestion of cane sugar over a period of many months without any suspicion of specific sensitivity, she did not expect a reaction from this test. Much to her surprise, however, she developed a dull frontal headache within five minutes after the second serving of 25.0 Gm. of this material. Within the following hour her headache became progressively more severe and within the second hour she developed anorexia, nausea, and considerable bloating. She remained extremely fatigued and "generally upset" for the following two days. The type of reaction that she experienced prompted her to accuse us of feeding her beet sugar.

On another occasion she was fed experimentally an "unknown" preparation having a salty taste, actually one-half teaspoonful of monosodium glutamate of beet origin in one-half glass of water. She complained of itching of the anterior neck and upper chest between eight and ten minutes after the test feeding. The pruritus spread rapidly to involve the entire neck, being particularly intense about the hair line, and the sites of the previous dermatitis of her fingers. Coincident with the onset of the pruritus she complained of a sharp pressure in the frontal and occipital areas of her head followed immediately by an intense, throbbing, generalized headache. At fifteen minutes she noted an inability to focus her eyes and for the following half hour was unable to read or to see objects clearly. She complained of mild abdominal cramps and nausea beginning at forty-five minutes which persisted for the following ten minutes. The intense headache as well as the blurring of vision had subsided sufficiently at sixty minutes that she was able to read ordinary print. She remained subject to palpitation on exertion and extreme fatigue for the remainder of the day. In retrospect, she likened the early phase of this reaction to the constitutional symptoms she had previously experienced from hay fever therapy, making the statement, "You know, it was the same type of immediate reaction that I get from a hay fever shot that subsequently developed into a severe reaction."

With the avoidance of incriminated foods the patient has had complete relief of her formerly troublesome allergic symptoms with the exception of ragweed hay fever. She refused specific therapy with ragweed extract in view of the repeated severe constitutional reactions that she had formerly experienced in this connection. She also refused to be tested with further unknown solutions and then specifically with monosodium glutamate of wheat and of corn origin when the nature of these proposed tests were explained to her. It is of interest that the detection of beet sensitivity and the avoidance of foods containing beet sugar completed her specific food diagnosis.

M. A., a 38-year-old woman, had been subject to chronic nasal symptoms, intermittent canker sores, and frequent severe incapacitating headaches all her life. The headaches were generalized in location, associated with pulling, drawing, and marked tenderness of the posterior cervical muscles, and commonly with nausea and vomiting. In addition she remained subject to marked fatigue, considerable irritability and nervousness, and a dull constant type of headache persisting between the more acute and incapacitating episodes of head pain.

*Prepared through the courtesy of Dr. Robert C. Hockett, Research Director, Sugar Research Foundation, New York City, N. Y.

Although she had suspected several minor foods of bringing on headaches and other allergic symptoms, their complete avoidance had failed to bring about a significant degree of relief.

When first seen in 1947 she was found to be moderately dust sensitive, but each of three attempts to measure her degree of house dust allergy by testing with serial intradermal doses in accordance with a previously described technique[9] resulted in immediate nasal stuffiness followed in one-half hour by the development of a headache. House dust therapy at very low levels was moderately effective in controlling her nasal symptoms but did not change the course of the headaches, fatigue, or myalgia.

As a result of performing individual food tests with several major foods she was found highly sensitive to potato, developing a very severe headache immediately following this test.

On a program of dust avoidance, specific low level therapy with house dust extract and the elimination of potatoes and other minor incriminated foods she remained free of troublesome symptoms until April, 1949. At this time she developed an attack of bronchitis after massive dust exposure. Intractable coughing persisted during the following four months in spite of repeated efforts to diagnose specific allergic reactions and various types of medications, including oft-repeated doses of Demerol and other opiates. The patient frequently lost consciousness in the more violent attack of coughing.

All sources of beet and cane were avoided simultaneously in preparation for individual food tests. Her coughing ceased for the first time in several months for twenty-four hours prior to the beet test. Eighteen minutes after the ingestion of 20 Gm. of granulated beet sugar the patient developed a severe paroxysm of coughing associated with generalized chilling and followed by the onset of nausea and a very severe headache. The coughing became progressively more severe during the following half hour and she passed into a state of semiconsciousness. Although it was possible to arouse her from the stupor, she subsequently had no recollection of the experience. Intractable coughing and headache persisted for the following three days and required massive doses of opiates and antihistaminics for partial relief. A similar but somewhat less severe clinical reaction followed the experimental ingestion of cane sugar.

With the avoidance of beet and cane in addition to other foods, the patient remained free of headaches and coughing for the following week.

She was then discharged from the hospital during the height of the ragweed pollenating season and drove a distance of 150 miles to her home. Midway on this trip she developed her initial attack of ragweed pollinosis and for the following week continued to have very severe hay fever although she remained relatively free of coughing and headache. She was then rehospitalized, and according to attendant nurses, sneezed, sniffled, or coughed a total of 640 times in a twenty-four-hour period prior to the administration of adrenocorticotropic hormone (ACTH, Armour). This aspect of her case has been reported elsewhere.[11] Suffice it to say that the hay fever and all other allergic symptoms completely subsided within twenty-four hours after the patient received the first dose of ACTH. She received a total of 125.0 mg. in five divided doses given at six-hour intervals.

Two weeks following the initial beet test, at a time when the patient was completely free of allergic symptoms, an individual food test with beet sugar was repeated and was not associated with reactive symptoms. The cane test was repeated similarly and tolerated. She was then returned to a general diet and continued on such for the following four months without developing any of her formerly troublesome allergic manifestations. The only other medication that the patient received during this interim was a continuation of the maintenance dose of estrogen that she had been receiving for many months prior to the diagnostic allergy studies and initial course of ACTH. However, this patient had a recurrence of allergic manifestations following massive dust exposure four months after the initial therapy with ACTH and again responded with symptomatic relief after additional therapy with ACTH.

DISCUSSION

Beet sensitivity is not an uncommon cause of chronic allergic symptoms. Although there is some reason to believe that beet allergy occurs most frequently in areas where beet sugar is used predominately, it should be pointed out that the use of beet sugar is more widespread than is commonly believed as it is employed in the preparation of commercially processed foods which have a national market. It is significant that beet sugar carries sufficient allergenicity to cause symptoms in many beet-sensitive patients.

In addition to beets as a vegetable and sugar derived from beets, another source of exposure to beet is the use of monosodium glutamate employed as a condiment or seasoning agent, some of which is derived from the protein-containing waste of beet sugar mills. A similar product from the chemical standpoint is manufactured from wheat, another from corn, and probably a smaller amount from soy beans. Samples of monosodium glutamate of beet, corn, and wheat origin have been shown to cause allergic symptoms in at least certain patients possessing high degrees of specific sensitivity to these foods. The amount of wheat monosodium glutamate ordinarily added to a steak has been incriminated as a cause of allergic symptoms in a few wheat-sensitive individuals. A steak of similar size and eaten under similar test conditions but prepared without the addition of wheat monosodium glutamate has not been followed by reactive symptoms in these individuals. Thus far we have not encountered detectable clinical reactions in beet-sensitive patients from the amount of monosodium glutamate of beet origin ordinarily added to a steak in the process of preparing it. Neither have 900 mg. of beet monosodium glutamate, the amount which may be added to a steak for instance, been followed by detectable symptoms when this amount is deliberately added to meat and fed "blindly" to a known beet-sensitive individual. This amount has recently been tested in three additional highly beet-sensitive individuals. However, as there is no doubt that larger amounts of monosodium glutamate of beet origin are capable of eliciting detectable reactions when added to water and ingested by certain beet-sensitive patients, the possibility that accidental exposures of this type in smaller amounts might be a cause of symptoms in certain individuals should be kept in mind.

The study of this problem has been handicapped by the failure of manufacturers to indicate the source materials employed in the manufacture of monosodium glutamate. Until this question can be answered, the absolute avoidance of beets in preparation for individual food tests should attempt to eliminate this possible source of contamination. The same holds for the avoidance of wheat, corn, and soy beans.

Similarly, the failure of federal labeling regulations to differentiate the types of sugar employed in processed foods, that is whether it is of beet, cane, or corn origin, is a further serious handicap to the allergist engaged in the problem of making specific food diagnoses. It is a particularly vexing problem for the allergic individual who happens to be sensitive to one or more of these specific sugars when he is unable to determine by the information on the

label of a given product whether it contains one or more of these particular sugars which he is attempting to avoid. This point of view has recently been presented before the Public Hearings of the Food and Drug Administration in respect to the Proposed Standards of Identity of Breads and Related Foods.[12]

Likewise, the utter lack of labeling regulations pertaining to the declaration of the specific food agents employed in the manufacture of various types of alcoholic spirits entails further difficulties for the beet-sensitive patient in view of the widespread preference for beet sugar in the preparation of liquors, cordials, and other rectified spirits. Beet sugar is preferred in this connection because, as a rule, it has a lower content of dextrans or polysaccharide contaminations which precipitate in the presence of alcohol. Cane sugar, ordinarily higher in dextran content,[13] tends to result in the development of slight turbidity when mixed with alcoholic spirits. Incidentally, beet sugar in the presence of alcohol seems to be particularly effective in producing the symptoms of beet sensitivity.

Several beet-sensitive patients have been observed to develop anthocyanimuria (vegetable pigments in urine) following the ingestion of beets. This confirms Zindler's[11] original observations of the frequency of beet pigments in the urine when beets are ingested by specifically sensitive allergic individuals.

Any of the various types of allergic manifestations may result from beet sensitivity; these include not only asthma, rhinitis, urticaria, atopic dermatitis, headache, and gastrointestinal symptoms but also allergic myalgia,[15, 16] fatigue,[17, 18] and certain mental symptoms of allergic origin.[19] The latter manifestations were illustrated in several of the case histories of this presentation. For reasons not yet apparent, refined sugars of beet or cane origin seem to be particularly effective in producing these constitutional symptoms of allergic etiology in specifically sensitized individuals.

SUMMARY

Clinical evidence bearing on the significance of beet sensitivity is presented. Specific symptoms in beet-sensitive individuals not only develop from the ingestion of beet as a vegetable but also occur from eating granulated beet sugar and large experimental doses of monosodium glutamate of beet origin prepared from the waste of sugar beet processing mills.

The widespread use of products derived from beets, particularly beet sugar, and inexact labeling regulations pertaining to commercially processed foods, alcoholic beverages, and pharmaceutical preparations makes the specific avoidance of beets a difficult task for the individual who happens to be specifically sensitive to beets.

REFERENCES

1. Duke, W. W.: Asthma, Hay Fever, Urticaria and Allied Manifestations of Allergy, St. Louis, 1926, C. V. Mosby Company.
2. Rinkel, H. J.: Personal communication.
3. Zindler, G. A.: Personal communication.
4. Randolph, T. G., and Yeager, L. B.: Corn Sugar as an Allergen, Ann. Allergy 7: 651, 1949.

5. Randolph, T. G., Rollins, J. P., and Walter, C. K.: Allergic Reactions Following the Intravenous Injection of Corn Sugar (Dextrose or Glucose). To be published.
6. Randolph, T. G., and Rollins, J. P.: Allergic Reactions From the Ingestion or Intravenous Injection of Cane Sugar (Sucrose). J. LAB. & CLIN. MED. 36: 242, 1950.
7. Rinkel, H. J., Randolph, T. G., and Zeller, M.: Food Allergy, Springfield, Ill., Charles C Thomas. In press.
8. Flavor and Acceptability of Monosodium Glutamate, Proceedings of the Symposium, March 4, 1948, The Stevens Hotel, Chicago, Ill., sponsored jointly by: The Quartermaster Food and Container Institute for the Armed Forces and Associates, Food and Container Institute, 1940, West Pershing Road, Chicago 9, Ill.
9. Rinkel, H. J.: Inhalant Allergy. III. The Coseasonal Application of Serial Dilution Testing (Titration), Ann. Allergy 7: 639, 1949.
10. Randolph, T. G.: House Dust Allergy. To be published.
11. Randolph, T. G., and Rollins, J. P.: Relief of Allergic Diseases by ACTH Therapy, Proceedings of the First ACTH Conference, Philadelphia, 1950, The Blakiston Company, p. 479.
12. Docket No. FDC—31 (B) Before the Administrator, Federal Security Agency, In the Matter of a Definition and Standard of Identity for Bread and Related Products, Aug. 3, 1949, pp. 14,593-14,628.
13. Neill, J. M., Sugg, J. Y., Hehre, E. J., and Jaffe, E.: Serological Studies on Sugar. II. Reactions of the Antiserums of Type 2 Pneumococcus and of Leuconostoc mesenteroides With Cane and Beet Sugars and With Cane Juice, Am. J. Hyg. 34: 65, 1941.
14. Zindler, G. A.: Anthocyanuria and Beet Allergy. To be published.
15. Randolph, T. G.: Allergy as a Cause of Nuchal Myalgia and Associated Headache, Arch. Otolaryng. 50: 745, 1949.
16. Randolph, T. G.: Allergic Myalgia. To be published.
17. Rowe, A. H.: Allergic Toxemia and Migraine Due to Food Allergy, California & West. Med. 33: 785, 1930.
18. Randolph, T. G.: Fatigue and Weakness of Allergic Origin; To Be Differentiated From "Nervous Fatigue" or Neurasthenia, Ann. Allergy 3: 418, 1946.
19. Randolph, T. G.: Mental Symptoms of Allergic Origin. To be published.

Arch. Int. Med. 69:670-690. 1942.

RENAL LESIONS FOLLOWING THE INTRAVENOUS INJECTION OF A HYPERTONIC SOLUTION OF SUCROSE

A CLINICAL AND EXPERIMENTAL STUDY

R. H. RIGDON, M.D.
AND
E. S. CARDWELL, M.D.
MEMPHIS, TENN.

Sucrose has been widely used for tissue dehydration in such conditions as cerebral edema associated with trauma,[1] chronic hypertension,[2] acute alcoholism,[3] status asthmaticus,[4] postoperative anemia,[5] edema of nephrosis and nutritional edema.[6]

The quantity and the concentration of sucrose given to patients have varied widely. Dyar and Matthew [7] gave 400 cc. of a 25 per cent solution intravenously. These investigators found no contraindication to the use

From the Department of Pathology, University of Tennessee.

This study was aided by a grant from the University of Tennessee.

1. Hahn, E. V.; Ramsey, F. B., and Kohlstaedt, K. G.: Clinical Experience in the Use of Sucrose Instead of Dextrose in the Osmotic Therapy of Increased Intracranial Pressure Occurring in Cases of Acute Brain Injury, J. A. M. A. **108**:773 (March 6) 1937. Jackson, H.; Dickerson, D., and Gunther, A.: The Reduction of Intracranial Pressure in Cerebral Injury by the Intravenous Use of Hypertonic Sucrose Solution, Ann. Surg. **106**:161, 1937. Gotten, N.: Head Trauma, J. A. M. A. **110**:1727 (May 21) 1938.

2. Murphy, F. D.; Hershberg, R. A., and Katz, A. M.: The Effect of Intravenous Injections of Sucrose Solution (50 Per Cent) on the Cerebrospinal Fluid Pressure, the Blood Pressure and Clinical Course in Cases of Chronic Hypertension, Am. J. M. Sc. **192**:510, 1936.

3. Hilton, J. P., and Alderson, D. M.: Sucrose Solution in the Treatment of Alcoholism and Confusional States, Rocky Mountain M. J. **35**:227, 1938.

4. Keeney, E. L.: The Effectiveness of Intravenous Hypertonic Sucrose and Adrenalin in the Treatment of Status Asthmaticus, J. Allergy **9**:497, 1938.

5. Strahm, J. G., and Osgood, S. B.: Intravenous Sucrose as a Diuretic, Northwest Med. **35**:89, 1936. Helmholz, H. F.: Sucrose as a Diuretic, J. Lab. & Clin. Med. **25**:1180, 1940.

6. Lowenburg, H., and Nemser, S.: Intravenous Injection of Fifty Per Cent Solution of Sucrose in Edema, Arch. Pediat. **53**:762, 1936. Lowenburg, H., and Miller, A. B.: A Case of Nutritional Edema, ibid. **55**:297, 1938.

7. Dyar, E. W., and Matthew, W. B.: Use of Sucrose Preparatory to Surgical Treatment of Glaucoma, Arch. Ophth. **18**:57 (July) 1937.

of sucrose except perhaps the presence of markedly low renal function. Masserman [8] has given intravenously as much as 500 cc. of a 50 per cent solution of sucrose. He expressed the opinion that "comparatively large amounts of sucrose in the circulation are not toxic and cause no serious disturbances in the chemistry or cytology of the blood." Keith and Power [9] found "the total excretion of the various urinary constituents during a day in which sucrose is injected but slightly altered aside from changes that may be attributed to diuresis." Murphy and his associates [2] gave 300 to 500 cc. of a 50 per cent solution of sucrose to 21 patients with chronic hypertension. The patients improved, and no unfavorable results were noted from the treatment.

Cutler [10] apparently was the first to give any information as to the quantity of sucrose that may safely be administered to patients. He concluded from his histologic studies of human kidneys that sucrose may produce renal lesions when approximately 6 to 10 Gm. per kilogram of body weight is given in a week.

The renal lesions that may occur in man after the injection of sucrose have been described by Helmholz,[11] Cutler,[10] Anderson and Bethea [12] and Anderson.[13] The pathologic process has been nicely described and well illustrated by the two last-named authors. The epithelial cells in the convoluted portion of the renal tubules become markedly swollen, and the cytoplasm is foamy. This change has been observed both as a focal and as a diffuse lesion.

In reviewing the clinical cases reported by Anderson and Bethea [12] and Anderson [13] we have been impressed with the fact that other investigators have given more sucrose to their patients without the development of any renal lesions. Anderson [13] discussed the probability that "pre-existing renal lesions may enhance the effects of sucrose on

8. Masserman, J. H.: Effects of the Intravenous Administration of Hypertonic Solutions of Sucrose, Bull. Johns Hopkins Hosp. **57**:12, 1935.

9. Keith, N. M., and Power, M. H.: The Urinary Excretion of Sucrose and Its Distribution in the Blood After Intravenous Injection into Normal Men, Am. J. Physiol. **120**:203, 1937.

10. Cutler, H. H.: Effect of Sucrose on the Kidney, Proc. Staff Meet., Mayo Clin. **14**:318, 1939.

11. Helmholz, H. F.: Renal Changes in the Rabbit Resulting from Intravenous Injection of Hypertonic Solution of Sucrose, J. Pediat. **3**:144, 1933.

12. Anderson, W. A. D., and Bethea, W. R., Jr.: Renal Lesions Following Administration of Hypertonic Solutions of Sucrose, J. A. M. A. **114**:1983 (May 18) 1940.

13. Anderson, W. A. D.: Sucrose Nephrosis and Other Types of Renal Tubular Injuries, South. M. J. **34**:257, 1941.

the tubules so that changes are produced from relatively small amounts." He asserted:

> Sucrose nephrosis was found to be severe in kidneys involved by marked vascular disease. It may well be that interference with renal circulation by vascular change, or a similar effect produced by thickening of glomerular basement membrane, will markedly enhance the damaging effect of sucrose. Particularly if there was a reduction in filtration rate, it would operate to prolong the exposure of the epithelial cells to the sucrose in the filtrate.

In considering Anderson's explanation of the effect of previous renal disease on the development of tubular lesions following the injection of sucrose, it must be remembered that Murphy, Hershberg and Katz [2] gave 300 to 500 cc. of a 50 per cent solution of sucrose to 21 patients with chronic hypertension. This treatment relieved such symptoms as headache, vomiting, vertigo, twitching and dizziness. No unfavorable results were observed in any of the 21 patients.

Lindberg and associates [14] gave 200 cc. of a 50 per cent solution of sucrose to 15 persons without any renal damage.

> In no case was there evidence of marked impairment of renal function. An occasional specimen of urine after the injection showed a 1 plus reaction for albumin or hyaline casts. The urea clearance remained essentially stationary, and in some cases it was increased by 3 to 5 per cent . . . Five patients with known renal damage were similarly studied. In these, too, the urine and urea clearance showed no significant changes as a result of the injection of 200 cc. of sucrose intravenously.

The following factors have impressed us in reviewing the autopsy reports on a group of patients who were given sucrose: (1) the quantity of sucrose administered; (2) the state of dehydration at the time the sucrose was injected, and (3) the fluid intake and output during the period in which sucrose was given. The following case reports will illustrate these factors.[15]

REPORT OF SIXTEEN CASES

Case 1.—A white man aged 64 became comatose two days before admission. He lived four hours after hospitalization and only three hours after the intravenous injection of 200 cc. of a 25 per cent solution of sucrose.

Generalized arteriosclerosis, with an occlusion in the left coronary artery and a recent myocardial infarct, was evident at autopsy. Scars were also present

14. Lindberg, H. A.; Wald, M. H., and Barker, M. H.: Renal Changes Following Administration of Hypertonic Solutions, Arch. Int. Med. 63:907 (May) 1939.

15. Cases 1, 2, 3, 4, 6, 7 and 10 have been included in previous papers by Anderson and Bethea [12] and Anderson.[13] Dr. W. D. Forbus, professor of pathology, Duke University, has permitted us to include cases 13 to 16. Dr. A. J. Gill, of Duke University, summarized the histories of these cases.

in the myocardium. The cavities of the heart were dilated, and the viscera were congested. Bronchopneumonia was present. The renal arteries were sclerotic. The epithelial cells in the convoluted portion of the tubules were swollen, and their cytoplasm was foamy.

The three hour interval between the injection of sucrose and death was the shortest period which has elapsed between injection of sucrose and postmortem study of the kidneys in this series. It illustrates the rapidity with which the renal lesion may develop. The histologic changes in this case were similar to those observed in cases in which death occurred several days after the injection of sucrose. The fluid intake no doubt was small before hospitalization, since the patient was unconscious. The amount of fluid lost through the skin should have been large, since he died in June. This case illustrates the role that dehydration may play in the subsequent development of tubular changes following the injection of sucrose.

There was no evidence from the histologic studies that a pathologic condition existed in the kidneys before the patient received the sucrose.

Case 2.—A Negro aged 67 gave a history of a severe headache of three months' duration. He became drowsy, and the muscles of the upper extremities began to twitch. He became unconscious three hours before admission and remained comatose until death. He died thirty-six hours after hospitalization.

The temperature on admission was 104 F., the pulse rate 130 per minute, the respiratory rate 32 to 40 per minute and the blood pressure 140 systolic and 85 diastolic. The spinal fluid pressure equaled 218 mm. of water. The specific gravity of the urine was 1.023, and the albumin present gave a 2 plus reaction. Occasional white blood cells and casts were found in each high power field. The blood contained 30 mg. of nonprotein nitrogen per hundred cubic centimeters. The numbers of white cells and red cells and the quantity of hemoglobin in the blood were normal.

During the thirty-six hours of hospitalization the patient was given a total of 1,000 cc. of a 25 per cent solution of sucrose intravenously and 14 cc. of a 50 per cent solution of magnesium sulfate intramuscularly.

Generalized arteriosclerosis was observed at autopsy. Small hemorrhages were present in the endocardium. The prostate gland was hypertrophied. Bronchopneumonia was present. The cortex of the kidneys was slightly scarred. Apparently every epithelial cell in the convoluted portion of the renal tubules was markedly swollen, and the cytoplasm was foamy. An albuminous precipitate was present in Bowman's space in some of the glomeruli and in the lumen of some of the tubules.

This patient apparently had some renal insufficiency before receiving the sucrose. There are not enough data to determine the state of hydration.

Case 3.—A white man aged 74 gave a typical history and showed the classic signs of myocardial failure on admission. A systolic murmur could be heard over the entire precordium. The temperature was 96.4 F., the respiratory rate 30 per minute, the pulse rate 88 per minute and the blood pressure, 130 systolic and 70 diastolic. The patient lived only a day and a half in the hospital. On admission the blood contained 30 mg. of nonprotein nitrogen per hundred cubic centimeters. Eleven and a half hours before death he was given 200 cc. of a 25 per cent solution of sucrose intravenously.

Healed rheumatic endocarditis of the aortic and the mitral valve, with stenosis, was evident at autopsy. The heart was hypertrophied, and the cavities

were dilated. Chronic passive congestion and generalized arteriosclerosis were present. Precipitated protein was present in Bowman's space in some of the glomeruli. Apparently, every epithelial cell in the convoluted portion of the renal tubules was swollen, and the cytoplasm was foamy.

The presence of a normal amount of nonprotein nitrogen in the blood on admission would suggest a relatively normally functioning kidney. Protein in Bowman's spaces, observed histologically, may have resulted from the chronic passive congestion. This case illustrates the diffuse tubular injury that may follow the intravenous injection of sucrose. It is the only case in this series in which the patient had severe edema at the time the sucrose was given.

Case 4.—A white woman aged 69 became unconscious at 7:30 a. m., while eating breakfast, and thereafter was unable to speak. She vomited dark, blood-like material on several occasions. Thirty minutes after this accident the patient was admitted to the hospital. She was unconscious but apparently not completely paralyzed. The temperature was 97 F., the pulse rate 96 per minute, the respiratory rate 36 per minute and the blood pressure 145 systolic and 68 diastolic. The spinal fluid was bloody. She was given 2 cc. of a 50 per cent solution of magnesium sulfate intramuscularly and 100 cc. of a 25 per cent solution of sucrose intravenously. Death occurred fourteen hours after the cerebral accident and twelve hours after the intravenous injection of sucrose.

A ruptured aneurysm at the bifurcation of the right internal carotid artery was evident at autopsy. Blood had dissected into the ventricles of the brain. The cavities of the heart were dilated, and the viscera showed the changes associated with venous congestion. The epithelial cells in the convoluted portion of the renal tubules were swollen and granular. The cytoplasm of these cells was not as "foamy" as has been observed in other patients given sucrose. There were a few scars in the cortex and focal collections of lymphocytes in the interstitial tissues.

The quantity of sucrose given to this patient was only half that given to others in whom tubular lesions occurred. Nothing in the kidneys indicated previous renal disease. The tubular changes which may have resulted from the administration of sucrose were indefinite. This case is important only because of the small quantity of sucrose which was administered and the absence of definite tubular lesions attributable to the sucrose.

Case 5.—A white man aged 68 fell unconscious approximately three hours before admission to the hospital. After a few minutes consciousness returned; however, within thirty minutes he again lapsed into coma. He was paralyzed on the right side of the body. Coma persisted, and death occurred forty-eight hours after admission to the hospital.

The patient was given 84 ounces (2,484 cc.) of milk and fruit juices through a Levin tube during hospitalization. Twelve hours before death he was given 100 cc. of a 25 per cent solution of sucrose intravenously.

A generalized arteriosclerotic process, with a cerebral hemorrhage in the area of the right internal capsule, was apparent at autopsy. The heart was hypertrophied, and the cavities were dilated. The viscera were congested. Arteriosclerosis was present both in the large and in the small arteries of the kidneys. The arterioles also were thickened. Scars and collections of lymphocytes were present in the interstitial tissue of the renal cortex. The epithelial cells lining the convoluted portion of the tubules were swollen, and the cytoplasm was finely

granular. The changes resembled those observed in cloudy swelling more than they did the tubular lesions that occur after the injection of sucrose.

The tubular lesions in this case may have been the result of the administration of sucrose; however, at this time there is nothing by which we can differentiate them from cloudy swelling. There are insufficient data to determine the degree of renal function and the state of hydration at the time the sucrose was given.

CASE 6.—A Negro aged 33 gave a history of severe, generalized headache and dizziness of three months' duration, spots before the eyes and progressive failure of vision. Massive hemorrhages, papilledema and exudates were present in the retina.

The blood pressure was 220 systolic and 163 diastolic, the temperature 99 F., the pulse rate 100 per minute and the respiratory rate 32 per minute. The urine had a specific gravity of 1.016 and gave a 2 plus reaction for albumin. Microscopic examination of an uncentrifuged specimen showed 5 to 8 white cells, an occasional red cell and 1 granular cell per high power field. The spinal fluid pressure equaled 300 mm. of water. There was moderate anemia. The hemoglobin content was 11.5 Gm. per hundred cubic centimeters of blood. On the third day in the hospital the patient became nauseated and vomited; he appeared lethargic and progressively became comatose. He died eight hours after the last injection of sucrose. The following data appear to be important:

Date	Course of Illness	Magnesium Sulfate, Orally, Oz.	25% Solution of Sucrose, Intravenously, Cc.	5% Solution of Dextrose, Intravenously, Cc.	Nonprotein Nitrogen in Blood, Mg. per 100 Cc.	Output of Urine, Cc.
5/2/29	Admission	...	..	.....	...	.....
5/4/29	.....	1.5	...	....	75	2,600
5/4/29	.....	...	...	2,000	80	900
5/5/29	.....	1.5	750	.....	...	3,500
5/6/29	.....	...	..	.....	..	400
5/7/29	.....	1.5	...	500	...	1,300
5/9/29	Death	...	500	1,500	130	700

Severe malignant arteriosclerosis was evident at autopsy. The heart was hypertrophied, and the cavities were dilated. Chronic passive congestion of the viscera and bronchopneumonia were present. The epithelial cells of many of the renal tubules were swollen, and the cytoplasm was foamy. The cells lining other convoluted tubules were small and stained deeply with hematoxylin. These did not show the swelling that may accompany the injection of sucrose.

The clinical and pathologic findings indicated severe nephritis, with renal insufficiency. This was present when the patient was admitted. The quantity of sucrose and magnesium sulfate given to this man apparently was sufficient to produce some dehydration. The most interesting pathologic process observed in this case resulted from the distribution of the renal lesions. The low cuboidal cells lining the convoluted portion of some renal tubules were not affected, while corresponding cells in other tubules were swollen and the cytoplasm was foamy.

CASE 7.—A Negress aged 60 complained of a sharp pain in the right temporal region on the day preceding admission to the hospital. At 2 a. m. on the day of admission she was found unconscious in bed. She was admitted to the hospital sixteen hours afterward. Coma was present and persisted until death, which

occurred twelve hours later. All the muscles were spastic. The reflexes were hyperactive in the upper extremities. The temperature was 101 F., the pulse rate 120 per minute, the respiratory rate 38 per minute and the blood pressure 200 systolic and 120 diastolic. The urine gave a 4 plus reaction for albumin. On admission the patient was sweating profusely. Her husband stated that she also sweated much during the night.

The patient was given 4 cc. of a 50 per cent solution of magnesium sulfate intramuscularly and 250 cc. of a 25 per cent solution of sucrose intravenously immediately after admission to the hospital. She died eleven hours after the injection of the sucrose.

Necropsy showed generalized arteriosclerosis and a cerebral hemorrhage into the right internal capsule. The blood had dissected into the ventricles. The heart was hypertrophied. There was an extensive arteriolosclerotic process in the kidneys. Some of the glomeruli were fibrosed. Areas of fibrosis and foci of lymphocytes were present in the cortex. In some focal areas the convoluted portion of the tubules were swollen and the cytoplasm was foamy. In other areas the epithelial cells were small and pyknotic.

There was both clinical and pathologic evidence of renal insufficiency. It was present preceding the injection of the sucrose. The absence of tubular lesions in those portions of the convoluted tubules in which the cells appeared to be of the regenerative type was interesting in view of the fact that a similar process was observed in cases 6 and 8. This patient, no doubt, was dehydrated on admission, since she had been unconscious for about sixteen hours. There was also a history of profuse sweating.

Case 8.—A Negro aged 65 was paralyzed on the left side of the body on admission. No history was obtained. The spinal fluid was xanthochromic. The temperature was 99 F., the pulse rate 66 per minute, the respiratory rate 18 per minute and the blood pressure 134 systolic and 100 diastolic. The Kahn reaction of the blood was negative. A specimen of urine obtained by catheterization had a specific gravity of 1.024 and showed neither albumin nor sugar. The blood contained 30 mg. of nonprotein nitrogen per hundred cubic centimeters. There was severe anemia.

On the seventh day before his death the patient was given 100 cc. of a 50 per cent solution of sucrose intravenously and on the following day an additional 50 cc. Clinical signs of bronchopneumonia developed on the third day before death. The urinary output for six of the seven days preceding death was as follows: seventh day, 550 cc.; sixth day, 300 cc.; fifth day, 850 cc.; fourth day, 550 cc.; third day, 440 cc., and second day, 1,300 cc.

Generalized arteriosclerosis was evident at autopsy. A hemorrhage in the area of the internal capsule on the right side had ruptured into the lateral ventricle. Bronchopneumonia was present. Some of the glomeruli were fibrosed. The walls of a few of the arterioles were thickened. Hyaline casts were present in the lumens of some of the tubules. The epithelial cells in the convoluted tubules in focal areas of the kidneys were swollen, and the cytoplasm was foamy.

There was little if any evidence from either the clinical or the autopsy observations to indicate that renal disease existed at the time the patient was admitted to the hospital. The urinary output was low. This apparently was the result of a low intake of fluid. The tubular lesions were characteristic of those that may follow the injection of sucrose. The focal distribution of these lesions was interesting, since they occur often uniformly throughout the kidneys.

CASE 9.—On admission to the hospital a Negro aged 65 gave a history of severe headaches, occasional attacks of dizziness and pain in the back of three weeks' duration. The muscles of the upper extremities and the shoulders contracted involuntarily. The blood pressure was 270 systolic and 140 diastolic. A specimen of urine obtained by catheterization had a specific gravity of 1.012 and gave a 3 plus reaction for albumin. Microscopic examination of an uncentrifuged specimen showed 3 to 5 white cells per high power field. The white cell count was 21,100 and the red cell count 3,890,000 per cubic millimeter. There was 9.5 Gm. of hemoglobin per hundred cubic centimeters of blood. The Kahn reaction was negative.

The patient became stuporous and then comatose. He died six days after admission.

The following pertinent data were recorded day by day:

Date	Course of Illness	50% Solution of Magnesium Sulfate, Intramuscularly, Cc.	35% Solution of Sucrose, Intravenously, Cc.	Solution of Dextrose			Non-protein Nitrogen in Blood, Mg. per 100 Cc.	Output of Urine, Cc.
				Amount, Cc.	Concentration	Route *		
4/20/39	Admission	16	200	2,000	5%	I.V. S.C.	105	8,800
4/23/39	.....	16	400	4,000	5%	I.V. S.C.	...	3,400
4/24/39	.....	8	950	4,000	5%	I.V. S.C.	...	5,350
4/25/39	.....	12	750	3,000	5% 10%	S.C. I.V.	...	2,600
4/26/39	.....	8	400	3,000	5% 10%	S.C. I.V.	107	2,600
4/27/39	.....	4	600	2,000	5% 10%	I.V. S.C.	...	400
4/28/39	Death	8	225	2,000	5% 10%	I.V. S.C.	...	...

* I.V. indicates intravenous; S.C., subcutaneous.

Autopsy revealed hypertrophy of the prostate gland, acute urethritis, acute cystitis and acute and chronic pyelonephritis. Pulmonary emboli and bronchopneumonia were present in both lungs. Arteriosclerotic nephritis was present, with red blood cells and albumin in the lumens of the renal tubules. Essentially every epithelial cell in the convoluted portion of the tubules was swollen, and the cytoplasm was foamy.

There was definite renal insufficiency at the time the patient was admitted to the hospital. The daily intake and output of fluid apparently were sufficient. The quantity of sucrose (3,625 cc.) that the patient received during a period of seven days apparently was too much regardless either of the fluid balance or of the state of renal function.

CASE 10.—A Negress aged 15 was approximately nine months pregnant. There was some edema of the feet and ankles. She gave a history of headaches for four or five months. A week before admission she had "blind staggers." Her family stated that the patient had had at least twenty-five convulsions on the day of admission. She was comatose, and edema of the lungs was present at the time of hospitalization.

The urine had a specific gravity of 1.009, gave a 3 plus reaction for albumin and a positive reaction for acetone and contained white and red blood cells. On

two occasions the blood level of nonprotein nitrogen was 26 and 30 mg. per hundred cubic centimeters. The white cell count was 21,000; the red cell count was 3,200,000, and the hemoglobin content was 9.4 Gm. per hundred cubic centimeters of blood.

A stillborn baby was delivered a few hours after admission. The following day the patient's condition was critical. The renal output was small. There were some edema of the extremities at this time and extensive edema of the face. During the patient's stay in the hospital the blood pressure varied markedly from time to time from a low level of 90 systolic and 30 diastolic to a high level of 180 systolic and 110 diastolic. Fever was constantly present, and the temperature varied from 101 to 108 F. Clinical signs of bronchopneumonia developed on the day preceding death.

The fluid intake and output and the quantity of sucrose and magnesium sulfate that this patient was given during hospitalization are tabulated as follows:

Duration of Medication	Fluid Intake, Cc.	Fluid Output, Cc.	Sucrose, 25 per Cent Solution, Cc.	Other Dehydrating Medication
1 day	850	450	50	20 cc. 20 per cent magnesium sulfate, I.V.
2 days	300	250	1,500	6 oz. saturated solution of magnesium sulfate, stomach tube
3 days	2,000	300	600	
4 days	3,000	300	.....	
5 days	3,240	250	.....	20 cc. 50 per cent magnesium sulfate, I.M.
6 days	1,650	250	.....	
7 days	3,050	700	300	
8 days	3,000	420	.....	
9 days	2,000	300	.....	
10 days	1,650	.....	.....	
Total	20,810	3,220+	2,450	

The anatomic diagnosis was as follows: eclampsia and spontaneous delivery; puerperal infection and extensive necrosis of the cervix and the uterine mucosa (a smear of material from the uterus showed Bacillus welchii, Bacillus coli, Staphylococcus aureus and a nonhemolytic streptococcus); fresh thrombi in the blood vessels and the wall of the uterus and in the broad ligament; embolic abscesses in the lungs; acute fibrinous pleurisy in the left lung; focal areas of necrosis in the mucosa of the colon; petechiae in the mucosa of the duodenum; an acute ulcer in the duodenum (traumatic?); hydropic degeneration of the epithelial cells of the kidneys (due to sucrose); decubitus ulcers over the skin of the body; surgical incisions over blood vessels in several areas of the body, and septicemia (causative organisms, Staph. aureus and a nonhemolytic streptococcus).

Essentially every epithelial cell in the convoluted portion of the tubules was swollen, and the cytoplasm was foamy. The lumens of the tubules were occluded. A small amount of albumin was present in some of Bowman's spaces. Hyaline droplets were present in the cytoplasm of some of the tubular epithelial cells. There were no significant changes in the glomeruli.

It is difficult to determine the role that each of the several disease processes may have played in this patient. It may be said, however, that the function of

the kidneys was abnormal on admission, as shown by the specific gravity of the urine and the excessive excretion of albumin. The effect of the bacterial infection would certainly help to lower the renal reserve. It is of interest to observe the small fluid output and the large intake beginning on the third day. Swelling of the epithelial cells, as a result of the injection of the sucrose on the first and the second day of hospitalization, may have been sufficient to reduce the renal output. Edema became more extensive during hospitalization.

Case 11.—A Negress aged 8 gave a history of headache, stiffness of the neck and paralysis of the right side of the body of one week's duration and paralysis of the left side of two days' duration. She had been unable to swallow any food during the past four days and was unable to talk at the time of admission. The spinal fluid showed a trace of sugar and contained 660 mg. of chlorides per hundred cubic centimeters. There were 250 white cells per cubic centimeter of spinal fluid. These were primarily lymphocytes. Cultures of the spinal fluid were sterile. The white cell count was 6,500; the red cell count was 3,050,000, and the hemoglobin content was 10 Gm. per hundred cubic centimeters. The urine was normal. The patient died three days after admission to the hospital. The diagnosis was tuberculous meningitis.

This patient was given sucrose intravenously, and a series of determinations of the nonprotein nitrogen were made. The following data show the amount of fluid the patient received during the period of observation and the nonprotein nitrogen content of the blood at frequent intervals:

Date	Hour	Course of Illness	Administration of Fluids			Nonprotein Nitrogen in Blood, Mg. per 100 Cc.
			Fluid	Amount	Method	
9/15/40	.....	.....	Milk, fruit juice 5% solution of dextrose	960 cc. 2,000 cc.	Levin tube	..
9/16/40	.....	.....	5% solution of dextrose Milk, fruit juice	1,000 cc. 640 cc.	.....	..
	2:15 p.m.	.....	25% solution of sucrose	100 cc.	Intravenous injection	27
	4:30 p.m.	.....	Orange juice	240 cc.	.....	..
	4:45 p.m.	.....	5% solution of dextrose	1,000 cc.	Intravenous injection	..
	8:00 p.m.	.....	Water	6 oz.	Levin tube	31
9/17/40	7:20 a.m.	.....	5% solution of dextrose	2,000 cc.	Intravenous injection	24
			Fruit juice, milk Water	220 cc. 6 oz.	Levin tube Levin tube	
9/18/40	7:30 a.m.	.....	Milk, fruit juice	720 cc.	Levin tube	..
	5:25 p.m.	Death				

The amount of sucrose given to this child was only half the quantity that produced renal lesions in some of the adults included in this series. There was no evidence of renal injury either before or after the injection of sucrose. The fluid intake apparently was adequate.

Case 12.—A Negress aged 27 gave a history of headache of nine days' duration. The spinal fluid pressure was increased. There were 600 white cells, with 92 per cent lymphocytes, per cubic centimeter of spinal fluid. The fluid contained 30 mg. of sugar, 680 mg. of chlorides and 82 mg. of total protein per hundred cubic centimeters. The white cell count was 19,000 and the red cell count only 3,220,000. The urine was normal. A diagnosis of tuberculous meningitis was made. The

patient was given sucrose for its possible effect on the retention of nonprotein nitrogen in the blood. The following data show the quantity of fluids given:

Date	Hour	Course of Illness	Administration of Fluids			Nonprotein Nitrogen in Blood, Mg. per 100 Cc.
			Fluid	Amount, Cc.	Method	
9/14/40	.....	.....	10% solution of dextrose	1,000	.....	..
9/15/40	1:25 p.m.	.....	25% solution of sucrose	100	Intravenous injection	..
	1:35 p.m.	.....	10% solution of dextrose	1,000	Intravenous injection	..
9/16/40	9:50 a.m.	.....	10% solution of glucose	1,000	Intravenous injection	..
	2:30 p.m.	.....	25% solution of sucrose	100	Intravenous injection	28
	5:00 p.m.	.....	.....	.....	.....	33
9/17/40	8:00 a.m.	.....	10% solution of dextrose	1,000	Intravenous injection	33
9/18/40	8:00 a.m.	.....	.....	.....	.....	24
9/19/40	.....	.....	Liquids	.....	Oral administration	..
9/20/40	.....	.....	Liquids	.....	Oral administration	..
9/21/40	.....	.....	.....	.....	.....	25
	8:00 a.m.	.....	.....	.....	.....	23
9/27/40	.....	Death				

The quantity of fluid that this patient received during medication with sucrose was apparently adequate. There was no evidence of renal insufficiency either before or after the injection of sucrose. Each injection of sucrose given to this patient was only half that given to other adults in this series in whom tubular lesions developed.

Case 13.—A white man aged 38 gave a history of a severe headache of two weeks' duration. The patient was semicomatose, mentally confused and extremely dehydrated. He was given 200 cc. of a 50 per cent solution of sucrose and within twenty minutes was able to answer questions intelligently.

There were signs of increased intracranial pressure. A diagnosis of either a brain tumor or a brain abscess was made. On the day following admission he was given 50 cc. of a 50 per cent solution of sucrose at 3:30 p. m. and a second injection of 200 cc. at 10 p. m. A cerebral abscess caused by Pneumococcus type III was drained on the third day of his stay in the hospital. On this same day he was given 200 cc. of a 50 per cent solution of sucrose, and an additional 100 cc. of sucrose solution was administered on the fourth day of hospitalization.

On the third and the fourth day of hospitalization he was also given 200 cc. of a 50 per cent solution of dextrose and 900 cc. of a 10 per cent solution of dextrose. Antipneumococcus serum, sulfamethylthiazole (2-[paraaminobenzenesulfonamido]-4-methylthiazole) and sodium sulfapyridine (the sodium salt of 2-[paraaminobenzenesulfonamido]-pyridine) were given after the evacuation of the abscess. Fluids were not forced because of the patient's condition. The fluid intake apparently reached its low point, 1,204 cc., on the fourth day of hospitalization. The patient died on the fifth day of hospitalization.

Essentially every epithelial cell lining the convoluted portion of the renal tubules was swollen. The cytoplasm was foamy. Some of these cells showed a greater

swelling than did others in corresponding portions of the tubules. There were a few scars in the kidney.

The patient was dehydrated at the time he was given the first injection of sucrose. A hypertonic solution of sucrose was administered on each day of hospitalization. Death occurred on the fifth day. The renal lesions were similar to those observed in rabbits given sucrose during a period of restricted fluid intake.

CASE 14.—A white woman aged 48 gave a history and presented the physical signs of an acoustic nerve tumor. At operation a large tumor was found and was resected piecemeal. The postoperative course was good until the third day. At this time the pulse and respirations became rapid and irregular. It was considered likely that some edema had developed in the region of the medulla and cerebellum. An intravenous injection of 200 cc. of a 50 per cent solution of sucrose was given at this time. The patient died eighteen hours later.

The patient was given several blood transfusions and an average daily injection of 1,500 cc. of saline solution.

Apparently every epithelial cell in the convoluted portion of the renal tubules was swollen. The cytoplasm was granular. Frequently these granules in the cytoplasm were widely separated by clear spaces. The lumens of the tubules were decreased in size, and some precipitated albumin was present in the tubules and in Bowman's spaces.

The renal lesions may be confused in this case with those of advanced cloudy swelling; however, they are consistent with the changes that occur in the rabbit after the injection of sucrose. Lesions such as these apparently represent a "physiologic-pathologic" process in a cell that may readily revert to normal function.

CASE 15.—A Negro aged 25 gave a history of headaches and convulsive seizures during the past five years. A diagnosis of an intracranial neoplasm was made, and at operation a large parasagittal meningioma was removed.

The patient was given several transfusions immediately after the operation. Frequent injections of solutions of dextrose in concentrations of 5 and 10 per cent were given intravenously. The fluid intake appears to have been adequate, especially during the later days of life.

Thirteen days after operation the patient was given 100 cc. of a 50 per cent solution of sucrose intravenously. On the twenty-third day after operation he was given 200 cc. of a 50 per cent solution of sucrose intravenously. Death occurred on the twenty-fourth day after operation and the first day after the second injection of sucrose.

Necropsy showed some precipitated albumin in the interstitial tissue in the area of the medulla of the kidney. Small collections of polymorphonuclear leukocytes were also present in the interstitial tissue. The epithelial cells of the tubules were greatly swollen, and their cytoplasm was foamy. The cytoplasm of some of these epithelial cells was homogeneous and stained deeply with eosin. The nuclei of many of the epithelial cells in the convoluted portion of the tubules were pyknotic, shrunken or absent. Some of the epithelial cells lining Bowman's spaces were swollen, and the cytoplasm was foamy. It was of interest to note that some of the epithelial cells in the convoluted portion of the tubules were apparently smaller than normal and stained more deeply with hematoxylin. These cells suggested those described in other cases as regenerative cells and did not show changes similar to those observed in the other tubules. Albumin, casts and occasional red and white blood cells were present in the lumens of the tubules. The glomeruli were frequently swollen.

The renal lesions were consistent with those that may occur after the injection of large quantities of sucrose.

Case 16.—A white man aged 29 gave a history of chronic sinusitis with severe headache during the past month. After the patient's admission to the hospital signs of a brain abscess developed. At operation the abscess was drained. On the fifth day after operation the patient suddenly became comatose, the temperature rose to 39.3 C. (102.8 F.) and the respirations became rapid and shallow. An opening was made with a trephine over the midfrontal parietal region for the purpose of drainage. The patient died three hours after this operation.

On the day of death the patient was given 200 cc. of a 50 per cent solution of sucrose at 1:30 a. m. and a second injection of 200 cc. at 10:30 a. m. Death occurred at 2:20 p. m. He was given large amounts of dextrose solution in concentrations of 5 and 10 per cent for purposes of combating dehydration. Staphylococcus antitoxin was also given. He received one transfusion of 500 cc. of blood, without a reaction.

Essentially every epithelial cell lining the convoluted portion of the renal tubules was swollen, and the cytoplasm was foamy. Albuminous precipitate was present in the lumens of many of the tubules and in some of the spaces of Bowman.

The intake of fluid apparently was adequate. The patient was not appreciably dehydrated at any time. The renal lesions were similar to those that may occur after the injection of sucrose in experimental animals.

EXPERIMENTAL STUDY

Renal lesions have been produced in rabbits by Helmholz [11] and in dogs by Lindberg, Wald and Barker.[14] The quantity of sucrose administered and the length of time during which the animals were treated varied. Helmholz gave 1 rabbit 3,025 cc. of a 20 per cent solution of sucrose in twenty-one injections over a period of one hundred and eighty-two days. On the day after the last injection the blood contained 116 mg. of urea per hundred cubic centimeters and the excretion of phenolsulfonphthalein was only 5 per cent in two hours. The rabbit was weak and was killed. Focal nephritis was present. The author stated: "The protoplasm of the cells of the convoluted tubules was finely granular and the cells were swollen so as to occlude the lumens of a few of the tubules."

Lindberg and associates [14] gave 100 cc. of a 50 per cent solution of sucrose intravenously to dogs each day for as long as fourteen days. For 1 of these animals, treated for ten days, the microscopic diagnosis was as follows: "Few glomeruli showed retrogressive changes; some hemorrhages into glomerular tufts and changes; early degeneration of convoluted tubules." They described the kidneys from a second dog treated in the same way: "The tubules essentially normal; no marked changes seen in glomeruli." For the kidneys from a third dog given one injection of sucrose and killed twenty-four hours later the following description was given: "Moderate hyperemia of glomerular tuft; proximal and distal convoluted tubules showed intense swelling nearly obstructing tubules; glomeruli were swollen and hemorrhagic."

This wide variation in the susceptibility of dogs and rabbits to the intravenous injection of sucrose is very likely similar to the variations in the frequency of the occurrence of renal lesions in man following the intravenous injection of a hypertonic solution of sucrose. The clinical data suggest that the development of the tubular changes may be influenced by the state of hydration before and during the time sucrose is given. Apparently the factor of "hydration" has not been considered in the experimental production of renal lesions by the injection of sucrose. The experimental study reported here was made to determine the effect of restriction of fluid on the development of renal lesions when a hypertonic solution of sucrose is given intravenously.

Method and Material.—Normal rabbits, weighing 1.5 to 3.0 Kg. each, were used. A 50 per cent solution of sucrose [16] was injected into the marginal veins of the ear in various quantities and at different times and intervals. Blood for the determination of nonprotein nitrogen [17] was taken from the heart. Frequent observations were made on the animals' weight.

The rabbits were fed cabbages, oats and water. In those experiments in which the fluid was restricted only oats were fed.

The rabbits were killed at various intervals. The kidneys were removed and fixed immediately in a 10 per cent concentration of solution of formaldehyde U. S. P. A complete autopsy was performed on some of the animals. Material for sectioning was embedded in paraffin, and the sections were stained with hematoxylin and eosin.

Experiment 1.—A group of 8 rabbits were given various quantities of sucrose, as shown in table 1. Two animals in this group which died within the first twenty-four hours are not included in the table. The intervals during which fluid was restricted were only twenty-four to forty-eight hours. There is some indication, however, from these data that restriction of fluids may affect the retention of the nonprotein nitrogen.

Rabbit 97 on the day before death was drowsy and inactive. No muscular twitching or convulsions were observed. This animal refused water on the morning before his death in the afternoon. At autopsy the kidneys were swollen and pale. The epithelial cells in the convoluted tubules were swollen, and the lumens were essentially occluded as a result of this swelling. There was little abnormal to be seen in the cytoplasm of the epithelial cells. A few pink threads of fibrin-like material were present. The nuclei were pyknotic, with irregular peripheries. No changes were observed in the glomeruli. Precipitated albumin-like material was present in Bowman's space in some of the glomeruli.

Experiment 2.—In a second group, of 18 rabbits, cabbage and water were removed from the diet for a much longer time before and during the period the sucrose was given. Table 2 shows the intervals during which the rabbits were given only oats and the quantity of sucrose administered to each animal. This table also gives the amount of nonprotein nitrogen in the blood. Each of the 6 rabbits (116, 117, 118, 122, 123 and 124) given only oats for four days before

16. Eli Lilly & Co. supplied the sucrose used in these experiments.

17. Mrs. R. L. Wyatt, technician in the chemistry laboratory of the Division of Medicine, determined the nonprotein nitrogen in the blood.

TABLE 1.—*Retention of Nonprotein Nitrogen in Rabbits Given Sucrose* *

Duration of Experiment, Day	Rabbit					
	96	97	98	8-1	8-2	8-4
1	11:05 a.m. 15 cc. 4:00 p.m. 54 mg.	11:05 a.m. 15 cc. 4:00 p.m. 58 mg.	10:45 a.m. 34 mg. 10:45 a.m. 15 cc. 1:30 p.m. 15 cc. 4:15 p.m. 34 mg.	9:00 a.m. 37 mg. 9:00 a.m. 10 cc. 11:15 a.m. 10 cc. 3:30 p.m. 20 cc.	9:00 a.m. 45 mg. 9:00 a.m. 10 cc. 11:15 a.m. 10 cc. 3:30 p.m. 20 cc.	9:00 a.m. 48 mg. 9:00 a.m. 10 cc. 11:15 a.m. 10 cc. 3:00 p.m. 20 cc.
2	.....	.....	9:00 a.m. 28 cc. 1:15 p.m. 30 cc. 3:45 p.m. 35 mg.	10:00 a.m. 45 mg.	10:00 a.m. 64 mg.	10:00 p.m. 66 mg.
3	.....	.....	.....	8:00 a.m. 30 mg.	8:00 a.m. 32 mg.	10:00 p.m. 45 mg.
4	10:30 a.m. 28 mg. 10:30 a.m. 15 cc.	10:30 a.m. 46 mg. 10:30 a.m. 15 cc.	.....	8:30 a.m. 20 cc. 10:45 a.m. 10 cc. 11:30 a.m. 20 cc.	8:30 a.m. 20 cc. 10:45 a.m. 20 cc. 11:30 a.m. 20 cc.	8:30 a.m. 20 cc. 10:45 a.m. 20 cc. 11:30 a.m. 20 cc.
5	9:30 a.m. 15 cc. 1:15 p.m. 20 cc. 3:55 p.m. 28 mg.	9:30 a.m. 20 cc. 1:15 p.m. 20 cc. 3:45 p.m. 29 mg.	9:00 a.m. 29 cc.† 11:30 a.m. 20 cc. 1:30 p.m. 20 cc. 4:00 p.m. 45 mg.	.....	.....	.....
6	.....	.....	10:00 a.m. 45 mg.	8:15 a.m. 47 mg.	8:15 a.m. 94 mg.	8:15 a.m. 30 mg.
7	.....	.....	.....	8:00 a.m. 53 mg.	8:00 a.m. 44 mg.	8:00 a.m. 50 mg.
8	9:00 a.m. 20 cc. 11:30 a.m. 20 cc. 1:30 p.m. 20 cc. 4:00 p.m. 52 mg. Killed	9:00 a.m. 20 cc.† 11:30 a.m. 20 cc. 1:30 p.m. 20 cc. 4:00 p.m. 200 mg.	9:00 a.m. 187 mg.	8:30 a.m. 20 cc. 10:00 a.m. 20 cc.	8:30 a.m. 20 cc.† 10:00 a.m. 20 cc.	8:30 a.m. 20 cc.† 10:00 a.m. 20 cc.
9	.....	10:00 a.m. 363 mg. 4:30 p.m. Died	9:00 a.m. 360 mg.	8:30 a.m. 64 mg.	8:00 a.m. 43 mg.	8:00 a.m. 37 mg.
10	.....	.....	9:00 a.m. 178 mg.	8:15 a.m. 54 mg.	8:00 a.m. 38 mg.	8:15 a.m. 28 mg.
11	.....	.....	9:00 a.m. 143 mg.	8:00 a.m. 45 mg.	8:00 a.m. 45 mg.	8:00 a.m. 27 mg.
12	.....	.....	9:00 a.m. 88 mg.	.....	.....	.....
13	.....	.....	9:00 a.m. 66 mg.	.....	8:00 a.m. 34 mg.	8:00 a.m. 28 mg.
14	.....	.....	.....	9:30 a.m. 20 cc.† 11:00 a.m. 20 cc. 1:30 p.m. 20 cc. 4:00 p.m. 32 cc.	9:30 a.m. 20 cc.† 11:00 a.m. 20 cc. 4:00 p.m. 36 mg.	4:00 p.m. 47 mg.†
15	.....	.....	9:00 a.m. 44 mg.	9:00 a.m. 51 mg.	9:00 a.m. 41 mg.	9:00 a.m. 47 mg.
16	.....	.....	9:00 a.m. 56 mg.	9:00 a.m. 31 mg.	9:00 a.m. 43 mg.	9:00 a.m. 36 mg.
17	.....	.....	8:00 a.m. Died			
22	.....	.....	.....	8:30 a.m. 60 cc.†	8:30 a.m. 60 cc.†	8:30 a.m. 60 cc.†
23	.....	.....	.....	8:30 a.m. 33 mg.†	8:30 a.m. 36 mg.†	8:30 a.m. 40 mg.†
24	.....	.....	.....	8:30 a.m. 23 mg.	8:30 a.m. 24 mg.	8:30 a.m. 28 mg.
28	.....	.....	.....	9:30 a.m. 20 cc.† 1:30 p.m. 20 cc. 4:00 p.m. 20 cc.	9:30 a.m. 20 cc.† 1:30 p.m. 20 cc. 4:00 p.m. 20 cc.	9:30 a.m. 20 cc.† 1:30 p.m. 20 cc. 4:00 p.m. 20 cc.
29	.....	.....	.....	8:00 a.m. 23 mg.	8:00 a.m. 35 mg.	8:00 a.m. 37 mg.
30	.....	.....	.....	8:00 a.m. 43 mg. Experiment discontinued	8:00 a.m. 28 mg. Experiment discontinued	8:00 a.m. 36 mg. Experiment discontinued

* All measurements in cubic centimeters refer to intravenous injection of a 50 per cent solution of sucrose, and all those in milligrams signify the amount of nonprotein nitrogen per hundred cubic centimeters of blood.

† No water for twenty-four hours.

‡ No water for twenty-four hours before and twenty-four hours after the injection of sucrose.

TABLE 2.—*Effect of Restriction of Fluids on Retention of Nonprotein Nitrogen in Rabbits Given Sucrose**

Diet	Rabbit	Duration of Experiment, Days									
		1	2	3	4	5	6	7	8	9	10
Only oats for 3 days before and during experiment†	110	3:30 p.m.‡ 66 mg.	.....	.....	.....	.....	.....	..	.....	.....	.....
	111	3:30 p.m.‡ 123 mg.	.....	.....	.....	.....	.....	..	.....	.....	.....
	112	3:30 p.m.‡ 43 mg.	.....	.....	.....	.....	.....	..	.....	.....	.....
Oats, water and cabbage for 3 days before and during experiment	113	3:30 p.m.‡ 46 mg.	.....	.....	.....	.....	.....	..	.....	.....	.....
	114	3:30 p.m.‡	.....	.....	.....	.....	.....	..	.....	.....	.....
	115	3:30 p.m.‡	.....	.....	.....	.....	.....	..	.....	.....	.....
Only oats for 4 days before and during experiment	116	9:30 a.m. 20 cc.	9:00 a.m. 20 cc.	10:00 a.m. 20 cc.	9 a.m. 20 cc. 5 p.m. 126 mg.	4:00 p.m. 122 mg.	.....	.	3:00 p.m. 316 mg.	5:00 p.m.‡ 429 mg.	.....
	117	9:30 a.m. 20 cc.	9:00 a.m. 20 cc.	10:00 a.m. 20 cc.	9 a.m. 20 cc. 5 p.m. 154 mg.	4:00 p.m. 95 mg.	.....	..	3:00 p.m.‡ 375 mg.	.....	.....
	118	9:30 a.m. 20 cc.	9:00 a.m. 20 cc.	10:00 a.m. 20 cc.	9 a.m. 20 cc. 5 p.m. 139 mg.	.....	.....	..	.....	.....	.....
Oats, cabbage and water for 4 days before and during experiment	119	9:30 a.m. 20 cc.	9:00 a.m. 20 cc.	10:00 a.m.‡ 38 mg.	.....	.....	.....	..	.....	.....	.....
	120	9:30 a.m. 20 cc.	9:00 a.m. 20 cc.	10:00 a.m. 20 cc.	9:00 a.m. 20 cc.	4:00 p.m. 87 mg.	.....	..	3:00 p.m. 36 mg.	.....	9:00 a.m.‡ 30 mg.
	121	9:30 a.m. 20 cc.	9:00 a.m. 20 cc.	10:00 a.m. 20 cc.	9:00 a.m. 20 cc.	4:00 p.m. 75 mg.	.....	..	3:00 p.m. 49 mg.	.....	9:00 a.m.‡ 34 mg.
Only oats for 4 days before and during experiment	122	9:00 a.m. 36 mg. 20 cc.	9:00 a.m. 20 cc.	9 a.m. 54 mg.‡ 9 a.m. 20 cc. 4 p.m. 50 mg.	.....	.....	.....	.	.....	.....	.....
	123	9:00 a.m. 50 mg. 20 cc.	9:00 a.m. 20 cc.	9 a.m. 64 mg. 9 a.m. 20 cc. 4 p.m. 88 mg.	.....	9:00 a.m. 200 mg.	.....	..	4:00 p.m.‡ 214 mg.	.....	.....
	124	9:00 a.m. 34 mg. 20 cc.	9:00 a.m. 20 cc.	9 a.m. 52 mg. 9 a.m. 20 cc. 4 p.m. 62 mg.	3:00 p.m. 20 cc.	9:00 a.m. 58 mg.	4:00 p.m.‡ 100 mg.	..	.....	.....	.....
Only oats for 4 days before experiment; cabbage added when sucrose was first injected	125	9:00 a.m. 54 mg. 20 cc.	9:00 a.m. 20 cc.	9 a.m. 49 mg. 9 a.m. 20 cc. 4 p.m. 43 cc.	3:00 p.m. 20 cc.	9:00 a.m. 30 mg.	Experiment discontinued	..	.....	.....	.....
	126	9:00 a.m. 20 cc.	9:00 a.m. 20 cc.	9 a.m. 42 mg. 9 a.m. 20 cc. 4 p.m. 41 mg.	3:00 p.m. 20 cc.	9:00 a.m. 30 mg.	4:00 p.m. 36 mg.	..	.....	.....	.....
	127	.....	.....	9 a.m. 41 mg. 9 a.m. 20 cc. 4 p.m. 38 mg.	3:00 p.m. 20 cc.	9:00 a.m. 20 mg.	4:00 p.m.‡ 56 mg.	..	.....	.....	.....
Only oats for 4 days before and during experiment	128	9:00 a.m. 50 mg.	9:00 a.m. 51 mg.	.....	.....	9:00 a.m. 53 mg.	Experiment discontinued	..	.....	.....	.....
	129	.....	9:00 a.m. 67 mg.	.....	.....	9:00 a.m. 66 mg.	Experiment discontinued	..	.....	.....	.....

* All measurements in cubic centimeters refer to intravenous injection of a 50 per cent solution of sucrose, and all those in milligrams signify the amount of nonprotein nitrogen per hundred cubic centimeters of blood.

† Rabbits 110 to 115 were given 15 cc. of sucrose solution at 10 a.m. and at 1 p.m. and were killed at 3:30 p.m.

‡ Killed.

the sucrose was injected showed a retention of nonprotein nitrogen either after the third or after the fourth injection. The controls (rabbits 119, 120, 121, 125, 126 and 127) did not exhibit a corresponding retention of nonprotein nitrogen. In fact, there was only a slight retention of nonprotein nitrogen by rabbits 120 and 121, and in the others this factor was normal.

Two rabbits (128 and 129), as shown in table 2, were given only oats for four days, and the nonprotein nitrogen in their blood was determined on the fourth, the fifth and the seventh day. It was only slightly elevated.

The rabbits given only oats and sucrose rapidly lost weight. Those given oats, cabbage and water during the time sucrose was injected showed insignificant variations in weight. In fact, some of the animals gained. Rabbits given only

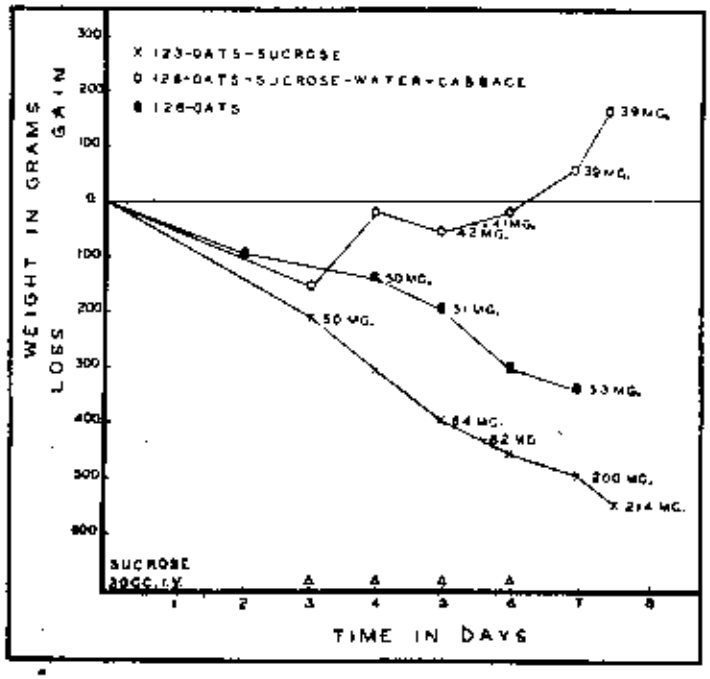


Fig. 1.—The effect of sucrose on the weight of rabbits and their retention of nonprotein nitrogen when the fluid intake is restricted.

oats lost weight; however, this was much less than when sucrose was also given. The variations in the weight of the different groups of rabbits are illustrated by the data in figure 1.

The kidneys in rabbits 110, 111, 112, 113, 114 and 115 showed essentially the same degree of tubular injury. The cells in the convoluted portion of the renal tubules were slightly swollen, and the cytoplasm was finely granular. The kidneys from rabbits 116, 117, 118, 122 and 123 showed the cells in the convoluted portion of the tubules to be greatly swollen, the cytoplasm vacuolated and the nuclei pyknotic. The lumens of the tubules were frequently occluded. Precipitated albuminous material was present in Bowman's space in some of the glomeruli and in the lumen in some of the tubules. No lesions were observed in the glomeruli, in Henle's loops or in the collecting tubules. The renal changes in rabbits 119,

Fig. 2.—*A* (rabbit 118), the lumens of tubules almost occluded by the greatly swollen epithelial cells. This rabbit was fed only oats for four days before and during the experiment. Twenty cubic centimeters of a 50 per cent solution of sucrose was injected intravenously each day for four days. Eight hours after the last injection the blood contained 139 mg. of nonprotein nitrogen per hundred cubic centimeters. The rabbit was killed on the fourth day of the experiment. *B* (rabbit 121), normal appearance of the kidneys. This rabbit was fed oats, cabbage and water for four days before and during the experiment. It was given the same amount of sucrose as was administered to rabbit 118. At the time rabbit 121 was killed, six days after the last injection of sucrose, the blood contained 34 mg. of nonprotein nitrogen per hundred cubic centimeters.

126 and 127 were similar to those in rabbit 110. It would appear that the lesion in the epithelial cells of the tubules in this group of rabbits varies only in degree from that in the rabbits given sucrose and fed oats, water and cabbage.

The degree of the pathologic change in the kidney of rabbit 124 appears to be about midway between that of rabbit 127 and rabbit 123. The nonprotein nitrogen value for rabbit 124 was 100 mg. per hundred cubic centimeters of blood, while that for rabbit 123 was 214 mg. and that for rabbit 127 was 55 mg.

There were no pathologic changes in the epithelial cells of the convoluted portion of the renal tubules in rabbits 120 and 121. The nonprotein nitrogen was also normal. It is important to observe in table 2 that the nonprotein nitrogen values for these 2 animals were 67 and 75 mg., respectively, per hundred cubic centimeters on the fifth day of the experiment. On the tenth day, when the animals were killed, the values were normal.

There were no pathologic changes in the other viscera of these animals that we can at present associate with the administration of sucrose.

Experiment 3.—Of a group of 5 rabbits, 2 were fed oats, cabbage and water and 3 were fed only oats. On the fourth day of the experiment each rabbit was given 25 cc. of a 50 per cent solution of sucrose. This injection of sucrose was repeated on the four following days. On the day following the last injection of sucrose the nonprotein nitrogen in the blood of the 2 rabbits given cabbages, oats and water was within the range of normal, while each of the other animals showed a retention of nonprotein nitrogen in the blood.

COMMENT

The quantity of sucrose given to the patients in cases 1, 3 and 7 was much less than that given to patients by Dyar and Matthew [7] and by Masserman.[8] Renal lesions were present in each of our patients. The investigators just mentioned failed to observe any "unfavorable" reactions in their patients, although a larger quantity of sucrose was injected by the same route. Anderson [13] concluded from his studies that previous renal lesions influenced the development of tubular lesions following the injection of sucrose. Murphy and his associates [2] gave 300 to 500 cc. of a 50 per cent solution of sucrose to a group of 21 patients with chronic hypertension and observed improvement, without unfavorable results. It appears that previous renal disease, such as glomerular and arteriolosclerotic nephritis, has little if any effect on the development of renal lesions following the injection of sucrose.

An insufficient amount of fluid apparently was given to some of our patients in whom tubular lesions developed after the injection of sucrose. In case 1 the patient was unconscious for approximately forty-eight hours before he was admitted to the hospital. He was given 200 cc. of a 25 per cent solution of sucrose one hour after admission and died three hours later. This case illustrates the rapidity with which this tubular lesion may develop. This has been discussed by Anderson.[13] The quantity of fluid given in case 10 after the third day apparently was adequate. The fluid output was low. It would seem that the epithelial

cells lining the convoluted tubules of the kidney may have become swollen on the second day after the injection of sucrose. The lumens of the tubules were blocked as a result of the swelling of these cells. Helmholz [11] discussed the occlusion of the lumen of the tubules in rabbits given sucrose.

Our experimental observations on the rabbit show that in animals given sucrose but no fluid renal lesions develop and nonprotein nitrogen is retained in the blood. The quantity of sucrose necessary to produce renal lesions in dehydrated rabbits is much less than that used by Helmholz [11] to produce renal lesions.

There are insufficient clinical data to indicate the effect of this renal lesion on renal function. The experimental results of Helmholz [11] and Lindberg and associates,[14] as well as ours, show that the renal function is definitely diminished after the injection of sucrose.

A knowledge of the quantity of sucrose that will produce renal lesions is important. In cases 4 and 5 the patients were given 100 cc. each of a 25 per cent solution of sucrose intravenously. No renal lesions were present. It is difficult to determine the degree of hydration of these patients at the time sucrose was given. The patient in case 11 was a child 8 years old. She was given adequate fluids during the time she received the sucrose. There was no retention of nonprotein nitrogen. In case 12 the patient was given adequate fluids and two intravenous injections of 100 cc. each of a 25 per cent solution of sucrose. No retention of nonprotein nitrogen occurred. The pathologic studies on rabbits suggest that there is a definite correlation between the development of tubular lesions and the retention of nonprotein nitrogen in the blood.

Multiple injections of sucrose were given to a group of patients by Hilton and Alderson.[3] The total quantity, however, was much less than that given to several of the patients included in the present series. It seems unlikely that the occurrence of these renal lesions is directly influenced by a previous injection of sucrose. The chemical and pharmacologic action of sucrose has been discussed by Keith and Power,[9] Keith [18] and Bullock, Gregersen and Kinney.[19]

The pathologic lesions observed in our experimental animals and in the patients appear to be identical and to be similar to those reported by other investigators. We did not observe glomerular lesions like those described by Lindberg and his associates.[14] The results of the histologic studies and the observations on the retention of nonprotein nitrogen suggest a correlation between these pathologic changes and renal func-

18. Keith, N. M.: Experimental Dehydration, Am. J. Physiol. **68**:80, 1924.

19. Bullock, L. T.; Gregersen, M. I.; and Kinney, R.: The Use of Hypertonic Sucrose Solution Intravenously to Reduce Cerebrospinal Fluid Pressure Without a Secondary Rise, Am. J. Physiol. **112**:82, 1935.

tion. It is suggested from the studies on rabbits 120, 121, 126 and 127 that the epithelial changes in the tubules may be transient and that the quantity of sucrose is important in the production of the renal lesions.

This renal lesion was diffuse in all of the rabbits and in a majority of the patients. In some of the patients with arteriolosclerotic nephritis the epithelial cells lining the tubules were smaller than normal in focal areas of the kidney. Dr. R. A. Moore and Dr. W. D. Forbus discussed this factor when Anderson [19] presented his paper at a meeting of the Southern Medical Association. The epithelial cells lining the convoluted portion of the tubules in those cases in which the changes are focal are frequently much lower and stain more deeply with hematoxylin than do corresponding cells in normal kidneys. The appearance of these epithelial cells and their failure to respond to sucrose as do other tubular epithelial cells may be similar to the effect of uranium nitrate on previously injured tubular epithelial cells, observed by MacNider.[20]

SUMMARY

A group of 16 clinical cases in which a hypertonic solution of sucrose was given intravenously is reported. In some instances the epithelial cells in the convoluted portion of the renal tubules were swollen. From a review of these cases it appears that dehydration is one of the most important factors in determining whether or not this tubular lesion will occur after the intravenous injection of sucrose. The quantity of sucrose administered during a given interval is also important.

Renal lesions similar to those in man may be produced experimentally by giving rabbits sucrose intravenously. The quantity of sucrose necessary to produce these lesions is much less when the fluid is restricted before and during the experiment. In fact, when fluids are given it is difficult to produce renal lesions in rabbits with sucrose unless large quantities are used.

20. MacNider, W. de B.: Urine Formation During the Acute and Chronic Nephritis Induced by Uranium Nitrate, in Harvey Lectures, 1928-1929, Baltimore, Williams & Wilkins Company, 1930, p. 82.

Further reproduction
prohibited without consent
of copyright owner

Reproduced by permission
of the copyright owner

Fd Cosmet. Toxicol. Vol. 8, pp. 135–145. Pergamon Press 1970. Printed in Great Britain

Research Section

Feeding Studies on Sodium Cyclamate, Saccharin and Sucrose for Carcinogenic and Tumour-promoting Activity

P. J. C. Roe, L. S. Levy and R. L. Carter

Chester Beatty Research Institute, Institute of Cancer Research, Royal Cancer Hospital, Fulham Road, London, S.W.3, England

(*Received 4 September 1969*)

Abstract—Groups of 50 female Swiss mice were fed for 18 months on a standard diet containing sucrose (10%), sodium cyclamate (5%) or saccharin (5%), while 100 control mice were fed on the standard diet alone. The survival times were comparable in all groups and no acute toxic effects were encountered. No local tumours in the gastro-intestinal tract, nor excess incidence of distant tumours, were found in animals fed on the various sweeteners. The mice fed on the diet containing 10% sucrose gained more weight than animals in the other groups, but did not show an increased incidence of neoplasms.

Comparable groups of mice were given a single intragastric instillation of 50 μg benzo[*a*]pyrene in 0·2 ml polyethylene glycol 400, 7 days before the start of feeding of the different test diets. Some mice in all these groups developed benign and malignant neoplasms of the forestomach epithelium, but treatment with the sweetening agents had no obvious effect on the incidence, number or histological type of the tumours or any other type of neoplasm.

No neoplasms of the urinary bladder were seen on careful macroscopic examination of mice at necropsy; the bladders were not examined microscopically.

It is concluded that, under the experimental conditions described, the three sweeteners examined showed no carcinogenic or tumour-promoting activity.

INTRODUCTION

The two non-caloric synthetic sweeteners that have found wide use as substitutes for dietary sugars are saccharin (2,3-dihydro-3-oxobenzisosulphonazole) and the sodium and calcium salts of cyclamate (*N*-cyclohexylsulphamate). Until recently saccharin and cyclamate were believed to be relatively inert pharmacologically, and over the wide range of doses that have been studied they produced no acute toxic effects apart from slight softening of the stools (Fitzhugh, Nelson & Frawley, 1951; Richards, Taylor, O'Brien & Durscher, 1951; Schoenberger, Rix, Sakamoto, Taylor & Lorenz, 1953; Taylor, Richards, Wiegand & Weinberg, 1968). The risks of long-term exposure [illegible] were thought to be uncertain. Stein, Scrogin & Coulston (1967) saw no [illegible] or pathological changes in monkeys fed sodium cyclamate at [illegible] months; in a number of studies no tumours have been reported when cyclamate or its metabolite cyclohexylamine, have been fed to experimental animals (see Taylor, Richards, Wiegand & Weinberg, 1968, for review) though a related compound—dicyclohexylamine—has been stated to be weakly carcinogenic to rats (Fiss, 1958; Shabad, 1964; [illegible], 1964; Pavlovskaya, 1965). Fitzhugh *et al.* (1951) had observed an "increased incidence of the uncommon condition of abdominal lymphosarcoma" in rats maintained for life on a diet containing 5% saccharin.

After the present paper had been prepared for publication, we became aware of a report by Bajusz (1969) on the occurrence of myocardial lesions in hamsters given high doses of calcium cyclamate and of the then unpublished observation of an increased risk of neoplasms of the bladder in rats fed on a high concentration of a saccharin–cyclamate mixture (Price, Biava, Oser, Vogin, Steinfeld & Ley, 1970).

At the time the experiments reported in this paper were begun, the evidence relating to the carcinogenicity of sugars and sweeteners in general was difficult to interpret. This applied particularly to the induction of subcutaneous sarcomas in rats by repeated subcutaneous injections of various sugars, including sucrose (Hueper, 1965; Grasso & Golberg, 1966; Carter, 1970). Hueper (1965) suggested that such tumours arose because the test carbohydrate was contaminated with polycyclic aromatic hydrocarbons during preparation, while Grasso & Golberg (1966) stressed that non-specific physico-chemical factors such as repeated injection of hypertonic solutions played an important part in sarcoma development. Few, today, would accept that such tumours reflect 'inherent' carcinogenic activity in the sugars themselves. On a more general level, there is some evidence that increased caloric intake *per se* may favour the development of tumours in experimental animals (Tannenbaum & Silverstone, 1953). In theory, therefore, the substitution of non-caloric sweeteners such as saccharin and cyclamate for sucrose should lead non-specifically to a reduced risk of neoplasia.

In view of these conflicting observations, it seemed worthwhile to re-appraise the possible carcinogenic activity of cyclamates and other sweeteners added to the diet of experimental animals. In addition to the tests for complete carcinogenic activity, we explored the possibility that the addition of sweetening agents to the diet promoted tumour development after oral treatment with a known carcinogen (benzo[a]pyrene: 3,4). The need to examine food additives for co-carcinogenicity as well as for carcinogenicity has been stressed elsewhere (Roe, 1969) but sweetening agents have not, to our knowledge, been tested from this viewpoint.

EXPERIMENTAL

Materials. The sweetening agents used were sucrose (British Drug Houses Ltd., Poole, Dorset), saccharin (British Saccharin Sales Co. Ltd., Nottingham) and sodium cyclamate (Abbott Laboratories Ltd., Sittingbourne, Kent). TP was obtained from Koch-Light Laboratories, Ltd. (Colnbrook, Bucks.) and polyethylene glycol (PEG) of average molecular weight 400, from British Drug Houses Ltd. (Poole, Dorset).

Animals and diets. Female mice of a random-mated Swiss albino strain (8–14 wk of age) were used. The standard diet used for both control and treated groups was Oxoid Breeding Diet, as supplied by Oxo Ltd. (London). This diet included crude oil (3·3%), crude protein (20·5%), crude fibre (2·6%), digestible crude oil (2·1%), digestible crude protein (17·9%) and digestible crude fibre (1·2%). It also contained a mixture of vitamins, including A, B_1, B_2, B_6, B_{12}, D_3, E, nicotinic acid, pantothenic acid, biotin and all 'essential nutritional factors', inositol and choline, and necessary minerals. The diets were prepared by blending the standard diet and sweeteners in a powdered form in a food mixer. Approximately 5 g dry powdered food, moistened with water, was allocated daily to each mouse, this being rather more than the amount they regularly consumed.

Experimental design and conduct. The mice, divided into eight groups, received various treatments which are summarized in Table 1. On day 0, animals were given a single intra-

gastric instillation of either 0·2 ml PEG or 50 μg BP in 0·2 ml PEG after overnight starvation. All mice then received the standard diet only (i.e. without sweeteners) for 7 days, before being transferred to their various test diets. Throughout, water was given *ad lib.* They remained on these diets until the experiment terminated 18 months after pretreatment with BP or PEG. The mice were weighed fortnightly for the first 4 months of the experiment and monthly thereafter. After the first 6 wk all mice were examined at weekly intervals for obvious tumour development and daily for general health. Sick animals were killed. Post-mortem examinations were carried out on mice killed or found dead during the experiment

Table 1. *Survival of mice given various sweetening agents in the diet with or without oral pretreatment with BP*

Group no.	No. of mice/group	Pretreatment* plus dietary sweetener (%)	No. of survivors at month 12	15	18
1 H†	100 48	PEG only	85 40	77 35	65 32
1 L†	52		48	42	33
2 H	100 39	BP + PEG only	90 33	81 31	61 21
2 L	61		57	50	40
3	50	PEG + 5% cyclamate	45	42	34
4	50	BP + PEG + 5% cyclamate	48	43	41
5	50	PEG + 10% sucrose	45	34	27
6	50	BP + PEG + 10% sucrose	47	40	35
7	50	PEG + 5% saccharin	44	40	36
8	50	BP + PEG + 5% saccharin	38	37	32

* Mice were pretreated with an oral dose of 50 μg BP in 0·2 ml PEG or with 0·2 ml PEG alone, followed 7 days later by diets containing added sweetener.

† H and L refer to mice weighing > 36 g and < 36 g respectively at the start of the experiment (see text).

or killed terminally. Some 449 out of 500 mice were so examined; the remainder were either found in a state of advanced autolysis or had been eaten by cage mates. Post-mortem examination was carried out by a standardized procedure [illegible] on the completion of a form by the entry of negative as well as positive findings [illegible]. All major organs including the urinary bladder but excluding the brain, pituitary gland and spinal cord, were examined macroscopically in the present experiment. All neoplasms, or lesions suspected of being neoplasms, were removed and fixed in Bouin's fluid. Paraffin sections were prepared and stained with haematoxylin and eosin.

Because of a misunderstanding, mice were not allocated randomly to the eight treatment groups. The oldest, and therefore heaviest, mice were included mainly in the control groups (groups 1 and 2) while the lightest and youngest mice were allocated to test groups 3–8. In order to see if initial body weight influenced the subsequent incidence of tumours, a distinction was made between heavier (above 36 g) and lighter (below 36 g) animals, designated H and L, respectively, in the two control groups. The observations for these H and L sub-groups are recorded separately in the tables.

RESULTS

Survival

The numbers of survivors at 12, 15 and 18 months are shown in Table 1. Neither treatment with BP nor subsequent treatment with any of the sweetening agents appeared to

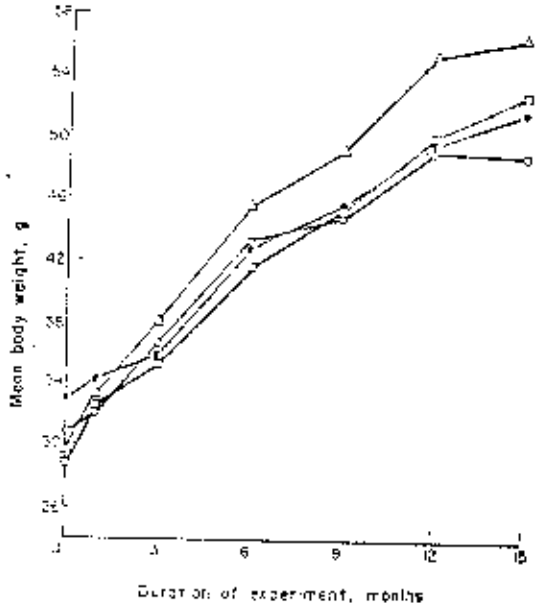


FIG. 1. Growth of female mice fed a basic diet containing 10% sucrose (△), 5% saccharin (□), 5% sodium cyclamate (○) or no added sweetener (●). Values for mice weighing more than 36 g or less than 26 g at the start of treatment were excluded.

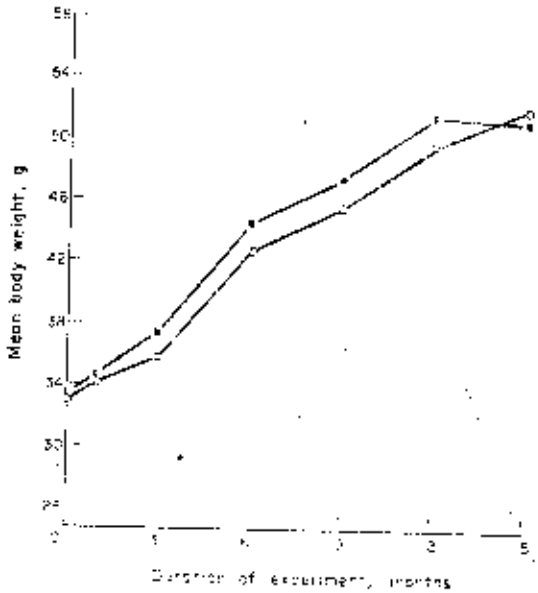


FIG. 2. Growth of female mice fed a basic diet with (●) or without (○) previous administration of a single intragastric dose of 50 μg benz[a]pyrene. Values for mice weighing more than 36 g or less than 26 g at the start of treatment were excluded.

Table 2. *Incidence of forestomach neoplasms and other neoplasms in mice that survived for 18 months* from the start of treatment*

Group	Treatment	No. of mice examined at autopsy at 18 months	No. of mice with: Squamous papillomas of forestomach	Squamous carcinomas of forestomach	Pulmonary tumours	Hepatomas	Malignant lymphomas	Other neoplasms	
H L†	PEG	65 {32, 33	2 {0, 2‡	0 {0, 0	15 {8, 7	5 {3, 2	3 {0, 3	1 {1, 0	subcutaneous sarcoma
H L	BP/PEG	61 {21, 40	20 {8, 12	1 {1, 0	18 {6, 12	9 {3, 6	0 {0, 0	2 {2, 0	subcutaneous sarcoma and sarcoma of uterus
	PEG/Cyclamate	34	1	0	6	1	1	0	
	BP/PEG/Cyclamate	41	4	0	10	6	0	2	subcutaneous sarcoma and mammary carcinoma
	PEG/Sucrose	27	0	0	5	1	3	2	both spindle-cell tumours of uterus
	BP/PEG/Sucrose	35	8§	2§	5	4	0	1	haemangioma of liver
	PEG/Saccharin	36	0	0	8	1	2	2	haemangioma of liver and retroperitoneal sarcoma
	BP/PEG/Saccharin	32	10*§	1§	7	2	1	2	granulose cell tumour of ovary and haemangioma of liver

* [illegible] that died after 15·5 months had a small papilloma of the forestomach. Otherwise no stomach neoplasms were seen [illegible]

† [illegible] 36 g respectively [illegible] the start of the experiment (see text).

‡ [illegible] with a solitary [illegible]

§ [illegible] with both a papilloma and a carcinoma.

Table 3. *Incidence of neoplasms in mice that failed to survive for 18 months from start of treatment*

Group	Treatment	No. of mice dying between 9 and 18 months without post-mortem examination	No. of mice dying: Before 12 months: No. examined *post mortem*	No. of mice dying: Before 12 months: No. with neoplasms	No. of mice dying: Between 12 and 15 months: No. examined *post mortem*	No. of mice dying: Between 12 and 15 months: No. with neoplasms	No. of mice dying: Between 15 and 18 months: No. examined *post mortem*	No. of mice dying: Between 15 and 18 months: No. with neoplasms
1	PEG	9 (5)	9 (3)	2 (malignant lymphoma)	8 (4)	1 (lung adenoma)	9 (7)	2 (lung adenoma) 2 (hepatoma) 1 (undifferentiated tumour of lung)
		4	6	2 (malignant lymphoma)	4	1 (subcutaneous sarcoma)	2	1 (reticulum cell sarcoma and hepatoma)
2	BP, PEG	9 (5)	6 (3)	0	8 (6)	2 (lung adenoma) 1 (hepatoma) 1 (localized lymphosarcoma)	16 (7)	3 (lung adenoma) 1 (malignant lymphoma)
		4	3	1 (malignant lymphoma)	2	1 (pulmonary adenoma)	9	1 (malignant lymphoma) 1 (hepatoma) 1 (granulosa cell tumour) 1 (subcutaneous haemangioendotheliosarcoma)

3	PEG/Cyclamate	3	5	0	1	0	7	3 (lung adenoma) 1 (lung adenocarcinoma) 1 (subcutaneous fibromyxosarcoma) 1 (reticulum cell sarcoma) 1 (localized lymphoma)
4	BP/PEG/Cyclamate	4	0	–	1	0	4	1 (malignant lymphoma) 1 (subcutaneous sarcoma and lung adenoma)
5	PEG/Sucrose	7	2	0	5	1 (lung adenoma) 1 (malignant lymphoma) 1 (granulosa cell tumours of both ovaries)	6	2 (lung adenoma) 1 (hepatoma) 1 (subcutaneous sarcoma)
6	BP/PEG/Sucrose	3	2	0	2	1 (lung adenoma)	5	1 (malignant lymphoma) 3 (lung adenoma)
7	PEG/Saccharin	3	3	0	1	0	2	0
8	BP/PEG/Saccharin	8	6	0	0	– –	4	1 (lung adenoma) 1 (granulosa cell tumour of ovary) 1 (hepatoma) 1 (forestomach papilloma)

[a] H and L refer to mice weighing > 36 g and < 36 g respectively at the start of the experiment.

influence survival up to 18 months, nor was there any consistent difference in survival between the L and H sub-groups of groups 1 and 2.

Body weight

Mice fed on the sucrose diet gained weight faster than mice fed on other diets (Fig. 1); at 15 months, sucrose-fed mice weighed on average 4 g more than was to be expected on the basis of their average weight at day 0. A single dose of BP had no effect on the rate of weight gain in mice fed the standard diet (Fig. 2). Pretreatment with BP did not influence the weight gain in mice fed on diets containing added sucrose or cyclamate but was associated with reduced weight gain in saccharin-fed mice. At the start of treatment the average weight of mice in group 7 was 29·0 g and of mice in group 8, 28·9 g; at 6 months, mice in group 7 weighed 41·7 g on average, but those in group 8 only 38·7 g; and at 12 months the average weights were 50·1 g and 44·8 g, respectively. We can think of no logical explanation of this finding and believe it may be fortuitous.

Neoplasms and hyperplastic changes in the forestomach

The incidence of forestomach neoplasms in mice that survived for 18 months is given in Table 2. Clearly, treatment with BP [illegible] the incidence of neoplasms arising in the forestomach epithelium. The [illegible] of sweetening agents did not increase the incidence of such tumours, whether BP was given or not. Focal and more generalized epithelial hyperplasia and hyperkeratosis were seen in some BP-treated mice but the sweetening agents exerted no [illegible] effect on the incidence or severity of these changes.

Neoplasms at sites other than the forestomach

The incidence of hitherto unknown neoplasms, other than those of the forestomach, in animals killed at 18 months is [illegible] in Table 2, and of those in animals dying before 18 months, in Table 3. The incidence of [illegible] was greater in animals treated with BP, but the incidence of pulmonary tumours and malignant lymphomas was similar in all groups. It is noteworthy that [illegible] malignant and benign tumour incidence in the nasal passages, gallbladder, [illegible] and [illegible] large intestine. Totally macroscopic examination of the bladder revealed [illegible] in more than any group.

DISCUSSION

The four main findings in this investigation are negative. First, the addition of 10% sucrose, 5% sodium cyclamate or 5% saccharin to a standard diet given to female mice was not associated with an increase either in [illegible] arising in the gastro-intestinal tract or in distant tumours at other sites. Secondly, none of the sweetening agents showed any co-carcinogenic activity when tested in experiments in mice which had previously received a single oral dose of 50 μg BP. Thirdly, [illegible] the incidence, number and degree of malignancy of [illegible] neoplasms in mice fed on any of the sweetening agents. Finally, [illegible] showed a greater [illegible] than the other groups [illegible] of the sucrose diet [illegible] and was not impeded by any increase in tumour incidence. The [illegible] (1963), who found that [illegible] tumours were more common in these groups. Lastly, none of the sweeteners had any discernible toxic effects and none

appeared to have an adverse effect on survival up to 18 months from the start of the experiment.

In spite of the non-random allocation of mice to the various treatment groups, our experience with this strain and consideration of the results as a whole strongly suggest that the three sweetening agents tested possess neither carcinogenic nor tumour-promoting activity under the conditions of the experiment.

Differences between species in susceptibility to cyclamate have not been adequately explored. The need for such work is illustrated by the recent report by Bajusz (1969) which showed that large doses of calcium cyclamate, given orally to hamsters for many days, rapidly induced calcification and necrosis of cardiac and skeletal muscle, coronary sclerosis of the Mönckeberg type and nephrocalcinosis. Equimolecular amounts of calcium given as chloride, aspartate, acetate and ascorbate were inactive. It would seem that the cyclamate part of the calcium cyclamate molecule is necessary for the production of this toxic effect. But at present it appears that the effect is species-specific since calcium cyclamate, given in equivalent doses to rats, did not lead to extensive calcification. We recorded no evidence of ectopic calcification in the mice of groups 3 and 4.

The findings reported in the present paper are consistent with the view that neither saccharin nor cyclamate are carcinogenic for mice when administered by mouth. This would have been our conclusion had it not been for our becoming aware of the observed increase in the incidence of bladder tumours in rats fed on a sodium cyclamate–sodium saccharin (10:1) mixture in high concentrations (Price et al., 1970) and the suspicion that *in vivo* conversion of cyclamate to cyclohexylamine (Hagan & Hansbrough, 1966; Lesko, Wakefield & Taylor, 1967; Oser, Carr, [illegible]) was involved in the induction of these tumours. By the time this information became available the mice in our experiment were dead, but Unilever Research Ltd. (Colworth House, Sharnbrook, Bedfordshire) kindly examined mice from our colony [illegible] for ability to convert cyclamate to cyclohexylamine. [illegible] from the age of 6–8 wk for a period of 2 months on a basic laboratory diet. [illegible] given water containing 0.5% sodium cyclamate. [illegible] contained cyclohexylamine at a level indicative of a 2–3% conversion rate.

In view of these later studies, it is likely that mice in groups 1 and 2 of the main experiment also converted some cyclamate to cyclohexylamine. [illegible] of this, we can not exclude the possibility that [illegible] of mucosal epithelial tumours were present in the bladders of the mice of any group. We can, however, with full confidence state that our use of a systematic necropsy regime excludes the possibility that bladder tumours of a size visible to the naked eye were present in any of the 440 mice [illegible] *post mortem*.

Acknowledgements—We are indebted to Mrs. Norma [illegible] and to Miss Janice Hepburn for her help and patience in the preparation of this manuscript.

This investigation was supported by grants to the Cancer Research Unit [illegible] by the Medical Research Council and the [illegible] Research.

REFERENCES

Bajusz, E. (1969). Myocardial lesions: A reassessment of [illegible] *Nature, Lond.* 223, 406.

Carter, R. L. (1970). Induced [illegible] Edited by J. J. C. Rose. Blackwell Scientific Publications, Oxford.

Field, W. F. H. & Rose, F. J. C. (1968). [illegible] oral administration of [illegible] *J. Pathol. Bacteriol.* [illegible]

Fitzhugh, O. G., Nelson, A. S. & Frawley, J. P. (1951). A comparison of the chronic toxicity of synthetic sweetening agents. *J. Am. Pharm. Ass.* 40, 553.
Grasso, P. & Golberg, L. (1966). Subcutaneous sarcoma as an index of carcinogenic potency. *Fd Cosmet. Toxicol.* 4, 297.
Hueper, W. C. (1965). Are sugars carcinogenic? An experimental study. *Cancer Res.* 25, 440.
Kimura, S. & Ishibagase, H. (1966). Studies on synthetic sweetening agents. VIII. Cyclohexylamine, a metabolite of sodium cyclamate. *Chem. Pharm. Bull. Tokyo* 14, 471.
Peatty, J. S., Wakefield, M. & Taylor, T. (1967). Clinical excretion of cyclohexylamine following oral administration of sodium cyclamate in man. *Fd Cosmet. Toxicol.* 5, 427.
Lemeshova, G. V. (1955). Toxicity of cyclohexylamine and dicyclohexylamine. *Trans. Proc. Transl. Surgl.* 24, T95.
Oser, B. L., Carson, S., Vogin, E. E. & Sanders, R. C. (1965). Conversion of cyclamate to cyclohexylamine in rats. *Nature, Lond.* 220, 175.
Price, G. B. (1958). The carcinogenic activity of dicyclohexylamine and its nitrite salt. *Proble. Oncol.* 4, 569.
Price, J. M., Biava, C. G., Oser, B. L., Vogin, E. E., Steinfeld, J. & Ley, H. L. (1970). Development of bladder tumors in rats fed cyclohexylamine or high doses of a mixture of cyclamate and saccharin—A preliminary report. *Science, N.Y.* 167, 1131.
Richards, R. K., Taylor, J. D., O'Brien, J. L. & Duncan, H. D. (1951). Studies on cyclamate sodium (sucaryl sodium), a new non-caloric sweetening agent. *J. Am. Pharm. Ass.* 40, 1.
Roe, F. J. C. (1965). Spontaneous tumours in rats and mice. *Fd Cosmet. Toxicol.* 3, 707.
Roe, F. J. C. (1968). Carcinogenesis and safety. *Chem. Toxicol.* 6, 455.
Schoenberger, J. A., Rim, D. M., Sauerwald, A., Taylor, J. D. & Kirk, R. M. (1969). Metabolic effects, toxicity and excretion of calcium N-cyclohexylsulphamate (calcium cyclamate) in man. *Am. J. med. Sci.* 225, 551.
Shanad, L. [illegible] (1969). [illegible] and principles of prevention. *Acta Unio. Cancr.* 19, 458.
Stein, A. A., Serrone, D. M. & Coulson, E. (1967). Metabolism and biochemical studies of sodium cyclamate. *Toxic. appl. Pharmac.* 10, 281.
Tannenbaum, A. & [illegible] (1951). [illegible] in mice and cancer. *Adv. Cancer Res.* 1, 45.
Taylor, J. D., Kirk, R. M., Schoenberger, J. A., Rim, D. M., Sauerwald, A. & Duncan, H. D. (1969). Metabolism of calcium N-cyclohexylsulphamate (calcium cyclamate) and its sodium salt in animals. *Proc. Soc. exp. Biol. N.Y.* 131, 510.
Taylor, J. D., Richards, R. K., [illegible] (1969). The metabolism and toxicity of cyclamate and saccharin. *Fd Cosmet. Toxicol.* 7, 313.

Étude, par des expériences de nutrition, de l'action irréversible au développement des tumeurs du système de la sacchrine et du cyclamate

Résumé—Des groupes de 50 souris femelles [illegible] ont été soumis [illegible] à un régime alimentaire standard contenant [illegible] de cyclamate de sodium (5%) ou de la saccharine (5%) [illegible] du cyclamate [illegible] effet de toxicité [illegible] du système urinaire [illegible] et [illegible] adultes. Le gain [illegible] était [illegible] chez les [illegible].

[illegible] régimes [illegible] Des [illegible] groupe [illegible] la [illegible] des tumeurs.

Verfütterungsversuche mit Natriumcyclamat, Saccharin und Sucrose und Prüfung auf carcinogene und tumorfördernde Aktivität

Zusammenfassung – Gruppen von 50 [illegible] ein Standardfutter mit einem Zusatz von [illegible] (10%) Natriumcyclamat ([illegible]) [illegible] Saccharin (5%), während 100 Kontrollmäuse das Standardfutter [illegible] zeiten wurden in allen Gruppen [illegible] Erkrankungen beobachtet. Es wurden keine [illegible] Lebermassiges Auftreten [illegible] Süssstoffen erhielten [illegible] Sucrose gefütterten Mäuse [illegible] an Gewicht zu als die Tiere in den anderen Gruppen, zeigten aber kein vermehrtes Auftreten von Neoplasmen.

Vergleichbare Gruppen von Mäusen erhielten 7 Tage vor dem Beginn der Verfütterung der verschiedenen Süssstoffe mit der Magensonde eine einzelne Dosis von 50 µg Benzfalsyren in 0,2 ml Polyethylenglycol 400. Einige Mäuse in allen diesen Gruppen [illegible], wie erwartet, benigne und maligne Neoplasmen im Vormagenepithel. Jedoch hatte die Verfütterung der Süssstoffe keinen offensichtlichen Einfluss auf Häufigkeit, Zahl oder Histologie dieser oder jeder anderen Neoplasmatype.

Bei der Sektion der Mäuse unter [illegible] wurden mikroskopischer Untersuchung keine Neoplasmen der [illegible] Eiter [illegible] untersucht.

Es wird daraus der Schluss gezogen, dass unter den [illegible] Versuchsbedingungen die drei untersuchten Süssstoffe keine carcinogene oder tumorfördernde Aktivität zeigten.

Zucker 1970, 23(9), 247-57

IS SUGAR HARMFUL FOR THE HUMAN ORGANISM?

by

H. Schiweck

Delivered at the course of higher education for children's dentists, organised by the Federal Association of Dentists of the Public Health Service e.V. -- state of Hessen -- on January 16, 1970, in Friedberg/Hessen.

If we wish to attempt to answer the question, "Is the consumption of saccharose, or the food product that we ordinarily call sugar, adverse to human health, or does sugar -- consumed in excess -- possibly even damage health?", then we must concern ourselves chiefly with the examinations and opinions published and presented for discussion on the one hand by the research group of Yudkin and on the other by Cleave and Campbell in their book "Saccharidosis".

In his lecture, Mr. Schöll underlined the fact that we can speak medically of a pathological picture of saccharidosis. Allow me, therefore, as a layman, to assume a somewhat different perspective in relation to this theme, since I by no means feel competent enough to say anything that would be significant in a purely medical sense. If one examines the book "Saccharidosis" by Cleave and Campbell thoroughly, one can find the following opinions expressed above all. I should like to examine these opinions individually in rather more detail:

Sugar is a "refined" food product, during the production of which an unnatural concentration of carbohydrate forms, because approximately 90% of the weight of the sugar beet is eliminated (p. 22). As a result of this, the tongue and the appetite are deceived during the consumption of sugar, and the result is an overconsumption (pp. 24 and 80). The authors are correct when they write that about 14 kg sugar are obtained on the average from 100 kg of sugar beets, but they have at the same time overlooked the fact that 100 kg of sugar beets contain about 76.5 kg water. Now, thus far, we have not succeeded in making sugar out of water, and there is little prospect that we would ever seriously attempt this, for it would mean an inexcusable relapse into the seventeenth century, when alchemists believed that they could make gold from other substances. In Table 1 there is represented a quantitative diagram of the production of sugar from sugar beets. As can be seen from this diagram, only about 0.75 kg of dry substance -- if we disregard the evaporation of water -- is eliminated during the obtaining of sugar; that is, about 3% of the total dry substance or 10% of the non-saccharose dry substance, or 35% of the soluble non-saccharose dry substance

of the sugar beet.

The other substances of the sugar beet -- insofar as they are not sugar -- are precipitated as by-products and are chiefly utilised as cow feed, as they are unsuited to human nourishment. The remains consist, namely, chiefly of cellulose, pektin and the polysaccharides arabane and galactane; they are, in other words, the remains of the epidermis and plate substance of the cell wall of the sugar beet, and they can be tolerated only by the enzyme system of a ruminator. I certainly need say nothing here about the "nutritional value" of molasses, since some of you probably tried to eat molasses as a spread instead of marmalade at the end of the second World War. If, therefore, the non-saccharose substances of the sugar beet have of themselves no health-promoting effect, it is difficult to understand why, together with sugar and water in the sugar beet -- thus in the natural state, as the authors express it -- they are so healthful that they can cancel the allegedly negative effect of sugar. For that is what Cleave and Campbell claim, namely that sugar in the natural state is not harmful. For instance, on pages 55-56, in connection with the question of diabetes, they write the following:

"Although the decisive question concerning the nourishment of the Zulus living in the country and in cities will be discussed later, we note here that these Zulu and Pondo sugar cane workers, among which there is in principle no diabetes, consume large amounts of natural sugar cane. About 2 kg are chewed daily, whereby some large fibers are later discarded. This corresponds to about 350 g white sugar. They also consume about 60 to 85 g ordinary sugar per day. However, this portion is really small in relation to the enourmous amount of consumed maize, beans and other natural carbohydrates, which is necessary for fulfilling their great energy need. It must namely be emphasized that there are few other occupations in the world that require such intense physical exertion as the cutting, transporting and piling of sugar cane.

Although these workers were always able to eat as much sugar cane as they desired, in the last 10 years they were allotted only 60 to 85 g real sugar daily -- thus since there has been overproduction. This fact is of great importance, since we will later show that it is often 20 years or more before diabetes results as a consequence of the consumption of refined carbohydrates. We must therefore direct our attention to the appearance of cases of diabetes in the next 10 years. In the meantime, however, the present absence of diabetes among these workers -- despite their constant massive consumption of sugar cane -- supports our assumption, that unrefined carbohydrate carriers are just as little dangerous as the refined carbohydrates are dangerous. This is true for all manifestations of saccharidosis, as we will later show. These sugar cane workers are in like manner unplagued by dental caries and paradentosis (Chapter VI)."

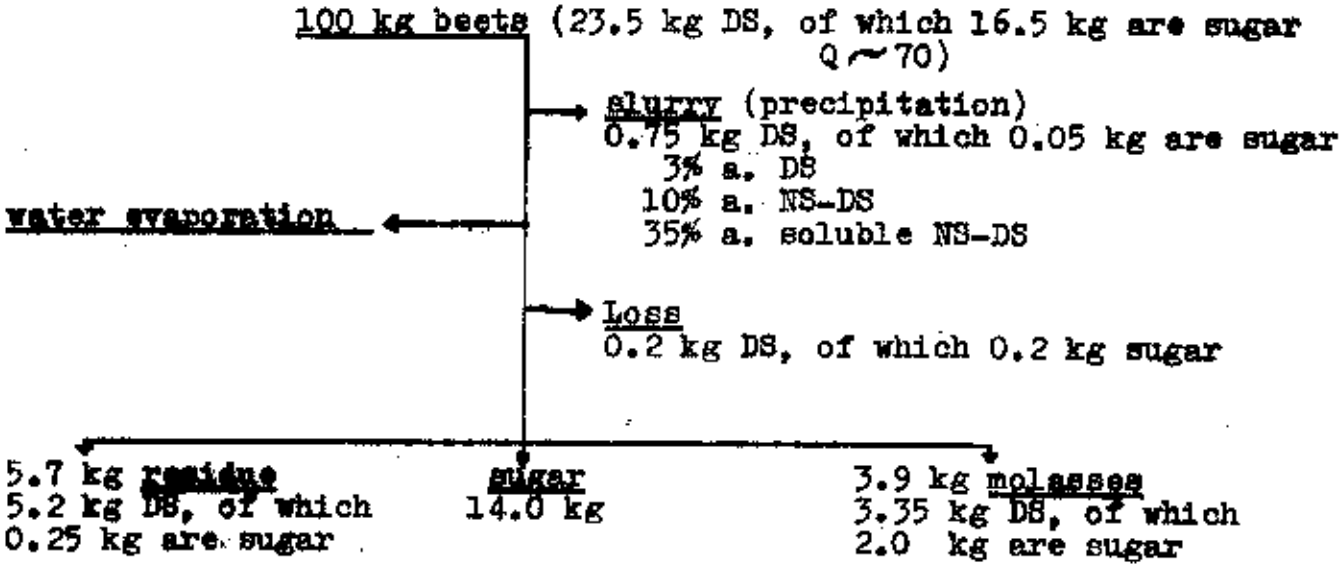
Before I speak in more detail about the cited rule of 20 years, I should like to discuss briefly the reasons why Cleave and Campbell claim that sugar in the form, for example, of honey

or dried dates is not a natural food product. On pages 88-89 they write:

"Thus, honey and certain fruits such as dates, for example, appear at first glance to be natural food products. However, a closer examination will alter this view considerably. Until a short while ago -- from an evolutionary viewpoint -- honey was only rarely available. Of course, since the discovery of fire, men have theoretically been in a position to smoke out the nests of wild bees, and there is archaeological evidence that this occasionally occurred. However, a little consideration shows that that such an undertaking must have been extremely dangerous, and that it was in any case so little profitable, that it was seldom worth the trouble. Therefore, man is much less accustomed to the consumption of honey that contains, practically speaking, no other nutritive substance except sugar, than he is to the consumption of factory sugar, as it is sold in stores. Every person who believes that honey is a natural food product that he can consume ad libitum might experience some annoying surprises. Solomon might not always have been wise, but he certainly demonstrated his wisdom when he advised men not to eat too much honey (Proverbs 25, 27). In short, honey should, from a practical point of view, be viewed as just as dangerous as sugar. This is true for all manifestations of saccharidosis, not only for obesity.

As concerns dates, they are certainly a natural food product -- but not for the white race!"

Table 1



DS = dry substance NS-DS = non-saccharose dry substance

Now it is hardly comprehensible why our forebears, who were capable of killing a bear, could not handle these so very dangerous bees, and we can hardly believe today in race-determined differences in the digestive and metabolic systems of men, for they have nowhere been proven scientifically. Naturally a

Japanese who comes to Europe for the first time has difficulty adapting to the food because of different composition and preparation of food products, just as we must accustom ourselves to foreign foods. However, this transition is in general made quickly, without any serious illness resulting from it. If one is of the opinion that organic food products are healthful, then one should at least examine to what extent the sugar cane and sugar beets of today are actually still natural plants, and to what extent products of breeding and cultivation. Then it will be established that sugar cane and sugar beets are not much older than the higher sugar consumption of the civilized world, namely about 150 years.

The use of sugar increased in the industrial nations in the last 150 years so rapidly, that the human body had no time to adapt to the alteration of its consumption habits. Thus, if we compare the state of health of the populace of industrial nations with that of population groups in regions that have preserved their natural living habits, then the industrial nations are seen in a more unfavorable light. Actually, not so much has changed in the manner of nourishment, but the consumption of "refined" carbohydrates is significantly higher in the case of industrial nations. If a rapid increase in the consumption of refined carbohydrates were to take place among the other population groups, we could not expect that their state of health would also immediately worsen; rather, we would have to reckon with the fact that the effects would become evident only within a space of time of some 20 to 30 years (the law of 20 years). To strengthen this opinion, investigations are now being conducted among Indians living in Natal, among Zulus, Eskimos, and other tribes living under natural conditions. One might wonder why the authors do not confirm their theories with statistical material from Europe, which is in any case more reliable than their own scanty efforts and investigations in Natal and India, which are in addition difficult to check.

For instance, if we compare the per capita sugar consumption for the last 150 years in England, the United States and Germany, we see on the one hand how different the development was and how varying in high degree the sugar consumption in England and Germany is even today (Figure 1). On the other hand, we do not find the differences in the frequency of illnesses that are purportedly due to the consumption of sugar. According to Cleave and Campbell (p. 63), the average per capita use of 30 kg per year is a kind of limit for the frequency of occurrence of diabetes. Therefore, although this limit has scarcely been surpassed in Germany, the number of diabetics in Germany is not smaller than in England.

Thus, for individual countries, the following numbers of diabetics per 1000 persons are given (2):

Denmark	1954	4 - 5
Sweden	1945	5.7
West Berlin	1953	5
France	1953	10
USA	1960	11
Great Britain	1947	2.3
	1960/1963	6.4

In the same work, WHO figures concerning diabetes mortality are also given. According to these figures, mortality in the individual countries is also of varying degree. At present, it is highest in Belgium, with 23.2 per 100,000, moderately high in the U.S.A with 16.7, in Switzerland with 14.7, in the Federal Republic of Germany with 13.6 (1962: 8,173 persons, 5445 female, 2728 male), in France with 12.6, for Central Europe 9.6, and for Australia 12.1... . It is low in the South American nations, with 2.7 to 7.5, in Asia with 0.4 to 4.4 (except for Ceylon: 7.3), lowest in South Korea with 0.4. There is a parallel between diabetes mortality and the social standard and average lifespan of a people. The death rates for those of younger age are very

Table 2
Sugar use per capita of population 1966/67[1]
(kg, raw value; according to F.O. Licht,
World Sugar Statistics 1967/68)

Australia	56.3	Spain	21.1
Denmark	53.5	Rumania	20.9
Great Britain	52.5	Turkey	17.1
Switzerland	49.2	U.S.A	47.4
Soviet Union	47.3	Canada	44.4
Norway	46.5	Argentina	40.3
Netherlands[2]	46.4	Chile	39.1
Sweden	45.3	Mexico	36.1
Czechoslovakia	43.2	Brazil[4]	35.9
Austria	42.8	Peru	28.1
Hungary	36.7	Colombia	22.9
France	36.4	South Africa	39.5
Poland	34.1	Egypt	18.6
Germany	34.0	Iran	23.6
Belgium	33.0	Japan[4]	19.1
Italy	28.2	India[3]	5.9

[1]Only centrifugal sugar [2]Sugar-containing goods not exported
[3]Only Khandsari [4]Any sort

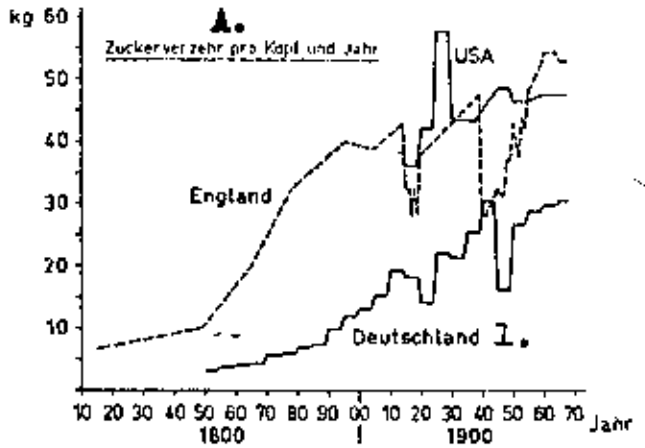
slight today, for the most part less than 0.5 per 100,000 population. In the age between 45 and 74, however, 10 to 20 per 100,000 are reported; herein the figures for women are higher, usually more than 20, than for men. In contrast to this, however, the per capita sugar use in, for example, Belgium, is lower than in Switzerland, the U.S. and Australia, and about equal to that in the South American countries of Argentina, Brazil and Chile (Table 2).

Although these figures concerning the frequency of diabetes might not, according to more recent investigations, be true any more for Germany at least (3) -- the collective investigation of the population of Munich showed that about 3% of all the populace suffered from diabetes --, the same is true for all figures pub-

lished thus far, because formerly the analytical possibilities of testing a large amount of people were simply not available.

Figure 1

Development of sugar use in the USA, England and Germany since the 19th century



Key:
A.= Sugar consumption per capita and year
1.= Germany

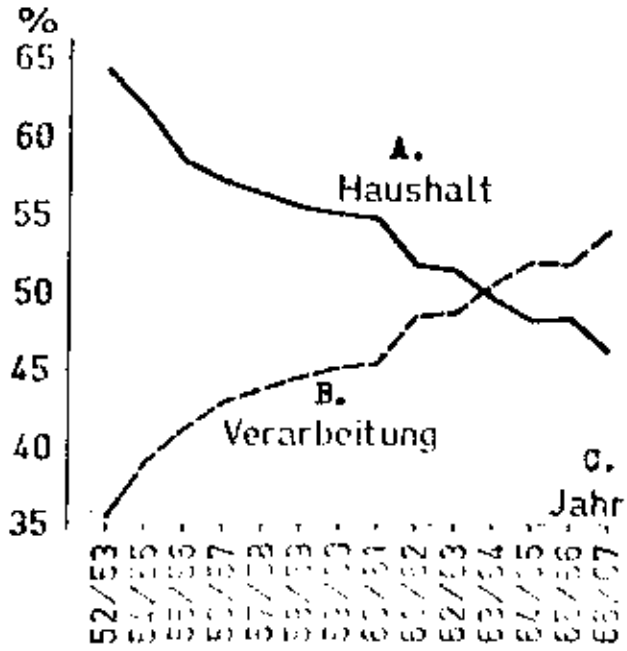
A glance at the graphic representation of the development of sugar use in the USA, England and Germany is also sufficient to allow for a judgment concerning the so-called 20 years law. The per capita sugar use in England passed the magic number of 30 kg per year around 1885, without subsequently ever sinking below this figure, while we in Germany did not reach this level of use until 1940/45, and since 1960, the use has been oscillating around this figure. Naturally, one would have to be blind to such facts if one wished to prove absolutely what Cleave and Campbell express on page 44 as follows:

"On the other hand -- and that is the departure point -- in relation to diabetes in this work it is our single goal to determine that this disease is caused by the consumption of refined carbohydrates, especially sugar, whereby the various components are destroyed in the sugar metabolism."

On the other hand, we certainly must not overvalue purely statistical methods of consideration, for in the case of such investigations, we often neglect to determine actually how large the variation width is between single districts, population groups or age classes in one country, and whether, for instance, the usage habits have changed over a longer period of time. This last should be demonstrated in two figures. Figure 2 shows the displacement in recent years of sugar use in Germany from the household to industry, a development that can be observed in all industrial nations. Thus, for example, industrial use of sugar in the USA today has reached a proportion of 70% of the total use.

Figure 2

Development of the sugar market for industrial consumers in contrast to direct consumption in the household in the Federal Republic of Germany since 1952/53



Key:

A.= household
B.= manufacture
C.= year

The consequence is that the greater portion of sugar consumption takes place in hidden form, combined with other food products, e.g. ice cream or chocolate with milk products and fat. Furthermore, the rate of growth of the individual industrial consumers is not equal, as shown in figure 3. We can clearly recognize the over-proportional growth of, for instance, the consumer group "drinks". In this case, too, a tendency common to all industrial nations is manifested.

Another danger inherent in purely statistical considerations lies in the fact that something is juggled with the figures, according to how exactly the figures are used. A typical example of this is provided by Yudkin (4):

"One has the impression that both disciples and opponents of the nourishment theory use only those data that support their own thesis. How misleading incomplete statistics can be may be demonstrated by two tables. Both show mortality due to myocardial infarction for the year 1960, related to every 100,000 men of an age between 55 and 64, and connected with fat consumption per capita and per day in grams of the country involved.

	1. Japan	2. Italien	3. Bundesrepublik	4. Schweiz	5. Neuseeland
A. Sterblichkeit	57	113	190	205	576
B. Fettverbrauch	16	57	101	113	149

Key:
A.= mortality
B.= fat consumption
1.= Japan 2.= Italy 3.= Federal Repuulic of Germany
4.= Switzerland 5.= New Zealand

There is seen a uniform correlation: the higher the fat consumption, the greater the mortality due to myocardial infarction. However, the picture changes if we compare five other countries to each other:

	1. Norwegen	2. Dänemark	3. England	4. Kanada	5. USA
A. Sterblichkeit	239	319	470	689	829
B. Fettverbrauch	125	140	123	123	135

Key:
A.= mortality
B.= fat consumption
1.= Norway 2.= Denmark 3.= England 4.= Canada 5.= U.S.A.

Despite the somewhat smaller daily fat consumption, the infarction mortality rate in the U.S.A is more than three times as high as in Denmark. Thus the correlation between fat consumption and coronary mortality is not valid in all cases."

Unfortunately, we miss the requirement for greater statistical objectivity that he himself expresses in later works of Yudkin, when, for example, he says in his lecture in Kiel in April of 1969 (5):

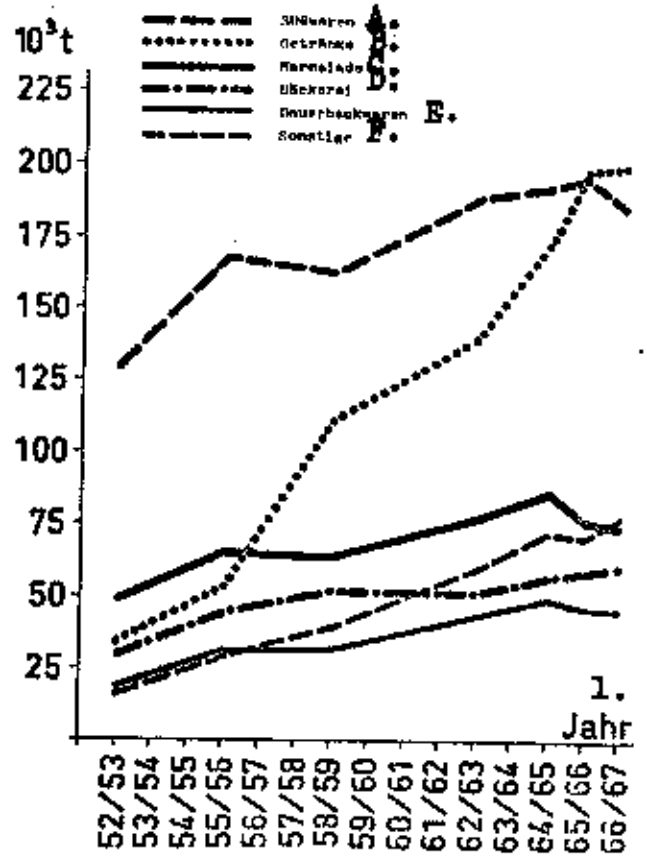
"Sugar consumption in England per capita and per year has increased from about 2 kg 250 years ago to about 10 kg in the middle of the 19th century and 50 kg in the post-war period, thus by a factor of 25 in all. Today we consume in two weeks as much sugar as our forebears consumed in one year. In the Federal Republic the increase set in later and somewhat more hesitantly. In spite of this, consumption today has risen already to more than

45 kg per year."

As can be seen from figure 2, sugar consumption in the Federal Republic of Germany amounts to only 30 kg per capita and year and -- unfortunately for us -- not, as Yudkin claims, 45 kg. He increased our consumption by a factor of 1.5, although it would have been easy enough to learn the actual consumption figure.

Figure 3

Industrial sugar consumption according to consumer groups in the Federal Republic of Germany since 1952/53



Key:

A.= sweet products
B.= drinks
C.= marmalade
D.= baked goods
E.= frozen baked goods
F.= other
1.= year

Cleave and Campbell doubt that a higher fat consumption could be a cause of diseases, especially coronary diseases, and they explain this as follows (p. 134):

"Animal fats, including those contained in milk and butter, have been present in the natural environment of man for hundreds and thousands of generations. Their consumption can therefore not represent a primary cause of coronary ailments, so long as it agrees with personal taste and appetite. This is also true

for the case that the fats, for example in butter, are concentrated, since such a concentration neither equals nor surpasses the concentration already present in the pure fat of meat (see Chapter II, page 26). With respect to coronary vessel ailments, plants and fish oils, which often -- even though not nearly so often as supposed -- contain large amounts of highly unsaturated "essential" fatty acids, in no sense offer advantages over animal fats, unless, of course, the palate has a preference for them."

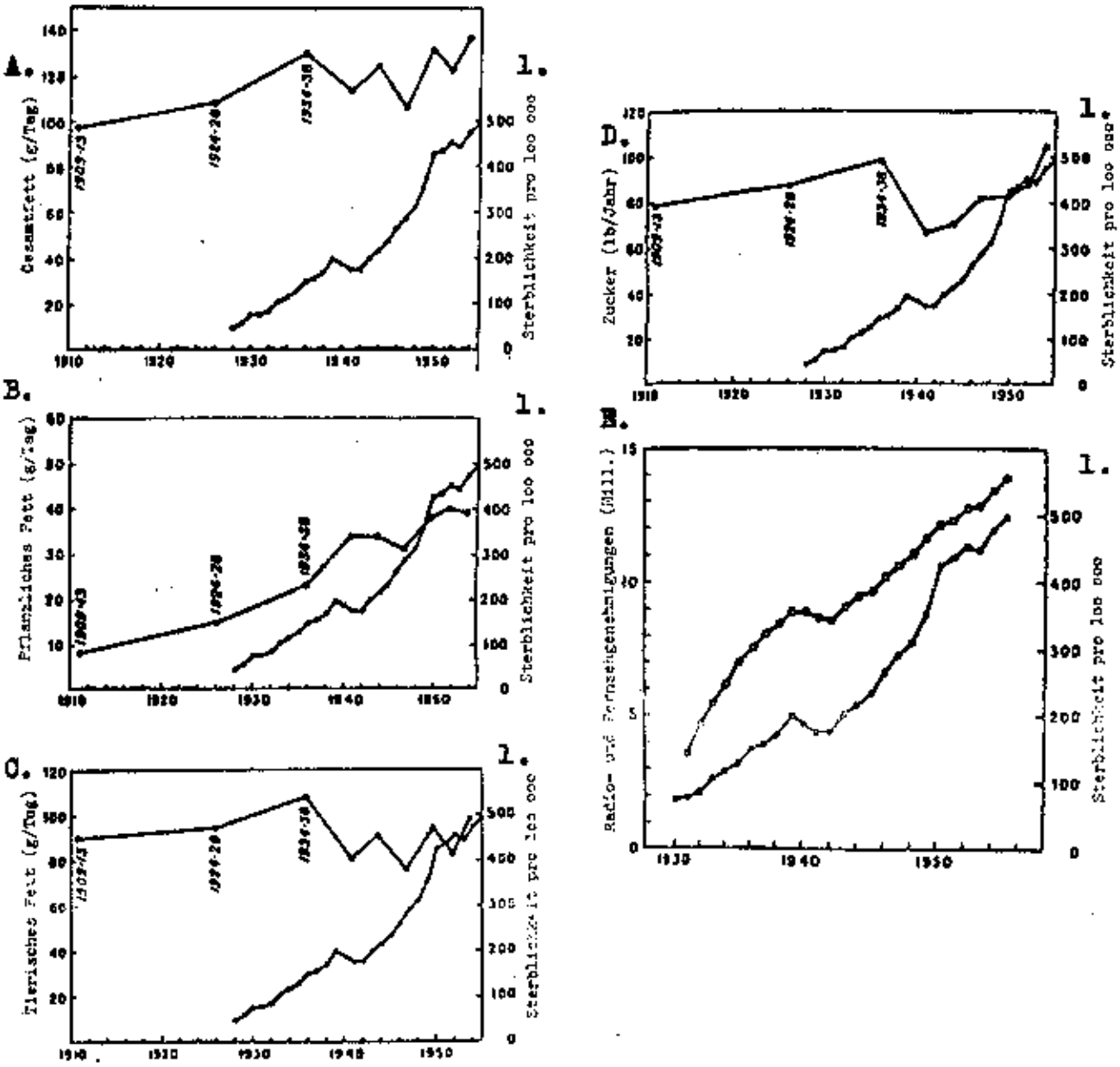
Yudkin argues similarly (5) in the above-mentioned Kiel lecture, when he writes in his summary:

"From an evolutionary historical viewpoint, the fact can be cited that our forefathers lived for millions of years off meat and animal fat, but in contrast ate hardly any sugar. From this the conclusion can be drawn that man is biologically more suited to meat than sugar. Upon historical consideration it is revealed that the increase in the frequency of coronary thrombosis in the last 50 to 100 years runs parallel to a similarly marked increase in sugar consumption. Thirdly, from an epidemiological viewpoint, there is a narrow correlation between the sugar consumption of the inhabitants of some countries and their susceptibility to these ailments."

Now, we are lucky enough to be able to allow Yudkin to speak against Yudkin on just this theme. In a work from the year 1957, Yudkin (4) attempted to relate coronary deaths to the consumption of food products, and found thereby, as can easily be seen from figure 4, that it is difficult to find a correlation between cases of death and the consumption of a single food product. He therefore supposed that overconsumption in general, combined with lesser physical activity, was one of the causes of heart disease. The closest correlation found in his examinations was that between coronary deaths and the number of radio and television observers. In another work (6) from the year 1964 we find a summarization (Table 3), which repeatedly appears in Yudkin's subsequent works. In this table, the daily calorie intake and the average consumption of basic foods such as fat, protein, total carbohydrates and sugar are shown in relation to the gross national product per person. Although it is clearly seen from these figures that sugar and fat consumption are practically parallel, there is found here the otherwise unsupported claim that there is a causal connection between heart diseases, diabetes, and sugar consumption. In the same work there is also a graphic representation (figure 5) that strengthens this narrow connection between fat and sugar consumption. The available figures from 41 countries were analyzed therein.

Figure 4

Connection between coronary deaths and fat and sugar consumption as well as the number of radio and television permits



Key:
A.= total fat (g/day)
B.= vegetable fat (g/day)
C.= animal fat (g/day)
D.= sugar (lb/year)
E.= Radio and television permits (millions)
1.= mortality per 100,000

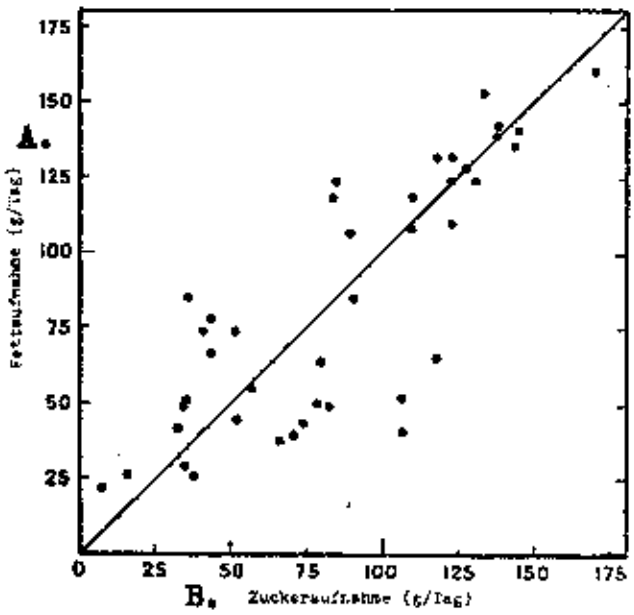
Table 3

Influence of the average national income on calorie, protein, fat, total carbohydrate, and sugar intake

Yearly income (£)			Daily intake				
range	ave.	no. of countries	kcal	g fat	g protein	g carbohydrates	g sugar	sugar in % of carbo.
to 50	38	6	2060	34	52	394	35	9
51-100	82	6	2500	61	68	437	59	13.5
101-200	148	7	2700	73	78	456	86	19
210-350	292	7	2970	118	86	419	102	24
350 up	460	8	3200	140	92	422	139	33

Figure 5

Correlation between fat and sugar consumption in 41 countries according to Yudkin



Key:
A.= fat intake (g/day)
B.= sugar intake (g/day)

In 1964, the famous work of Yudkin and Roddy (7) appeared, in which the above-mentioned claim, among others, was to be documented. The sugar consumption of two groups of diseased persons was compared with that of healthy persons. The patients of the first group suffered from heart ailments, and those of the second group from Bürger's disease, a related peripheral circulatory disorder. The first group of patients, with 20 men aged 45 to 65 (average age 56.4 years), had a sugar consumption of 132 g/day average; the second group, consisting of 25 men with an average age of 56.5 years, had a sugar consumption of 141 g/day average, and the control group with 25 men of an average age of 56.0 years a daily sugar consumption of 77 g. In a later work, Yudkin and Moorland (8) obtained nearly identical results in a later investigation similar to this first. It should be added that the daily sugar consumption of the two groups of patients with 132 or 141 g was indeed significantly higher than that of the control group, with 77. Nonetheless, it is worthy of note that the sugar consumption of the two groups of patients is on the same level as the average sugar consumption in England. Thus the conclusion may probably be drawn, that the sugar consumption of the two groups of patients can not be viewed as especially high, but rather that the amount of sugar consumed by the control group is extraordinarily low for England. In order of magnitude, it is close to the average German per capita consumption. In the work last cited, the authors relate to the animal experiments of MacDonald (9). That author states first of all that fructose or saccharose consumption has no influence on the cholesterin level, as other authors found. He does however confirm an effect on the triglyceride level, whereby, in the second paragraph of the summary, he points out stress due to excessively high carbohydrate intake. As a rule, such eruptions in the lipide metabolism can actually be achieved only with amounts of 300 to 500 g sugar per day in man. Yudkin and Szanto (10) were able themselves to confirm this fact in the work they published in September, 1969.

As can be seen from Table 4, which was taken from this last-cited work, only the triglyceride content and the plasma insulin content in the blood for the group with a saccharose consumption of 438 g/day are significantly higher than they are in the case of the control group, with a sugar consumption of 164 g/day, or the average English consumption. The experiments reported in Table 4 were conducted with two groups each of 9 or 10 healthy students; each of the groups was an average of 28 years old, 175 cm tall, and the average weight was 73 kg. The experimental conditions were the following: In the first week all the subjects ate their normal food, in the second and third week, the starch portion of the food was replaced as much as possible by sugar. In the fourth and fifth weeks, the groups returned to their normal eating habits, and in the sixth and seventh weeks did as much as possible without sugar.

I will later return to this research, but will first further discuss the works of Yudkin in chronological order.

Thus, we note first of all that Yudkin becomes more careful with his statements. Thus, for example, in 1967 he comes to the conclusion that the significance of sugar for the formation of arteriosclerosis is greater than for the formation of diabetes, and

that much more comprehensive data is needed before we can make quantitative determinations and further statements (11).

Table 4

Effect of sugar consumption on 19 male experimental subjects (Average values)

	Pre-examination period	Sugar consumption period	Pause	Starch consumption period	Pause
kcal/day	3390	3350	—	3370	—
Weight (kg)	73.0	74.0[1]	73.4	73.6	73.5
Sugar (g/day)	164	438	—	10	—
Lipides (mg/100 ml)					
Cholesterin	249	248	250	243	248
Phosphatides	230	231	231	230	230
Triglycerides	143	150[1]	142	140	142
Blood glucose (mg/100 ml)					
after 0 minutes	69	69	68	69	70
after 30 minutes	99	99	98	99	100
after 120 minutes	68	68	68	67	68
Serum insulin content (μU/ml)					
after 0 minutes	22	28[2]	23	22	21
after 30 minutes	63	86[2]	67	64	64
after 120 minutes	34	49[2]	36	32	32
Thrombocyte adhesiveness (%)	67	65	69	67	67
Fibrinogen (mg/100 ml)	255	256	259	257	255
Disintegration time for blood clots (h)	5¾	5	5	5¼	5¼

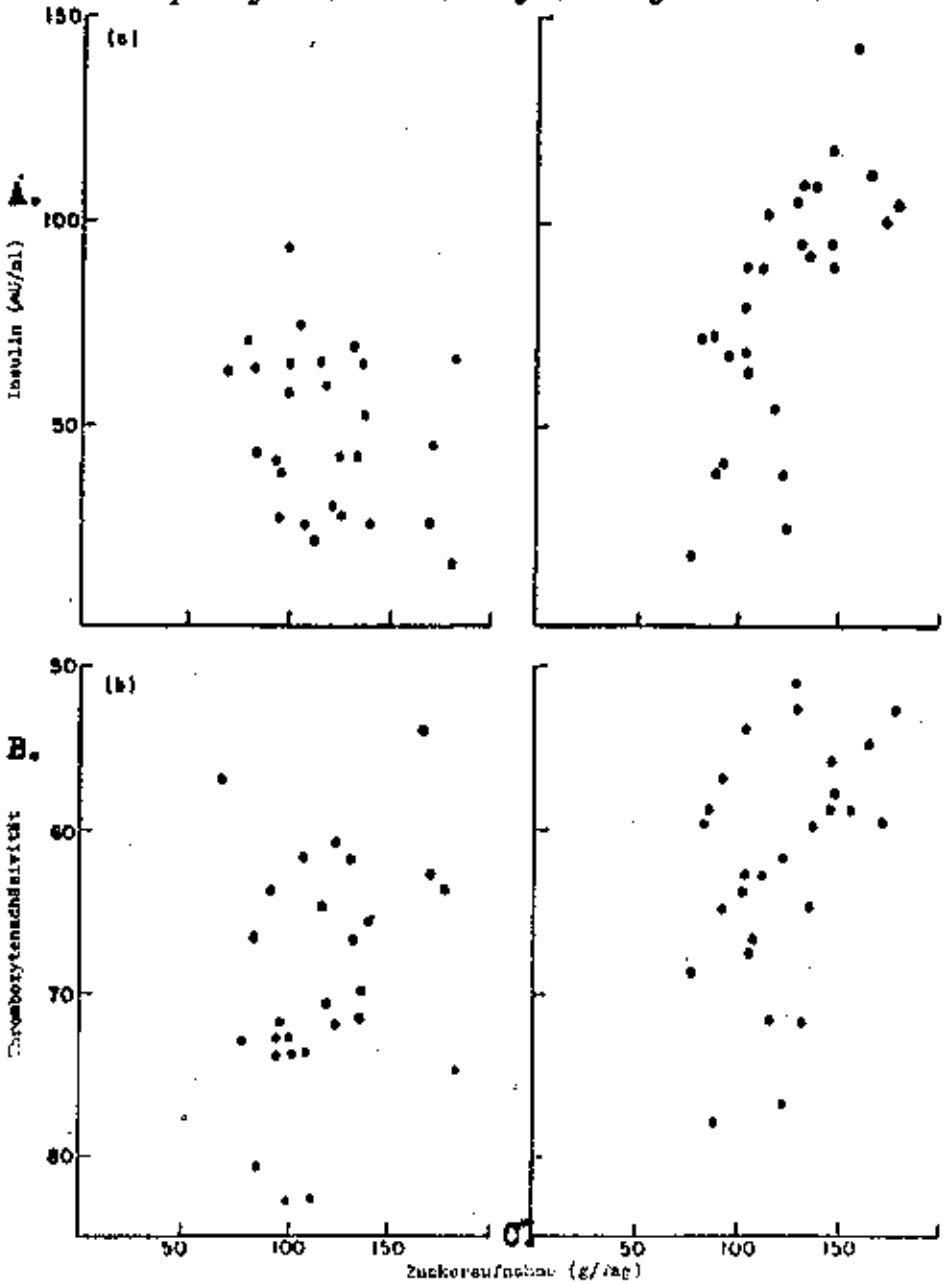
1 and 2 Significant differences between these values and those of the preceding and following periods: [1] $P < 0.05$; [2] $P < 0.01$.

In 1968, numerous discussion between Yudkin and other researchers were published in the magazine "Lancet". In his replies, Yudkin had become more differentiated, when, for example, he again agrees with Professor Paul that the etiology of heart ailments is indeed very complex, or that sugar consumption among the youthful is greater than among older persons, and that thus far it has not been sufficiently proved that heart ailments can be influenced by certain means of nourishment (12).

From the many scientific works that have opposed the statements of Yudkin critically, I should like here to select a few for discussion, among them the investigations of Little, which were conducted on a statistically selected group of researchers, and the work by Stare of Boston, who is recognized as a sober and critical nourishment physiologist. In the work of Little, Shanow and Cisma (13) it is clearly established that only the fat in the nourishment has a significant effect on the serum lipides. Saccharose revealed no significant reactions. No convincing correlation could

be found between carbohydrates or saccharose and the serum lipide status.

Figure 6
Correlation between sugar consumption in g/day and serum insulein content (a) and thrombocyte adhesiveness (b) in the case of healthy persons and patients with peripheral coronary artery ailments



Key:
A.= insulin (U/ml)
B.= Thrombocyte adhesiveness
C.= sugar intake (g/day)

Stare's research group subjected all hitherto appeared works to an extraordinarily detailed and convincing examination and analysis, the result of which is summarized as follows (14):

"The findings available thus far allow only conjecture concerning the connection between nourishment on the one hand and development and course of arteriosclerotic processes on the other. There seems to be a possible influence on the serum lipide level and above all on the serum cholesterin content. However, the various biochemical mechanisms have not yet been clarified.

There is no doubt about the fact that the serum cholesterin level can be influenced by alterations in the fat and cholesterin content in the food. Epidemiological, animal experimental and clinical findings permit the conclusion that more favorable results can be achieved by lowering the portion of saturated fatty acids and the food cholesterin and by increased intake of poly-unsaturated fatty acids. Important suggestions for the food product industry can be found herein. By means of corresponding coordination of the various industrial branches, it is possible today to manufacture food products that can prevent arteriosclerosis.

Several results of human and animal experiments reveal that the type and amount of food carbohydrates affect the level of the serum lipides. However, the effect is only slightly significant. It is somewhat more marked in the case of food poor in fats. Since, however, types of diets that are simultaneously poor in fat and rich in saccharose are normally rarely used, the practical significance of the carbohydrate effect on the cholesterin content is slight in comparison to the influence of the food fats."

Nothing need be added to this summarization. It agrees completely with our previously proven knowledge, and is moreover reconfirmed by two statistical investigations that were published last year. Howell and Wilson (15) summarized their comparative five-year statistical investigations as follows:

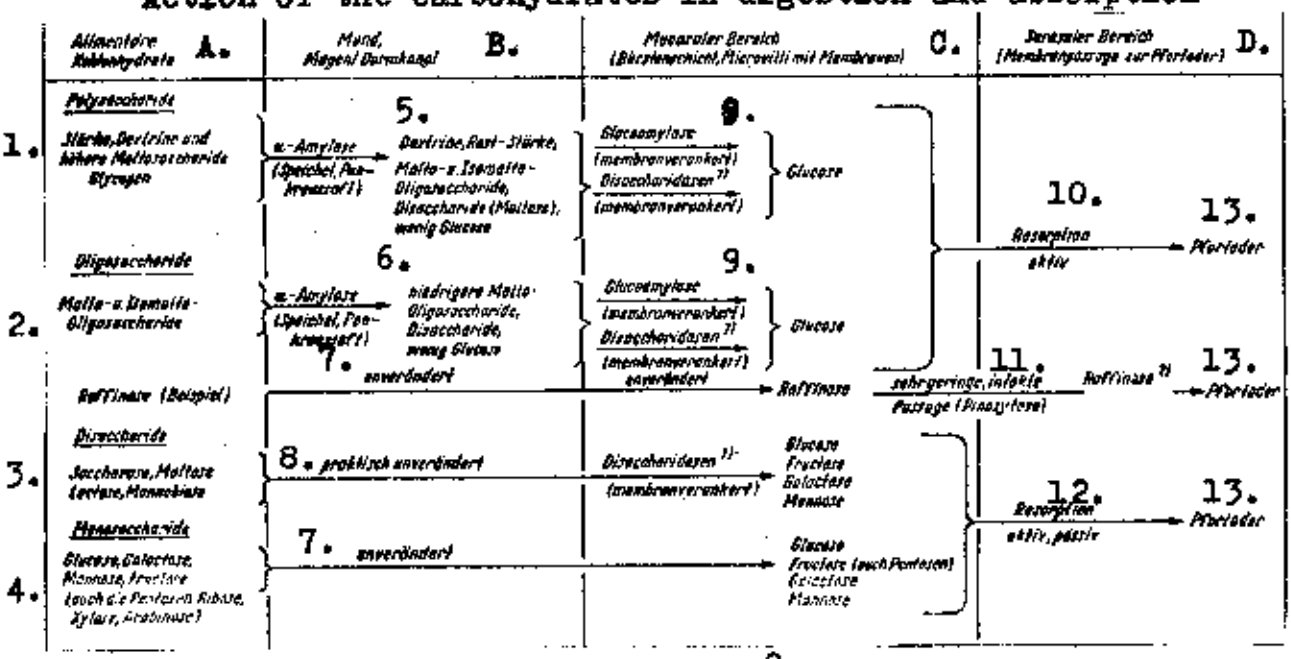
"The sugar consumption of 1,158 persons who believed to be free of ischemic heart ailments was compared with that of 170 patients who either had ischemic heart ailments, or were suspected of having them. In no group was any significant correlation between sugar consumption, serum cholesterin content, number of white blood corpuscles, hemoglobin content, β-lipoproteins, or uric acid found.. There was also no correlation between sugar consumption and the weight reached after the twenty-fifth year. These results confirm the view that more data are required before Yudkin's hypothesis that a higher sugar consumption is an important factor for the formation of ischemic heart ailments can be accepted."

Burns-Cox, Doll and Ball (16) arrive at similar results, extensively using the method described by Yudkin in 1964 and 1967 for determining the sugar consumption of persons questioned in their investigations. The authors summarize their results as follows:

"We attempted to reproduce Tudkin's research on the question of whether daily sugar consumption is greater in the case of patients with heart ailments than in the case of healthy persons. For this, 80 men under 65 years of age who had suffered a myocardial infarction were compared with 160 men of the same age and same origin, who were in the clinic because of other illnesses. It was found that the infarction patients consumed only as little more sugar in their hot drinks and only a little more sugar per day than the control patients. However, none of the observed differences was statistically significant."

Figure 7

Action of the carbohydrates in digestion and absorption



[1]α-glucosidases, β-galactosidases [2]Intact passage is possible for all saccharides, even starch; usually occurs in small amounts; maximum 2% of blood glucose values (4), but upon change of the intestinal mucosa for greater permeability, can increase significantly.

Key:
A.= food carbohydrates B.= mouth, stomach/intestinal tract
C.= mucosal area (brush layer, microvilli with membranes)
D.= serosal area (membrane passage to portal vein)
1.= polysaccharides; starch, dextrin and higher maltosaccharides; glycogen
2.= Oligosaccharides; malto and isomalto-oligosaccharides; raffinose
3. Disaccharides; saccharose, maltose, lactose, mannobiose
4.= Monosaccharides; glucose, galactose, mannose, fructose (also the pentoses ribose, xylose and arabinose)
5.= α-amylase (saliva, pancreatic juice); dextrin, residuary starch, malto and isomalto-oligosaccharides, disaccharides (maltose), little glucose
6.= α-amylase (saliva, pancreatic juice); lower malto-oligosaccharides, disaccharides, little glucose
7.= unaltered 8.= practically unaltered

Key cont'd:
9.= glucoamylase (membrane-anchored); disaccharidases (membrane-anchored)

10= absorption, active
11.= very slight, intact passage (pinocytose)
12.= absorption active, passive
13.= portal vein

Let us return now to the most recent publications of Yudkin's research group. In table 4, the results of one of the works (10) are given. Yudkin interprets this experimental result in the following manner in his summary:

"Methods of nourishment with a high sugar proportion cause no alteration in the cholesterin and phospholipide levels, nor in the values for the glucose tolerance test; however, they significantly alter the triglyceride level in all 19 persons examined. In the case of 6 of them, the plasma insulin content increased simultaneously, especially duringthe glucose tolerance test. The same 6 persons also showed an increase in their body weight and a significant increase in the adherence of the blood platelets.(thrombocyte adhesiveness). There were no differences between these 6 persons and the remaining 13 as concerns any other experimental result; even the food which they ate during the experiment was not different from that of the other persons. The changes that were caused in these persons by the highly saccharose-containing food disappeared in about 14 days when they returned to normal food.

It is suspected that the ability of saccharose to overproduce insulin is more significant due to its possible role in the etiology of ischemic heart ailments than its effect on the blood lipides. It is further suspected that only a few individuals are susceptible to developing heart ailments from the consumption of sugar, and that the same persons also reveal a sugar-induced overproduction of insulin (hyperinsulinism)."

In another work also published in September of 1969, Yudkin, Szanto and Kakkar (17) attempted to correlate sugar consumption with the serum insulin content and the adherence of the blood platelets (thrombocyte adhesiveness) in healthy persons on the one hand and in persons with peripheral arterial ailments on the other. The individual values of this examination are represented graphically (figure 6). From the graphic representation alone it is difficult to see a correlation between these values. Computationally, however, the authors did find a correlation in the case of the patients with heart conditions, but not in the case of healthy persons. They summarize their results as follows:

"The results support the supposition of Szanto and Yudkin, that a high sugar consumption raises the insulin level in some persons, though not in all, and that these persons are also susceptible to arteriosclerotic ailments, caused by saccharose."

Let us therefore summarize. Yudkin could only prove that even in the case of a daily sugar consumption of 400 to 500 g --

an amount that is some 4.5 times greater than the average German consumption and certainly can be consumed by one person only in exceptional cases — there is an increase in the serum insulin level and an increase in the adherence of blood platelets (thrombocyte adhesiveness) only in some, and not all persons. He believes that these two factors can cause heart conditions. Proof of this, nonetheless, is still due. He no longer speaks of ailments in the case of a normal sugar consumption of 80 to 150 g per day. What a change in his views, at least in the purely scientific realm!

With this, I should like to leave the controversy surrounding Yudkin and briefly go into something which we assume is suited to strengthening additionally the argument that the conjectures of Yudkin and Cleave and Campbell are not so tenable scientifically.

In table 5, the carbohydrates most important for nutrition, their principal sources and their percentual proportion in total carbohydrate nutrition for Germany are shown (18). In figure 7, the decomposition of the individual carbohydrates in the single steps of digestion is represented schematically (18). As can be seen from this diagram, saccharose passes through the mouth and stomach tract practically unsplit — a maximum of 8% is split in this area by the hydrogen ion activity —, before it is split into the two components fructose and glucose in the enzyme system, which is located in the brush seam of the mucous cells in the small intestine, and is finally absorbed. Absorption of the glucose occurs actively in dependence upon the sodium ion concentration, the absorption of the fructose passively by means of diffusion.

If the enzyme system necessary for the splitting of the saccharose is absent from the mucous cells — according to the division of Prader, maltase 3 (saccharase 1) and maltase 4 (saccharase 2), then we have the so-called illness aspect of saccharose-isomaltose malabsorption. Saccharose malabsorption was first described in 1961 by Weijers et al. (19) and was recognized as what its name suggests. A little later, Prader et al. (20) were able to show that in all cases of saccharose malabsorption there is also isomaltose malabsorption. Thus far, about 70 such cases have been described (21). The anomaly is hereditary; the method of inheritance has not yet been explained completely. There is probably a recessive inheritance process. As long as the carriers of the anomaly are controlled and fed only with cow's milk, they are free of symptoms. As soon as saccharose, dextrin-maltose mixtures or large amounts of starch are added to the food, they soon excrete diarrhetic, acid feces. If the intolerable carbohydrates are not eliminated from the food, dehydration and dystrophy develop. In the case of most patients, saccharose malabsorption disappears between the ages of four and twelve; the additional isomaltose intolerability improves much earlier. In the case of some anomaly carriers, however, malabsorption persists into adulthood. Diagnosis is relatively simple; upon oral administration of the intolerable carbohydrates, blood sugar increase is missing (less than 20 to 25 mg% increase over values on an empty stomach). At the same time, the patient excretes thin, acid feces, in which increased acetic acid, lactic acid and saccharose can be detected. The diarrhea of the anomaly

carrier is due largely to the osmotic effect of the disaccharides that are unsplit because of a lack of maltase 3 to 5., The low molecular carbohydrate fractions that form in the colon as a result of bacterial influence and which are also responsible for the acidity of the feces, probably play no or only a small role in the genesis of diarrhea. Dietetic treatment poses no great difficulties; after elimination of saccharose, dextrin and maltose mixtures and limitation of the starch in food, the symptoms disappear rapidly. Pure glucose has proved to be a good sugar supplement and can be administered with no problem, since it does not need to be split before absorption. Thus, if saccharose is intolerable to a person until it is absorbed, this manifests itself in a throughly recognisable disease.

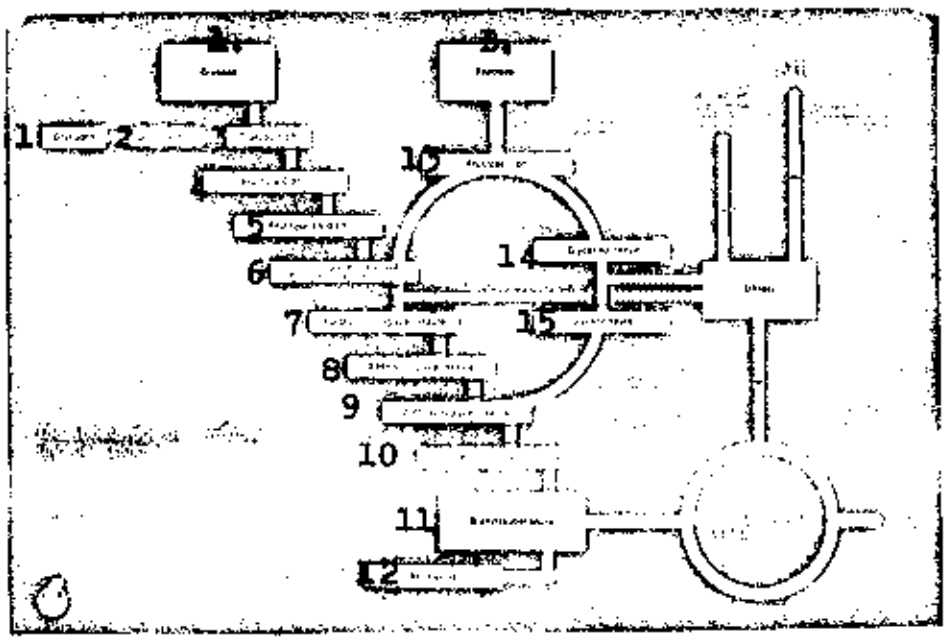
If the two monosaccharides formed from saccharose are absorbed first, then the intermediate carbohydrate metabolism begins for glucose and fructose. The human enzyme system can then no longer differentiate between the individual molecules of glucose or fructose and tell whether they come from saccharose, starch or a natural food product, such as, for example, an apple. This is not to say that there are no differences in the speed of absorption in the consumption of individual carbohydrates. They do exist, just like the mechanical passage, and the development of intestinal flora is dependent upon the composition of the food, especially the ratio of digestible food products to ballast substances such as cellulose, pectin, etc.

Figure 8 is intended to remind us briefly of the essential differences between the intermediate carbohydrate splitting of glucose and fructose (22). The principal difference of both courses consists in the fact that fructose is utilized independently of insulin, in contrast to glucose. The reduction course of the latter has been made known to us by the classic investigations of Cori, Embden, Meyerhof, Neuberg and Warburg. The reduction course of fructose is different starting from the entrance reaction; fructose is phosphorylized to fructose-1-phosphate by fructokinase, whereby ATP serves as the energy source. The reaction takes place in the liver about 10 times faster than the phosphorylization of glucose. Likewise, the splitting of fructose-1-phosphate into phosphordihydroxyacetone and glycerine aldehyde occurs more rapidly than the decomposition of fructose-1.6-diphosphate in the reduction of glucose. The glycerine aldehyde formed in the course of reduction of fructose is dehydrated into glyceric acid before, phosphorylized into 2-phosphorglyceric acid, it leads back into the normal reduction course of glucose (23).

Differences in the utilization of saccharose or glucose and starch can therefore be due to damages in some enzyme systems. That such enzyme damages can develop as the result of the consumption of a food product seems, however, very doubtful, since on the other hand, damages in the enzyme system of the carbohydrate reduction course are known, and lead to diseases with severe consequences. Only, these enzyme damages are mostly determined hereditarily, and the corresponding diseases, e.g. glucose intolerance or fructose intolerance, have been known for a long time (21).

Figure 8

Differences in the intermediate metabolism of glucose and fructose



Key:
A.= glucose B.= fructose
1.= glycogen 2.= glucose-1-ph 3.= glucose-6-ph
4.= fructose -6 ph 5.= fructose-1,6-ph
6.= illegible 7.= 1,3-diphosphoglyceric acid
8.= 3-phosphoglyceric acid
9.= 2-phosphoglyceric acid
10.= illegible
11.= pyruvic acid
12.= illegible
13.= fructose-1-ph
14.= glycerine aldehyde
15.= glyceric acid

If we consider further how narrowly connected the fat and carbohydrate reduction courses and even the protein metabolism are, then we find it difficult to believe that the civilization diseases of our time can be attributed to the consumption of a single food product. As a result, the various schools with their own ideas confront each other at these congresses and symposiums.

The Spiegel recently formulated this fact in a report on a convention as follows (24):

ARTERIOSCLEROSIS

Fatal Training

The number one killer of modern mankind hides in fatty foods and sweet snacks, it is swallowed down with many vitamin pills, inhaled with cigarette smoke, breathed in garages and in the streets. Sometimes it even pours out of the water faucet.

Wherever doctors search for sources of danger for the heart

and circulation among the living habits of middle-class citizens, they find it. Every few months, experts discuss at conventions the possible causes that lead to the calcium-fat deposits in the arteries. Nearly every second inhabitant of the western world dies from one of the results of this: myocardial infarction, pulmonary embolism or heart attack.

The heart-circulation death is thus far the inevitable price we pay for civilization. The participants of even the most recent conference on circulatory ailments could tell us nothing more than that. At the beginning of this month, the second International Symposium on Arteriosclerosis opened in Chicago; the weekend before last, the Annual Convention of the American Heart Association ended in Dallas, Texas.

Many lectures offered new evidence of the often-named connections between environmental influences and circulatory impairments. Several research reports concerning hitherto unsuspected sources of damage only confused the experts gathered in Chicago and Dallas, however. The infarction-promoting effect of psychological pressure and pressing deadlines (the so-called "manager's disease") was doubted neither in Dallas nor in Chicago. Perhaps some obscurities concerning other risk factors could be eliminated by different research reports. Several researches recently completed confirm the circulation-impairing effects of

> poor eating habits such as consumption of too-rich foods (and consequent overweight) or the custom of eating oneself full at only a few main meals (instead of eating less more frequently),
>
> a high proportion of animal fats (which are composed of so-called saturated fatty acids) in the diet,
>
> high sugar consumption in the form of sweets or certain types of fruits,
>
> nicotine -- insofar as it is inhaled (as especially by cigarette smokers), and
>
> soft drinking water.

There we have the summary of the Spiegel.

Or should we consider valid the suggestion of the famous Frankfurt physician Vollhardt, who often wrote on the prescriptions of his overweight patients: "FdH"?

LITERATURE

(1) Cleave, T. L.; Campbell, G. D.: Die Saccharidose. Bad Homburg 1969

(2) Gsell, O.: Epidemiologie des Diabetes unter besonderer Berücksichtigung von Morbidität und Mortalität. Dtsch. Med. Wschr. 93 (1968), 2446—2450

(3) Mehnert, H.: Weltstadt mit Zucker. Euromed. 6 (1966), 438

(4) Yudkin, J.: Diät und Koronarthrombose. Hypothesen und Tatsachen. Lancet 1967, Nr. 7, 155; ref. in: Pro Medico 1967, Nr. 9, 519

(5) Yudkin, J.: Die Rolle der Kohlenhydrate in der Ernährung. Milchwissenschaft 24 (1969), 401—405

(6) Yudkin, J.: Dietary fat and dietary sugar in relation to ischaemic heart-disease and diabetes. Lancet 1964, Nr. 7, 4—5

(7) Yudkin, J.; Roddy, J.: Levels of dietary sucrose in patients with occlusive atherosclerotic disease. Lancet 1964, Nr. 7, 6—8

(8) Yudkin, J.; Moorland, J.: Prevention of ischaemic heart-disease. Lancet 1966, Nr. 12, 1358

(9) MacDonald, I.: Dietary carbohydrates in normolipemia. Amer. J. clin. Nutrit. 20 (1967), Nr. 2, 185—190

(10) Yudkin, J.; Szanto, S.: The effect of dietary sucrose on blood lipids serum insulin, platelet adhesiviness and body weight in human volunteers. Postgrad. med. J. 45 (1969), 602—607

(11) Yudkin, J.: Evolutionary and historical changes in dietary carbohydrates. Amer. J. clin. Nutrit. 20 (1967), Nr. 2, 108—115

(12) Yudkin, J.; Watson, R. H. J.: Diet and cardiovascular disease. Lancet 1968, Nr. 12, 1297

(13) Little, J. A.; Shanoff, H. M.; Cisma, A.: Dietary carbohydrate and fat, serum lipoproteins, and human atherosclerosis. Amer. J. clin. Nutrit. 20 (1967), 133—138

(14) McGandy, R. B.; Hegsted, D. M.; Stare, F. J.: Dietary fats, carbohydrates and atherosclerotic vascular disease. New Engl. J. Med. 277 (1967), 186—192, 242—247

(15) Howell, R. W.; Wilson, D. G.: Dietary sugar and ischaemic heart disease. Brit. Med. J. 3 (1969), 145—148

(16) Burns-Cox, C. J.; Doll, R.; Ball, K. P.: Sugar intake and myocardial infarction. Brit. Heart J. 31 (1969), 485—490

(17) Yudkin, J.; Szanto, S.; Kakkar, V. V.: Sugar intake, serum insulin and platelet adhesiviness in men with and without peripheral vascular disease. Postgrad. med. J. 45 (1969), 608—611

(18) Täufel, K.: Forschungsergebnisse auf dem Gebiet von Nahrung und Ernährung, ausgewählt aus dem neuen Schrifttum. 6. Mitt.: Verdauung und Resorption von Kohlenhydraten. Nahrung 13 (1969), 271—283

(19) Weijers, H. A.; van de Kamer, J. H.; Dicke, W. K.; Ijsselstijn, J.: Diarrhoea caused by deficiency of sugar splitting enzymes. Acta Paediatrica 50 (1961), 55—71

(20) Auricchio, S.; Dahlqvist, A.; Mårsal, G.; Prader, A.: Isomaltose intolerance causing decreased ability to utilize dietary starch. J. Pediatrics 62 (1963), 165—170

(21) Mattern, M.: Mono- und Disaccharidintoleranz und Malabsorptionssyndrome und ihre diätetische Behandlung. Medizin und Ernährung 10 (1969), 1—9

(22) Schematische Darstellung von Stoffwechsel-Vorgängen. Hrsg.: Deutsche Laevosan-Gesellschaft Boehringer & Soehne, Mannheim

(23) Horecker, B. L.: Stoffwechselwege der Kohlenhydrate, ihre Bedeutung und physiologische Bedeutung. Medizin und Ernährung 10 (1969), 86—88, 90—98, 127—129

(24) „Der Spiegel" Nr. 48 vom 24. 11. 1969, 210—220

J. Dent. Res. 32:210-214. 1953.

Reproduced by permission of the copyright owner

THE INCIDENCE OF DENTAL CARIES IN CARIES-SUSCEPTIBLE AND CARIES-RESISTANT ALBINO RATS (RATTUS NORVEGICUS) WHEN FED DIETS CONTAINING GRANULATED AND POWDERED SUCROSE

W. H. STEWART, C. A. HOPPERT, AND H. R. HUNT

From the Departments of Zoology and Chemistry, Michigan State College, East Lansing, Mich.

INTRODUCTION

HUNT and Hoppert have established the fact that heredity is an important factor in the development of dental caries in albino rats. The diet they used consisted of 66 per cent coarsely ground rice, 30 per cent powdered whole milk, 3 per cent alfalfa meal, and 1 per cent sodium chloride.[1-6] The question arises as to whether the hereditary difference observed is specific for the above diet, or whether it persists when various other diets are used. This is the subject of the present investigation.

Hunt and Hoppert have observed more than 8,600 caries-resistant and caries-susceptible rats in their genetic study of dental caries. They have shown that use of molar teeth predisposes to caries,[7] that sex is not important,[8] and that age appears to increase slightly the resistance to caries in susceptible rats.[9]

Population studies have shown that there is an increase in the incidence of caries when sugar and refined carbohydrates are consumed by human beings.[12, 14] This would suggest that a high concentration of sugar in the diet would produce dental caries. However, some individuals are highly resistant to caries even when eating a large amount of sugar.[25]

Sognnaes found that a nutritionally adequate purified diet of high sucrose content produced noticeable caries if fed before tooth eruption in rats.[13] This fact raises the question as to whether the hereditary difference found between the Hunt-Hoppert caries-resistant and caries-susceptible strains of rats would persist when the animals are fed a diet similar to that used by Sognnaes except for its content of several natural products.

PROCEDURE

To produce rats for this investigation, breeders were obtained from Hunt and Hoppert's seventeenth and twenty-first generation resistant and susceptible rats, respectively, in October, 1950. The breeders were continued on the ration containing finely ground rice which has been used for the last six generations. Also, the same environmental controls prevailed which have been maintained during the fifteen years of the genetic study. The litters were put on the experimental diets at 25 days of age, the animals being divided into four groups as follows: (1) susceptible rats fed the sucrose diet

Received for publication, July 20, 1952.

group S_S), (2) susceptible rats continued on the ration used by Hunt and Hippert containing finely ground rice (group S_R), (3) resistant rats fed the sucrose diet (group R_S), and (4) resistant rats continued on the fine rice diet (group R_R). The numbers of rats in each group are given in the ordinants of *Fig. 1*. The composition of the granulated sucrose diet is given in Table I.

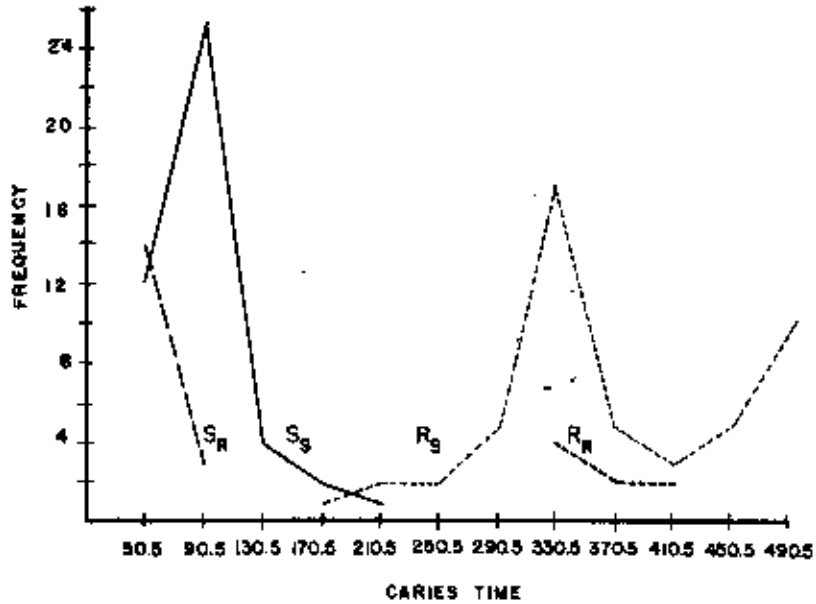


Fig. 1.—Comparison of susceptible and resistant rats fed diets containing granulated sucrose and fine rice.

Observations for cavities were made at two-week intervals by one of the authors (C. A. H.). The caries time in this experiment was the number of days elapsing from the date the rat was put on the experimental diet to the first positive evidence of caries in the lower molars. The rats were usually killed at the time of the first positive observation and the heads were preserved in alcohol. The soft tissue of the upper and lower jaws was removed and the molars were observed with a binocular microscope.

OBSERVATIONS

Fig. 1 shows the striking differences between the susceptible and resistant lines for both rations. The real differences between the two lines are underestimated because some of the resistant rats were still caries-free at the termination of the experiment when some of them had attained an average age of 500 days. In the calculations these animals were considered as having caries at the time of death. A summary of the observations is given in Table III.

F and T tests of the differences between groups S_S and R_S; S_R and R_R are as follows:

GROUP	F	T	MEAN DIFF.
S_S and R_S	7.71	19.89	275.5 days
S_R and R_R	9.72	28.04	313.8 days

TABLE I

GRANULATED SUCROSE DIET

	PER CENT
Granulated beet sugar (Michigan beet)	57
"Fine" rice*	10
Casein	18
Corn oil (Mazola)	2
Brewers' yeast	5
Alfalfa meal	6
Salt mixture†	3
	100

*The fine rice used contained approximately 2 per cent of particles retained by a 20 mesh screen.
†See Table II.

TABLE II

THE SALT MIXTURE*

	GM.
$CaCO_3$	544.08
$MgCO_3$	25.00
$MgSO_4$ (anhydrous)	16.00
NaCl	60.00
KCl	112.00
KH_2PO_4 (anhydrous)	212.00
$FePO_4 \cdot 4H_2O$	20.50
$MnSO_4$ (anhydrous)	.35
$Al_2(SO_4)_3 \cdot K_2SO_4$ (anhydrous)	.17
$CuSO_4$ (anhydrous)	.00

*This is a slight modification of the salt mixture used by Hubbell, Mendel, and Wakeman.[11]

TABLE III

GROUP	SOURCE OF CARBOHYDRATE IN THE DIET	NO. OF RATS	MEAN CARIES TIME (DAYS)
S_s	Sucrose and fine rice	44	92.25
S_R	Fine rice	17	58.29
R_s	Sucrose and fine rice	50	367.75
R_R	Fine rice	8	372.13

These mean differences are all significant past the 1 per cent level.[16] Thus, the variances and means are different for the resistant and susceptible rats on the sugar- and rice-containing diets.

A difference in the effect of the sucrose- and fine rice-containing diets is noted for the susceptible animals. Group S_R consisted of a random sample of rats from the litters to which group S_S belonged. The mean difference between groups S_S and S_R was 33.96 days caries time. F and T tests gave values of 5.32 and 4.27, respectively, which are significant.

THE POWDERED SUGAR DIET

After the experiment had been well under way it was decided to try a diet containing powdered rather than granulated sucrose, also omitting the fine rice. The diet listed in Table IV was fed to a group of susceptible animals.

This part of the experiment was conducted in the same manner as the previous one. Eleven susceptible rats on this powdered sucrose diet showed

TABLE IV

POWDERED SUGAR DIET

	PER CENT
Confectioners'* sugar	67
Casein	18
Corn oil (Mazola)	2
Brewers' yeast	5
Alfalfa meal	5
Salt mixture (modified Hubbell)	3
	100

*Confectioners' sugar is a powdered sucrose containing 3 per cent starch.

positive caries on the average at 65.7 days. The earliest observation of caries was 37 days after the rat was put on this diet. The number of animals in the experiment was small and more research is needed to test the effect of the diet. The data appear to conflict with the view that diets lacking hard coarse particles decrease the incidence of caries. It must be remembered that the animals had access to the ration containing finely ground rice until they had reached the age of 25 days. Whether caries was initiated by this early exposure to the fine rice diet is doubtful. Caries appearing in our susceptible rats on this diet earlier than 25 days of age has been rare. The presence of the 3 per cent of starch in the confectioners' sugar may have been a complicating factor. The capacity of bacteria to induce caries undoubtedly varies with different foods.

The ration containing the powdered sucrose was not fed to the resistant rats.

DISCUSSION

The hereditary difference between the Hunt and Hoppert strains of susceptible and resistant rats was still apparent on a diet containing 57 per cent granulated sucrose. The hereditary difference is not specific for only the fine rice-containing ration.

A difference between the effects of the granulated sucrose and fine rice diets was noted in the susceptible animals.

Examination of the molar teeth with a binocular microscope did not support the theory that tooth fracturing initiates caries. Small cavities were consistently seen deep in the sulci where fracturing by coarse food particles was improbable.

CONCLUSIONS

1. The manifestation of the hereditary difference between the Hunt-Hoppert caries-susceptible and caries-resistant albino rats is not confined to the diet containing ground rice. A highly concentrated sucrose diet also reveals the hereditary difference between the two lines.

2. A diet including confectioners' sucrose produced early caries in susceptible rats.

REFERENCES

1. Hoppert, C. A., Webber, P. A., and Canniff, T. L.: The Production of Dental Caries in Rats Fed an Adequate Diet, *Science* 74: 77, 1931.

2. Hoppert, C. A., Webber, P. A., and Cunniff, T. L.: The Production of Dental Caries in Rats Fed an Adequate Diet, *J. D. Res.* **12**: 161, 1932.
3. Hunt, H. R., and Hoppert, C. A.: Inheritance in Rat Caries, Genetics **24**: 76, 1939.
4. Hunt, H. R., and Hoppert, C. A.: Inheritance in Rat Caries, 1941, Year Book of the American Philosophical Society, p. 131.
5. Hunt, H. R., and Hoppert, C. A.: Inheritance of Susceptibility and Resistance to Caries in Albino Rats (Mus norvegicus), *J. Am. Col. Den.* **11**: 33, 1944.
6. Hunt, H. R., Hoppert, C. A., and Erwin, W. G.: Inheritance of Susceptibility to Caries in Albino Rats (Mus norvegicus), *J. D. Res.* **23**: 385, 1944.
7. Hunt, H. R., and Hoppert, C. A.: Occlusion as a Factor in Dental Caries of Albino Rats (Rattus norvegicus), *J. D. Res.* **27**: 553, 1948.
8. Hunt, H. R., and Hoppert, C. A.: Sex and Dental Caries in Albino Rats (Rattus Norvegicus), *J. D. Res.* **27**: 486, 1948.
9. Braunschweiger, G. E., Hunt, H. R., and Hoppert, C. A.: The Influence of Age in the Development of Dental Caries in the Rat (Rattus Norvegicus), *J. D. Res.* **27**: 154, 1948.
10. Hunt, H. R., and Hoppert, C. A.: The Distribution of Carious Cavities in the Lower Molars of Caries-Susceptible and Caries-Resistant Albino Rats (Rattus Norvegicus), *J. D. Res.* **29**: 157, 1950.
11. Hubbell, R. B., Mendel, L. B., and Wakeman, A. J.: A New Salt Mixture for Use in Experimental Diets, *J. Nutrition* **14**: 273, 1937.
12. Price, W. A.: Field Studies Among Some African Tribes on the Relation of Their Nutrition to the Incidence of Dental Caries and Dental Arch Deformities, *J. A. D. A.* **23**: 876, 1936.
13. Sognnaes, R. F.: Caries Conducive Effect of a Purified Diet When Fed to Rodents During Tooth Development, *J. A. D. A.* **37**: 676, 1948.
14. Bunting, R. W.: Textbook of Oral Pathology, Philadelphia, 1929, Lea & Febiger.
15. Gottlieb, B.: Dental Caries, Philadelphia, 1947, Lea & Febiger.
16. Snedecor, G. W.: Statistical Methods, Ames, Iowa, 1946, The Iowa State College Press.

Further reproduction prohibited without consent of copyright owner

Postgrad. Med. J. 45:602-607. 1969.

Reproduced by permission of the copyright owner

The effect of dietary sucrose on blood lipids, serum insulin, platelet adhesiveness and body weight in human volunteers

STEPHEN SZANTO
Ph.D., M.R.C.P.I.
Research Physician

JOHN YUDKIN
M.A., M.D., Ph.D.
Professor of Nutrition and Dietetics

Department of Nutrition, Queen Elizabeth College, University of London

Summary

A study was made of the effect of high or low intake of sucrose for periods of 14 days in a group of nineteen apparently healthy men.

The high sucrose diet produced no change in blood levels of cholesterol or phospholipids, or in glucose tolerance, but it produced a significant rise in triglycerides in all nineteen men. In six of them, there was in addition a rise in serum immunoreactive insulin, especially during the glucose tolerance test, and these same six subjects also showed a considerable increase in weight and a significant increase in platelet adhesiveness.

There was no difference between the six subjects and the remaining thirteen in any of the other measurements, or in the diets they consumed during the experiment. The changes produced by sucrose had disappeared, or nearly so, after 14 days of normal diet.

It is suggested that the effect of sucrose in producing hyperinsulinism may be more relevant to its possible role in the aetiology of ischaemic heart disease than its effect on blood lipids. It is further suggested that only some individuals are susceptible to the development of ischaemic heart disease by dietary sucrose, and that these may be identified as those that show 'sucrose-induced hyperinsulinism'.

Introduction

It is widely accepted that ischaemic heart disease (IHD) is a disease of affluence. Both longitudinal and cross-sectional studies have shown that the major dietary characteristic of affluence is a replacement of increasing amounts of starch by sugar (sucrose), so that in the United Kingdom during the last 200 years the proportion of sucrose has risen from less than 2% to about 35% of the total dietary carbohydrate (Yudkin, 1964). Relevant experiments, therefore, are those that study the effects of diets in which the relative proportion of these two major dietary carbohydrates is changed. We have conducted such an experiment on nineteen men aged 21–44 years.

Subjects and methods

The subjects were volunteers from the students and staff of two London University colleges. The purpose and design of the experiment was explained to them in detail, and they were each provided with food tables and given instruction in their use and in the recording of their food intake.

After a week's preliminary study, ten of the subjects (Group A) were asked for the next 2 weeks to take diets high in sucrose, the sucrose as far as possible substituting for starch so as not to change the caloric intake (Table 1). For the following 2 weeks—weeks 4 and 5—they reverted to their normal diets. For the next 2 weeks—weeks 6 and 7—they were asked to take diets with virtually no sucrose, making up in calories by increasing their starch intake. Finally they were again allowed to eat their normal diet. The remaining nine subjects (Group B) followed a similar procedure, but took the low-sucrose diets in weeks 2 and 3, and the high-sucrose diets in weeks 6 and 7. At the end of the 1st week, and thereafter at intervals of 2 weeks, the subjects were weighed, their glucose tolerance determined, the insulin response to this test measured, and the plasma lipids assayed. In addition, we measured platelet adhesiveness, plasma fibrinogen and clot lysis time.

TABLE 1. Experimental design

Period	No. of days	Group A	Group B
I	7	Preliminary	Preliminary
II	14	Sucrose	Starch
III	14	Rest	Rest
IV	14	Starch	Sucrose
V	14	Rest	Rest

Blood was taken from a forearm vein after an overnight fast. Then 50 g glucose was given by mouth

and further blood samples taken 30 and 120 min later. In all three samples, blood glucose was determined by the oxidase method of Huggett & Nixon (1957) and insulin by immunoassay (Hales & Randle, 1963). In the sample of fasting blood, cholesterol was measured by the method of Zlatkis, Zak & Boyle (1953) as modified by Henly (1957), triglyceride by the method of Handel & Zilversmit (1957), phospholipid by the method of Bartlett (1959), fibrinogen by the method of Ingram (1961) and clot lysis time by the method of Fearnley, Balmforth & Fearnley (1957).

Platelet adhesiveness was measured after 20 min rotation (Wright, 1941); no correction was made for the haematocrit values of the blood samples, since they all fell within the range 35–48%.

Each subject was given a specially prepared booklet of dietary instructions and a diary in which he recorded the foods and drinks he consumed and their quantities.

Results

We have combined the results of the different dietary treatments, so that we present them as if both groups followed the same experimental design. We feel justified in doing this because the initial values for the two groups of subjects were very similar (Table 2). Moreover, as we shall see, the effects of the high-sucrose diet had disappeared at the end of the subsequent 2-week rest period, except for a very small residual effect on serum insulin levels.

Neither the increase nor the decrease in sugar intake produced a significant change in the level of serum cholesterol or phospholipid, in glucose tolerance, in level of fibrinogen or in fibrinolytic activity (Table 3). On the other hand, the 14 days on the high-sugar diet produced a significant increase in the level of plasma triglycerides, in insulin response and in body weight, effects that were

TABLE 2. Initial findings in nineteen male subjects (mean and range)

	Group A (ten subjects)	Group B (nine subjects)
Age (years)	28 (23–44)	28 (22–41)
Height (cm)	175 (165–188)	174 (163–183)
Weight (kg)	73·7 (58·3–81·5)	72·4 (61·0–74·9)
Daily kCal	3420 (2520–4150)	3170 (3070–3440)
Diet (g/day)		
Protein	85 (72–108)	83 (71–96)
Fat	112 (98–182)	116 (100–149)
Carbohydrate	509 (301–599)	480 (402–656)
Sucrose	167 (98–209)	163 (93–248)
Serum lipids (mg/100 ml)		
Cholesterol	240 (199–272)	258 (213–281)
Phospholipid	222 (187–261)	238 (199–274)
Triglyceride	137 (98–168)	149 (122–176)
Blood glucose (mg/100 ml)		
0 min	68 (64–72)	70 (61–81)
30 min	94 (91–104)	104 (94–108)
120 min	66 (59–72)	70 (59–82)
Serum insulin (μU/ml)		
0 min	23 (17–26)	21 (14–25)
30 min	64 (49–86)	62 (53–75)
120 min	35 (29–46)	32 (23–41)
Platelet adhesiveness (%)	67 (59–81)	68 (59–82)
Fibrinogen (mg/100 ml)	231 (193–278)	283 (260–300)
Clot lysis time (hr)	5¾ (3¼–7¼)	5¾ (3¾–7¼)

TABLE 3. Effect of dietary sucrose in nineteen male subjects (mean)

	Preliminary	Sucrose	Rest	Starch	Rest
kCal/day	3390	3350	—	3370	—
Weight (kg)	73·0	74·0*	73·4	73·6	73·5
Sucrose (g/day)	164	438	—	10	—
Lipids (mg/100 ml)					
Cholesterol	249	248	250	243	248
Phospholipid	230	231	231	230	230
Triglyceride	143	150*	142	140	142
Blood glucose (mg/100 ml)					
0 min	69	69	68	69	70
30 min	99	99	98	99	100
120 min	68	68	68	67	68
Serum insulin (μU/ml)					
0 min	22	28†	23	22	21
30 min	63	86†	67	64	64
120 min	34	49†	36	32	32
Platelet adhesiveness (%)	67	65	69	67	67
Fibrinogen (mg/100 ml)	255	256	259	257	255
Clot lysis time (hr)	5¾	5	5	5¼	5¼

* or † Significant differences between these values and those for preceding and subsequent period.
*$P < 0·05$, †$P < 0·01$.

completely or almost completely reversed at the end of the next 14 days of the normal diet.

A more detailed analysis of these changes shows that the effect on serum insulin levels was confined to six of the subjects (Table 4). These same six subjects also showed an increase in platelet adhesiveness, as well as a much greater increase in body weight than did the other thirteen subjects (Tables 5 and 6, Fig. 1). The weight, platelet adhesiveness and insulin levels returned to their previous values during the next 14 days when the subjects were eating their normal diet. The changes in these three parameters were confined to six out of the nineteen subjects, whereas the change in triglyceride levels, with a rise during the high-sucrose diet and subsequent fall, were the same in the six subjects as in the remaining thirteen. Moreover, there was nothing in the experimental diets or in the original measurements—age, weight, height, blood pressure, smoking habits, blood lipids, diet, glucose tolerance or insulin response—that distinguished these six subjects from the other thirteen (Table 7).

TABLE 4. Effect of dietary sucrose on insulin response (mean, μU/ml)

	Period					
	Thirteen subjects			Six subjects		
	0 min	30 min	120 min	0 min	30 min	120 min
Preliminary	22	63	33	23	64	34
Sucrose	23	65	33	39*	131*	84*
Rest	22	65	34	25	72	40
Starch	22	64	33	22	64	32
Rest	21	64	31	23	63	34

*Significant difference between these values and those for preceding and subsequent period ($P < 0{\cdot}01$).

TABLE 6. Changes in platelet adhesiveness (%) in six subjects who developed hyperinsulinism and thirteen who did not (see Table 4)

	Period				
	Preliminary	Sucrose	Rest	Starch	Rest
Thirteen subjects	67	67	68	69	66
Six subjects	68	60*	71	65	68

*Significant difference between this value and those for preceding and subsequent period ($P < 0{\cdot}05$).

Discussion

Our primary object in carrying out this experiment was to discover a further link between the ingestion of sucrose and the production of ischaemic heart disease. The great difficulty that has been encountered in producing in experimental animals an exact parallel to human ischaemic heart disease, and to a lesser extent its precursor atherosclerosis, has directed research towards the discovery of factors that produce what are usually accepted as concomitants to the disease. This is the reason why

TABLE 5. Weight changes in six subjects who developed hyperinsulinism and thirteen who did not (see Table 4) [mean, kg (and range)]

	Average change during each week of experiment	After high-sucrose diet for 14 days	After reversion to normal diet for 14 days
Thirteen subjects	+0·1 (−0·5 — +0·4)	+0·4 (0 — +0·9)	+0·05 (−1·8 — +2·8)
Six subjects	0 (−0·1 — +0·05)	+2·1* (+1·4 — +2·7)	−2·0 (−3·2 — −1·4)

*Significant difference between this value and those for preceding and subsequent period ($P < 0{\cdot}01$).

TABLE 7. Findings in six subjects who developed hyperinsulinism and thirteen subjects who did not (see Table 4)

	Age (years)	Height (cm)	Weight (kg)	Daily diet			Serum triglycerids (mg/100 ml)	
				kCal	Pr.g.	Suc.g.	Mean	Range
Initial findings								
Thirteen subjects	27·5	175	72·5	3360	84	163	139	(98–169)
Six subjects	29·5	174	74·5	3460	84	166	151	(132–176)
High-sucrose diet								
Thirteen subjects	27·5	175	72·5	3330	83	431	146	(115–159)
Six subjects	29·5	174	74·5	3400	81	452	158	(139–179)

such a great deal of attention has been paid to raised levels of blood lipids, and why it is now common to ascribe aetiological significance in IHD, or therapeutic effect, to agents simply on the basis that they affect blood lipids.

That dietary sucrose can cause an increase in serum triglycerides in man is now well established, though most of the experiments hitherto reported have used diets that are quite different from those normally consumed (Macdonald & Braithwaite, 1964; Akinyanju *et al.*, 1968). Although the amounts of sugar eaten by our subjects during the high-sucrose period were three or more times as much as the average intake in this country, there are at least a few people who normally consume amounts of this order (Salter & Yudkin, unpublished).

It has been suggested that sucrose produces an increase in level of triglyceride because it produces an increase of weight (*Nutr. Rev.*, 1966). We have previously shown, with men consuming an 'atherogenic' diet, that the increase in the lipid can occur without weight gain (Akinyanju *et al.*, 1968). In our present experiment, thirteen subjects showed

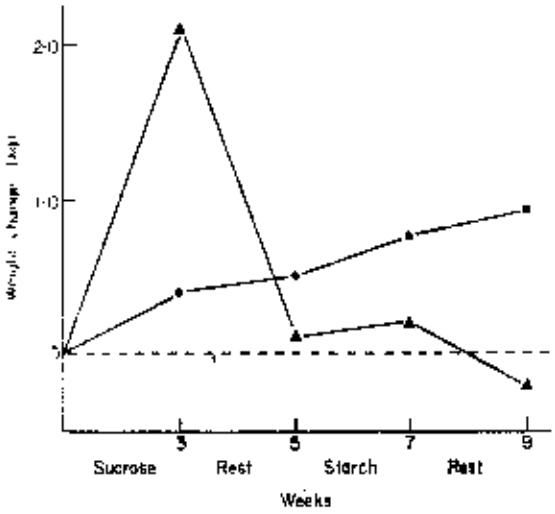


FIG. 1. Change in weight of the six subjects (▲) that showed hyperinsulinism, and of the thirteen subjects (●) that did not.

variable, small and usually insignificant changes of weight during the 9 weeks, while six subjects showed a considerable increase after 2 weeks on the high-sucrose diet, and a decrease to the previous value in the following 2 weeks. Nevertheless, in both sets of subjects there was a very similar rise in triglycerides induced by the high sucrose diet, and a fall to the previous level when the subjects reverted to their normal diet.

Much more striking, however, than the rise in serum lipids observed in all of our subjects was the great increase in levels of serum insulin in six of them the same six that showed a large increase in weight and an increase in platelet adhesiveness—during the 2 weeks of high-sucrose diet. In these subjects, the level of insulin was raised both in the fasting state and after the ingestion of glucose. Since the level of insulin rose to more than 100 μU/ml serum, these subjects can be said to have shown hyperinsulinism according to the definition of Grodsky *et al.* (1965). The high levels of insulin caused by the high sucrose diet were not associated with any change in the levels of blood glucose which remained within normal limits. It is likely that a decrease in glucose tolerance would have occurred if the high-sucrose diet had been continued for a longer time, as shown in the experiments of Cohen *et al.* (1966).

Although we do not yet know how much of the increase in weight was due to water retention and how much to fat synthesis, the association between the two features of weight gain and hyperinsulinism recalls the association between obesity and hyperinsulinism (Rabinovitz & Zierler, 1962; Samaan *et al.*, 1965).

The fact that all our subjects responded to dietary sucrose by a rise in triglyceride, but only some of them responded by a rise in insulin, supports the view of Nikkilä and his colleagues (1965) that 'disordered insulin–glucose homeostasis operates through a mechanism different from that of hyperlipidaemia'. That only about one-third of our subjects showed hyperinsulinism after the high-sucrose diet may be because only these were specially susceptible. This recalls the suggestion that only some people have a defect in one or other part of the insulin mechanism, which makes them susceptible to diabetes mellitus (Vallance-Owen & Ashton, 1963) or to obesity (Butterfield, 1967). It recalls also the experiments that demonstrate that sucrose may produce its effects upon enzymes and upon blood lipids through different mechanisms (Aitken, Robinson & Yudkin, 1967; Akinyanju & Yudkin, 1967).

Evidence is growing that hyperinsulinism may be a more relevant factor in the production of IHD than an increase in particular blood lipids. The injection of insulin into rats increases the incorporation of labelled glucose or acetate into aortic lipid (Stout, 1968), and oral administration of carbutamide to cockerels increases the degree of spontaneous atherosclerosis (Härtel, Punsar & Louhija, 1968). These facts support the view of Mahler (1965) that insulin may be an important factor in the development of atherosclerosis; it may indeed turn out that insulin is the beginning of the final common path to the production of IHD. If we accept the view that an increase in platelet adhesiveness is an im-

portant factor in the production of occlusive arterial disease, it would be relevant that sucrose produced in our experiment an increase both in insulin and in platelet adhesiveness in the same susceptible subjects.

One can now begin to explain the effects of the various factors known to be associated with IHD in terms of an association with levels of circulating insulin. Peripheral vascular disease, maturity-onset diabetes, obesity, smoking and hypertension all predispose to IHD, and all are associated with hyperinsulinism (Yalow & Berson, 1960; Rabinovitz & Zierler, 1962; Welborn *et al.*, 1966; Kingsbury & Jarrett, 1967; Szanto, 1967). Conversely physical exercise and weight reduction, which reduce the risk of IHD, are associated with a reduction in circulating insulin (Devlin, 1963; Szanto, 1968). Low levels of insulin are also found in the diabetes of chronic pancreatitis, in which vascular complications are said to be relatively uncommon (Joffe *et al.*, 1968).

We may now add sucrose to the list of agents—diabetes, obesity, smoking and hypertension—that produce hyperinsulinism. This is further evidence for the hypothesis that, like these other agents, sucrose increases an individual's proneness to occlusive arterial disease.

The hyperinsulinism caused by dietary sucrose may be responsible for many of the biochemical changes that this substance has increasingly been found to induce (see for example Yudkin, 1967). It will be useful we believe to bear this possibility in mind in future studies of the biochemical changes produced by sucrose. Similarly, if hyperinsulinism is a factor closely related to the underlying abnormality in IHD, we must not expect other changes—secondary and more remote—to be equally uniform in subjects with the disease.

In the light of this discussion, we suggest that only a proportion of the population is susceptible to the development of occlusive atherosclerotic disease following an habitual high consumption of sucrose. We believe that the sucrose leads, after many years, to an increase in insulin levels in those people that are susceptible. If these suggestions prove correct, such individuals might be identified before they develop overt disease by determining the effect upon their insulin levels of the challenge of a high consumption of sucrose for a few days; they might show what we may call 'sucrose-induced hyperinsulinism'. We would then have a relatively simple predictive test for the identification of susceptible persons who will develop IHD if they habitually consume large amounts of sucrose. We do not yet know whether these are the same persons as are susceptible because of cigarette smoking or some other predisposing factor.

There is a further consequence that we can d[illegible] from our hypothesis. It is that men with [illegible] occlusive arterial disease should include th[illegible] whose insulin levels have been increased by a h[illegible] continuing intake of sucrose. Although it will [illegible] include those whose insulin levels have been [illegible]creased by cigarette smoking or hypertension, [illegible] should nevertheless expect that persons with a h[illegible] sucrose intake will tend to congregate among th[illegible] with high serum insulin levels; those with low sucrose intake will be more likely to congregate among th[illegible] with low insulin levels. In general, that is, men w[illegible] occlusive arterial disease should show a correlation between serum insulin level and sucrose intake. On the other hand, men who by middle age have developed no symptoms or signs of atherosclerosis will be composed largely of the non-susceptible part of the population. Whatever their intake of sugar, this would not affect their serum insulin levels, so that we would expect this group to show little or no relationship between the insulin level and sucrose intake. This prediction of the presence or absence of a relationship between insulin and sucrose intake in these two groups has already been tested, and the results are reported in the following paper (Yudkin, Szanto & Kakkar, 1969).

Acknowledgments

We are grateful to the British Heart Foundation for a grant in support of this work.

References

AITKEN, J., ROBINSON, D. & YUDKIN, J. (1967) Effects of dietary carbohydrate on some liver enzymes. *Proc. Nutr. Soc.* **26**, xxx.

AKINYANJU, P.A., QURESHI, R.U., SALTER, A.J. & YUDKIN, J. (1968) Effect of an 'Atherogenic' diet containing starch or sucrose on the blood lipids of young men. *Nature (Lond.)*, **218**, 975.

AKINYANJU, P.A. & YUDKIN, J. (1967) Effects of dietary carbohydrates on the serum lipids of the rat. *Proc. Nutr. Soc.* **26**, xxxi.

BARTLETT, G.R. (1959) Phosphorus assay in column chromatography. *J. biol. Chem.* **234**, 466.

BUTTERFIELD, W.J.H. (1967) Third Symposium on Advanced Medicine. Proceeding of a Conference held at the Royal College of Physicians of London, 13th February to 17th February. *J. roy. Coll. Physns. Lond.* **1**, 276.

COHEN, A.M., TEITELBAUM, A., BALOGH, M. & GROSS, J.J. (1966) Effect of interchanging bread and sucrose as main source of carbohydrate in a low fat diet on the glucose tolerance curve of healthy volunteer subjects. *Amer. J. clin. Nutr.* **19**, 59.

DEVLIN, J.G. (1963) The effect of training and acute physical exercise on plasma insulin-like activity. *Ir. J. med. Sci.* 423.

FEARNLEY, G.R., BALMFORTH, G. & FEARNLEY, E. (1957) Evidence of a diurnal fibrinolytic rhythm; with a simple method of measuring natural fibrinolysis. *Clin. Sci.* 645.

[illegible], G.M., Karam, J.H., Pavlatos, F.C. & Forsham, P.H. (1965) Serum-insulin response to glucose in prediabetic subjects. *Lancet*, i, 290.

[illegible], C.J. & Randle, P.J. (1963) Immunoassay of insulin with insulin-antibody precipitate. *Biochem. J.* 88, 137.

Hendel, E. van & Zijverdraat, D.B. (1957) Micromethod for the direct determination of serum triglycerides. *J. Lab. clin. Med.* 50, 152.

Härtel, G., Pünsar, S. & Loubna, A. (1968) Insulin and atherosclerosis. *Lancet*, ii, 920.

Henly, A.A. (1957) The determination of serum cholesterol. *Analyst*, 82, 286.

Huggett, A. St G. & Nixon, D.A. (1957) Use of glucose oxidase, peroxidase, and o-dianisidine in determination of blood and urinary glucose. *Lancet*, ii, 368.

Ingram, G.I.C. (1961) A suggested schedule for the rapid investigation of acute haemostatic failure. *J. clin. Path.* 14, 356.

[illegible], B.I., Bank, S., Jackson, W.P.U., Keller, P., O'Reilly, I.G. & Vinik, A.I. (1968) Insulin reserve in patients with chronic pancreatitis. *Lancet*, ii, 890.

[illegible], K.J. & Jarrett, R.J. (1967) Effects of adrenaline [illegible] of smoking in patients with peripheral atherosclerotic [illegible] disease. *Lancet*, ii, 22.

[illegible], I. & Braithwaite, D.M. (1964) The influence [illegible] dietary carbohydrates on the lipid pattern in serum [illegible] adipose tissue. *Clin. Sci.* 27, 23.

[illegible], R. (1965) Diabetes and arterial lipids. *Quart. J. Med.* 34, 484.

[illegible], E.A., Miettinen, T.A., Vesenne, M.R. & Pelkonen, R. (1965) Plasma insulin in coronary heart-disease. *Lancet*, ii, 508.

[illegible] *Rev.* (1967) Dietary sucrose restriction and blood lipid levels. 25, 172.

Rabinowitz, D. & Zierler, K.L. (1962) Role of free fatty acids in forearm metabolism in man, quantitated by use of insulin. *J. clin. Invest.* 41, 2191.

Samaan, N., Brown, J., Fraser, R. & Trayner, I. (1965) Effect of obesity and of starvation on insulin activity. *Brit. med. J.* i, 1153.

Stout, R.W. (1968) Insulin stimulated lipogenesis in arterial tissue in relation to diabetes and atheroma. *Lancet*, ii, 702.

Szanto, S. (1967) Smoking and atherosclerosis. *Brit. med. J.* 3, 178.

Szanto, S. (1968) Effects of obesity and weight reduction on serum insulin levels. *Proc. Nutr. Soc.* 27, 11A.

Vallance-Owen, J. & Ashton, W.L. (1963) Cardiac infarction and insulin antagonism. *Lancet*, i, 1226.

Welborn, T.A., Breckenridge, A., Rubenstein, A.H., Dollery, C.T. & Fraser, T.R. (1966) Serum-insulin in essential hypertension and in peripheral vascular disease. *Lancet*, i, 1336.

Wright, H.P. (1941) The adhesiveness of blood platelets in normal subjects with varying concentrations of anticoagulants. *J. Path. Bact.* 53, 255.

Yalow, R.S. & Berson, S.A. (1960) Plasma insulin concentrations in non-diabetic and early diabetic subjects. Determinations by a new sensitive immuno-assay technic. *Diabetes*, 9, 254.

Yudkin, J. (1964) Patterns and trends in carbohydrate consumption and their relation to disease. *Proc. Nutr. Soc.* 23, 149.

Yudkin, J. (1967) Why blame sugar? *Chemy Ind.* 1464.

Yudkin, J., Szanto, S. & Kakkar, V.V. (1969) Sugar intake, serum insulin and platelet adhesiveness in men with and without peripheral vascular disease. *Postgrad. med. J.* 45, 608.

Zlatkis, A., Zak, B. & Boyle, A.J. (1953). A new method for the direct determination of serum cholesterol. *J. Lab. clin. Med.* 41, 486.

REPRODUCTIVE CAPACITY OF FEMALE RATS AS AFFECTED BY KINDS OF CARBOHYDRATES IN THE RATION [1]

By C. H. WHITNAH, *associate food analyst, Department of Chemistry,* and RALPH BOGART, *graduate research assistant, Department of Animal Husbandry, Kansas Agricultural Experiment Station*

INTRODUCTION

When experimental rats used in a study of galactose metabolism [2] failed to reproduce normally, it was decided to determine the character of the abnormality and also, if possible, the relation of the carbohydrate in the ration to the reproductive capacity of the rat.

LITERATURE

The effects on reproduction of moderate amounts of sucrose and lactose in the rations of rats have been reported by several workers. Mitchell (*6*) [3] found that lactose, when constituting not more than 30 percent of the ration or 50 percent of the total carbohydrate in the ration, did not interfere with growth or well-being. Skinner, Van Donk, and Steenbock (*8*) found that ovarian function, as measured by length and regularity of oestrus cycles, was improved by the addition of 10 percent of sucrose to a ration of mineralized milk. Keil, Keil, and Nelson (*4*), however, reported interference with reproduction as a result of the addition of 5 percent of sucrose to a differently mineralized milk ration.

Evans (*3*) stated that a lack of vitamin E has no effect on the ova or ovarian tissue. In a comparison of vitamin E deficiency with that of vitamins A and B, he stated that vitamin A deficiency is characterized by a continuous cornified smear.

Smith and Engle (*10*) found that anterior pituitary transplants stimulated the ovaries of immature and mature rats, producing an excess of follicles. Ovulation occurred in the young animals. In older animals there were some follicular cysts, and there was also some luteinization. The cysts were sometimes twice the size of normal follicles. Engle and Smith (*2*), in a study of corpus luteum formation in pituitary-stimulated animals, found that follicles did not rupture, but formed cysts which transformed directly into corpora lutea. Sometimes eggs were found "trapped" as a result of luteinization of the follicular wall. Smith (*9*) observed that hypophysectomy of the rat resulted in a regression of the ovaries. If corpora lutea were present, they persisted for a long time. Transplants of anterior pituitary stimulated these regressed ovaries to renewed activity but follicular and lutein cysts resulted. Casida and Hellbaum (*1*), using 21- to 25-day-old rats, produced large follicles and

[1] Received for publication April 11, 1936; issued November 1936. Contribution no. 296 from the Department of Chemistry and no. 110 from the Department of Animal Husbandry, Kansas Agricultural Experiment Station.

[2] These animals were under the care of G. N. Woodruff of Kansas State College.

[3] Reference is made by number (italic) to Literature Cited, p. 542.

corpora lutea by injections of extracts from adrenal glands of horses. They obtained ovulation in about 50 percent of the treated animals. They made no attempt to determine whether this effect was direct or indirect. Moore and Price (7) concluded from their work that the only gonad-stimulating hormones are hypophyseal or hypophyseallike substances.

MATERIALS AND METHODS

The rations fed were designed to be adequate in every known respect. One group of rations contained a minimum amount of galactose while the other group contained lactose which provided a liberal supply of readily available galactose.

The regular experimental rations (designated according to the characteristic carbohydrate) are shown in table 1.

TABLE 1.—*Composition of experimental rations*

Food product	Sucrose	Lactose	Starch
	Percent	*Percent*	*Percent*
Yeast	5	5	4
Osborne and Mendel salt mixture	4	4	5
Cod-liver oil	1	1	1
Casein	20	20	20
Butter oil	20	20	20
Starch	30	30	50
Sucrose	30		
Lactose		30	

Commercial lactose was used for all but one group of lactose-fed rats. The substitution of Mallinkrodt's "analytical reagent" lactose for this group produced no change in the results. No further attempt was made to differentiate lactose from factors which might be associated with it as the important constituent of the ration.

These rations were fed to many groups of rats during a period of more than 2 years. The early groups included several females for which the reproductive record is not complete enough to justify detailed description but which furnished the incentive for the detailed records set forth in this paper.

Some of the rats were mated in order to test fertility. Ages at which the vaginas opened were determined. Microscopic observations were made on the ovaries of some animals, and the uteri of these animals were examined macroscopically. Serial sections of the ovaries were made for microscopic study. In some cases one ovary and part of one horn of the uterus were removed by laparotomy. Since little difference was noted between the remaining ovaries of these animals and ovaries from other animals receiving the same feed, they have been included in the appropriate age and feed groups.

OBSERVATIONS

Seven animals fed lactose, five fed sucrose, and two fed starch were kept with fertile males for 40 days or until pregnancy was detected. During the time of this test all the animals were changed from the experimental rations to the regular stock ration. The results are presented in table 2.

TABLE 2.—*The effect of feeding various carbohydrates on the reproductive ability of rats*

Animal no.[1]	Carbohydrate fed	Age of animal		Size of litter	Condition of young
		When placed with male	When litter was born		
		Days	*Days*	*Number*	
70a	Lactose	79	107	10	Normal.
78a	do	79	105	5	All died within 3 days.
84b	do	79	102	7	Normal.
90b	do	79	105	5	Do.
121c	do	56	80	4	1 died, 3 normal.
125c	do	56	93	8	Normal.
129d	do	48	85	8	Do.
1	Sucrose	80		0	
2	do	70		0	
3 [2]	do	70	111	3	2 died, 1 weakly.
87b	do	79		0	
119c	do	56	80		Abortion.
123c	Starch	56	94	10	Normal.
127c	do	48	87	7	Do.

[1] The animals having like letters were litter mates.
[2] After producing 1 litter, this female was kept with a fertile male for 90 days without producing another litter.

As table 2 shows, each lactose- and starch-fed animal reproduced. All these litters, except possibly one born to a lactose-fed female, were normal. The one sucrose-fed female which produced a live litter was afterward sterile.

The age at which the vagina opened and the type of vaginal smears were determined for 17 sucrose-, 4 starch-, 6 stock-, and 18 lactose-fed animals. The results are presented in table 3. The sucrose-fed

TABLE 3.—*Effect of feeding various carbohydrates on age at which vaginas of rats opened, and types of smears secured*

Ration fed	Animals	Age at which vagina opened			Standard deviation	Vaginal smear record
		Youngest	Oldest	Average		
	Number	*Days*	*Days*	*Days*		
Sucrose	17	32	51	38	4.8	1-3 cycles, then dioestrum.
Starch	4	45	53	49	3.8	Not observed.
Stock	6	51	60	55	3.4	Normal cycles.
Lactose	18	45	64	59	6.7	Do.

animals showed sexual activity, as measured by the age at which the vaginas opened, earlier than any of the other groups. There is considerably less than 1 chance per 1,000 that this difference is due to chance. The smears of the sucrose-fed animals, however, showed only a few oestrus cycles. The lactose-fed animals were slightly late in reaching sexual maturity but showed normal cyclic changes in the smears. The starch- and stock-fed groups consisted of only a few animals, but these showed no abnormalities. They closely resembled the lactose group.

The observations made on the ovaries of the animals fed sucrose and those fed lactose are shown according to age groups in table 4.

TABLE 4.—*Effect of feeding sucrose and lactose in relation to microscopic observations of the ovaries of rats in various age classes*

Animals (number)	Age	Carbohydrate fed	Corpora lutea	Luteinization of follicles	Atresia
	Days				
3	37	Sucrose	+	+	-
2	37	do	−	+	−
1	37	Lactose	−	−	+
5	44-47	Sucrose	+	+	−
2	44-47	Lactose	−	−	+
10	64-72	Sucrose	+	+	−
10	64-72	Lactose	[2] +	−	+
6	64-72	do	−	−	+

[1] Atresia indicates that follicles are so degenerated that they should soon disappear.
[2] 3 animals in this group had fresh rupture points in some of the follicles. Eggs were found in the Fallopian tubes of 2.

Photomicrographs of typical sections from ovaries of sucrose- and lactose-fed animals of each age group are shown in plate 1, *A* to *F*. These sections all have the same magnification, but they do not represent the relative sizes of the ovaries from different animals.

The presence of luteinized follicles in the sucrose-fed animals of all age groups would account for the lack of atretic disappearance of follicles. Luteinization of immature follicles would also explain the failure of the follicles to rupture.

As table 4 shows, the sucrose-fed animals were precocious. Corpora lutea were found in the ovaries of some of these animals at 37 days of age (pl. 1, *A*). In sharp contrast to this, the lactose-fed animal of this age was practically in an infantile sexual condition. Small follicles only were present in the ovary (pl. 1, *B*).

At 44 to 47 days of age similar differences existed as in the 37-day-old animals but they were even more striking. The sucrose-fed animals showed many more corpora lutea, luteinization of the larger follicles, and only a few small follicles (pl. 1, *C*). There was some indication that the follicles were being transformed into corpora lutea without a rupture occurring; at least no rupture points were observed. Some of the large luteinized follicles contained eggs that were degenerating. The lactose-fed animals, on the other hand, contained ovaries with follicles of all sizes (pl. 1, *D*). There was no luteinization of follicles among lactose-fed animals.

In the age group 64 to 72 days, many large corpora lutea were found in the ovaries of the sucrose-fed animals. There were no cyclic ages of corpora lutea, and no small follicles were present. Some cysts due to luteinization of the large follicles were found, but none of the corpora lutea showed signs of degenerating as would be expected if normal cycles were occurring (pl. 1, *E*). There was considerable variation among the lactose-fed animals. Some had not yet ovulated, showing some indication of retardation. In most cases, however, fresh corpora lutea and normal follicles of different sizes were found (pl. 1, *F*). In some of the lactose-fed animals the rupture points were visible, and eggs were found in the tubes of two animals. More than one age of corpora lutea was observed in the ovaries of some animals. There was no luteinization of follicles.

A, Left ovary from a 35-day-old sucrose-fed rat, showing large corpora lutea (*cl*) and small luteinized follicles (*lf*). *B*, Left ovary from a 37-day-old lactose-fed rat, showing many small follicles (*f*). There is no luteinization of any follicles. Some atresia is present. *C*, Right ovary from the same animal as *A*, at 45 days of age. A sucrose-fed rat, showing large corpora lutea (*cl*) and luteinized follicles (*lf*). No normal-appearing follicles of any size. *D*, Right ovary from the same animal as *B*, at 45 days of age. A lactose-fed rat, showing follicles of different sizes (*f*). No corpora lutea and no luteinization of follicles. *E*, Ovary from 60-day-old sucrose-fed rat, showing many corpora lutea (*cl*) and luteinized follicles, some containing degenerating eggs (*de*). Some follicles are so heavily luteinized that cysts have resulted (*fc*). *F*, Ovary from 70-day-old lactose-fed rat, showing follicles of different sizes (*f*) and recent corpora lutea with blood clots (*cl*). There is no luteinization of follicles.

The uteri of the sucrose-fed animals of all age groups were generally larger than those of the corresponding lactose-fed animals. Some were distended with fluid and some were highly vascular.

Ovaries of three lactose-fed animals were observed about 30 days after the animals had given birth to litters. The normal cyclic ages of corpora lutea were present. Ovaries of four other animals aged about 200 days were also observed. These had been on a sucrose ration until about 70 days of age. Afterward they had been on the regular stock ration. The ovaries were made up almost entirely of masses of corpora lutea and follicular cysts. No evidence of cycles was seen, and there was indication of the cysts transforming into corpora lutea.

DISCUSSION

With our present knowledge of nutrition it is difficult to understand how the replacement of either the 20 percent lactose, or 20 of the maximum 50 percent starch, with 20 percent sucrose in the ration could have produced the observed sexual precocity and luteinization of follicles. A few instances have been reported in which sucrose in the ration was associated with abnormal reproduction. The observed abnormalities, however, were not linked with either abnormal ovarian structure nor with any pituitary disturbances. The absence of galactose might possibly be associated with the results produced by the sucrose ration. Sorensen and Haugaard (*11*) have shown that casein contains a small amount of galactose. It is possible that the additional cornstarch contained sufficient galactose to meet the need and make possible the observed relations of the three rations studied. It might, on the other hand, be assumed that the sucrose affected some gland which secreted a gonad-stimulating substance. Since most work indicates that the pituitary is the only gland which could have produced this effect, there is the possibility that the sucrose either directly or indirectly affected the activity of the pituitary.

The results obtained were similar to those produced by pituitary implants (*10*). One difference, however, is that no excessive number of follicles or corpora lutea were present. The luteinization of follicles and formation of cysts and the persistence of the corpora lutea were more like the results produced by implanting pituitaries into hypophysectomized rats (*9*). The transformation of follicular cysts into corpora lutea is similar to results obtained by Engle and Smith (*2*). The similarity of the ovarian abnormalities in the writers' animals to defects noted by other workers when the pituitary function was altered is strong evidence that some pituitary disturbance existed.

The possibility that a vitamin deficiency might have brought about the abnormalities noted is very small. The symptoms observed were not the characteristic symptoms of any vitamin deficiency. The tumor record and ovarian observations are far from indicating a deficiency of either vitamin A or E.

When the mixture of purified amino acids recently announced (*5*) becomes available as a substitute for proteins, it may be practical to determine more definitely what characteristics of the carbohydrates studied are associated with the results secured in this investigation.

SUMMARY

In this study of the relation of the carbohydrate in the ration to the reproductive capacity of rats, three carbohydrates were fed, sucrose, lactose, and starch.

The sucrose-containing ration was found to be inadequate for normal reproduction in rats, even when replaced during adult life by a normal ration.

Rats receiving the sucrose ration were precocious in sexual development.

Extreme luteinization of follicles and persistence of corpora lutea were characteristic of the ovaries of the sucrose-fed animals. This adequately explains the failure of these animals to reproduce.

The ovarian abnormalities of the sucrose-fed animals indicate pituitary disturbances.

Animals fed the lactose- and starch-containing rations reproduced normally and had normal ovarian structures.

LITERATURE CITED

(1) Casida, L. E., and Hellbaum, A. A.
1934. OVARIAN STIMULATION BY ADRENAL EXTRACTS. Endocrinology 18: 249-253.

(2) Engle, E. T., and Smith, P. E.
1929. THE ORIGIN OF THE CORPUS LUTEUM IN THE RAT AS INDICATED BY STUDIES UPON THE LUTEINIZATION OF THE CYSTIC FOLLICLE. Anat. Rec. 43: 239-240, illus.

(3) Evans, H. M.
1932. VITAMIN E. Jour. Amer. Med. Assoc. 99: 469-475.

(4) Keil, H. L., Keil, H. H., and Nelson, V. E.
1934. THE EFFECT OF ADDITION OF MINERALS AND SUCROSE TO MILK AS SHOWN BY GROWTH, FERTILITY AND LACTATION OF THE RAT. Amer. Jour. Physiol. 108: 215-220, illus.

(5) McCoy, R. H., Meyer, C. E., and Rose, W. C.
1935. FEEDING EXPERIMENTS WITH MIXTURES OF HIGHLY PURIFIED AMINO ACIDS. VIII. ISOLATION AND IDENTIFICATION OF A NEW ESSENTIAL AMINO ACID. Jour. Biol. Chem. 112: 283-302, illus.

(6) Mitchell, H. S.
1927. COMPARATIVE PHYSIOLOGICAL VALUES OF DIFFERENT AMOUNTS OF LACTOSE, BASED ON GROWTH AND FECAL ANALYSIS. Amer. Jour. Physiol. 70: 542-544.

(7) Moore, C. R., and Price, D.
1932. GONAD HORMONE FUNCTIONS, AND THE RECIPROCAL INFLUENCE BETWEEN GONADS AND HYPOPHYSIS WITH ITS BEARING ON THE PROBLEM OF SEX HORMONE ANTAGONISM. Amer. Jour. Anat. 50: 13-71, illus.

(8) Skinner, J. T., Van Dyke, E., and Steenbock, H.
1932. MANGANESE AS A FACTOR IN REPRODUCTION. Amer. Jour. Physiol. 101: 591-597, illus.

(9) Smith, P. E.
1930. HYPOPHYSECTOMY AND REPLACEMENT THERAPY IN THE RAT. Amer. Jour. Anat. 45: 205-272, illus.

(10) ——— and Engle, E. T.
1927. EXPERIMENTAL EVIDENCE REGARDING THE ROLE OF THE ANTERIOR PITUITARY IN THE DEVELOPMENT AND REGULATION OF THE GENITAL SYSTEM. Amer. Jour. Anat. 40: 159-217.

(11) Sorensen, M., and Haugaard, G.
1933. THE APPLICATION OF THE ORCINOL REACTION TO THE ESTIMATION OF THE NATURE AND AMOUNT OF CARBOHYDRATE GROUPS IN PROTEINS. Compt. Rend. Trav. Lab. Carlsberg 19, no. 21, 7 pp., illus. (Published simultaneously in German in Biochem. Ztschr. 260: [247]-277, illus.)

The Lancet 22: 155-162, 1957.

DIET AND CORONARY THROMBOSIS

HYPOTHESIS AND FACT *

JOHN YUDKIN

M.A., Ph.D., M.D. Camb., M.R.C.P., F.R.I.C.

PROFESSOR OF NUTRITION IN THE UNIVERSITY OF LONDON AT QUEEN ELIZABETH COLLEGE

MUCH has been written about the rôle of dietary factors in causing coronary thrombosis. In particular, many believe that the disease is related to the amount of dietary fat, or of a particular sort of fat. In support of these beliefs, we are presented with evidence of an epidemiological nature. We are told, for example, that the higher the average fat-consumption in a country, the higher the mortality due to coronary thrombosis (coronary mortality). Or we are told that the rise in the number of coronary deaths in this country was accompanied by a rise in the consumption of hydrogenated fats.

From time to time, however, it becomes evident that some of the epidemiological data do not fit these simple hypotheses. For example, in one investigation the chance of suffering from coronary disease was found to be twice as high in general practitioners as in other doctors (Morris et al. 1952), and it is difficult to see how this can be explained solely on the basis of differences in diet.

As more and more of these awkward facts turn up, one begins to have the uneasy feeling that both the proponents and opponents of a dietary hypothesis are seeking only those data which support their view. I thought it worth while therefore to draw together as many of these data as are reasonably available, and see what sort of picture emerged.

* Based on the chairman's address to the Nutrition Panel (Food Group) of the Society of Chemical Industry. A summary of this address appears in *Chemistry and Industry*, 1957, p. 627.

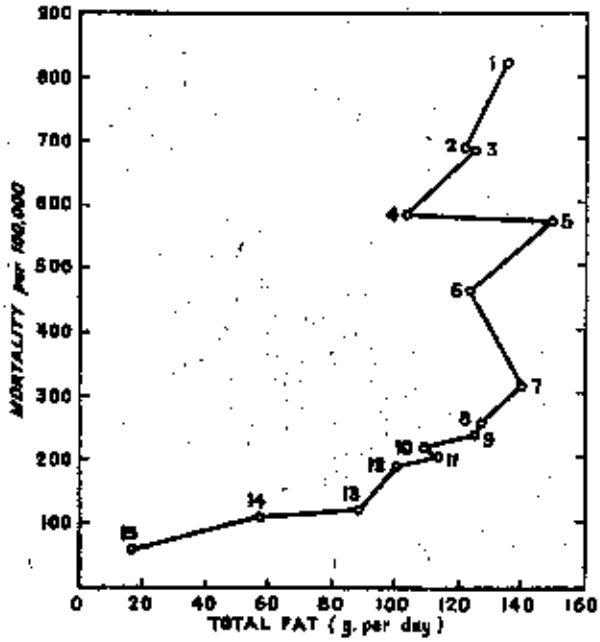


Fig. 1—Coronary mortality and total fat intake in various countries.

Mortality refers to number of men aged 55–64 in 100,000 certified as dying of coronary disease in 1952. The numbers refer to countries in order of decreasing mortality as follows: (1) U.S.A., (2) Australia, (3) Canada, (4) Finland, (5) New Zealand, (6) United Kingdom, (7) Denmark, (8) Sweden, (9) Norway, (10) Netherlands, (11) Switzerland, (12) Western Germany, (13) France, (14) Italy, (15) Japan.

In relating diet to coronary mortality, I have drawn on three different sorts of statistics. The first is a comparison between diets and coronary mortality in different countries for the same year; the most recent comprehensive figures available were for 1952, or 1951–52. The second comparison is between diets and coronary mortality in different classes in our own country. The third comparison is between trends in diets and trends in coronary mortality in this country since 1928, when the present basis of classification of deaths due to coronary thrombosis was introduced.

International Comparisons

Let us begin by looking at the sort of figures which have been quoted in support of the hypothesis that

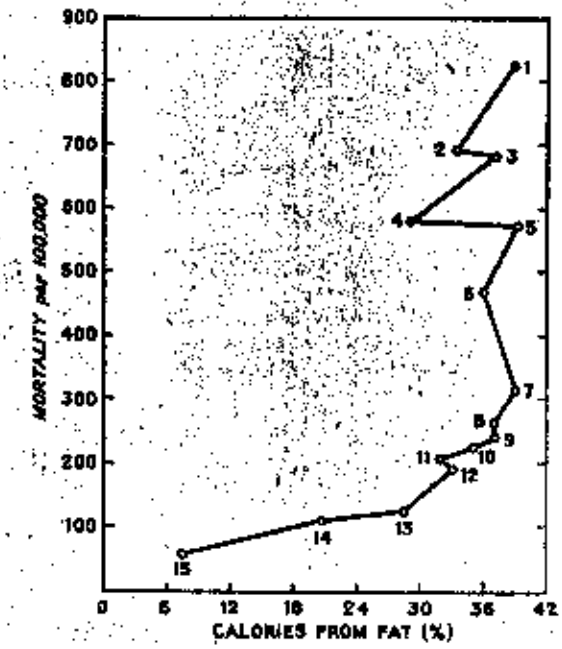


Fig. 2—Coronary mortality and percentage calories from fat in various countries. For key, see fig. 1.

coronary mortality is related to total fat consumption (Food and Agriculture Organisation 1955, World Health Organisation 1955, 1956). In 1952, the number of men aged 55–64, in every 100,000, who were certified as dying of coronary thrombosis in Japan, Italy, Western Germany, Switzerland, and New Zealand was 57, 113, 190, 205, and 576. The average daily intake of fat (in grammes) was reported as 16, 57, 101, 113, and 149. These figures show an excellent relationship between fat intake and coronary deaths. Yet we can select another group of five countries—Norway, Denmark, United Kingdom, Canada, and U.S.A.—where the figures for coronary mortality were 239, 319, 470, 689, and 829, and the daily fat intake (in grammes) 125, 140, 123, 123, and 135. Thus, with about the same intake of fat, there was a range of coronary mortality of nearly fourfold. If we combine all the available figures, we see that in general countries have a slowly increasing coronary mortality when the fat increases up to about 120 g. a day; with intakes of about 130 g. a day, there is no relationship with coronary mortality (fig. 1). The possible significance of this point will be discussed later.

Some workers have quoted fat intake, not as the absolute amount, but as a percentage of the total calories which are supplied by fat. I have not understood the reason for doing this. It would imply that a country like Italy, in which the average intake of calories is

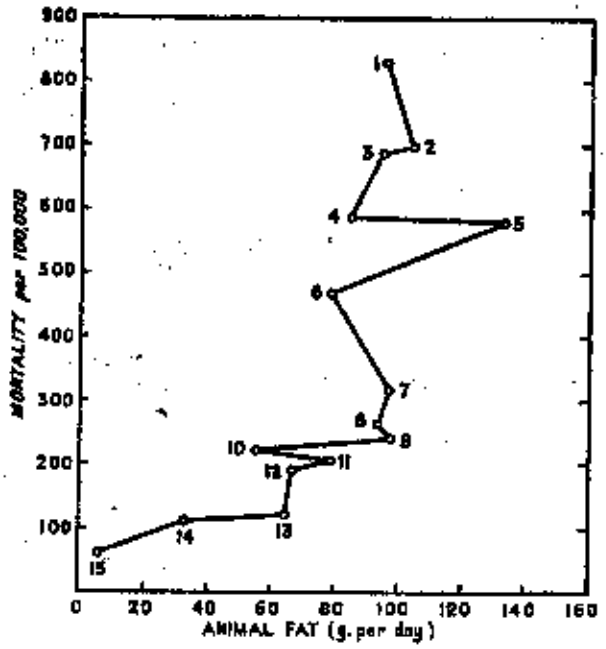


Fig. 3—Coronary mortality and animal-fat intake in various countries. For key, see fig. 1.

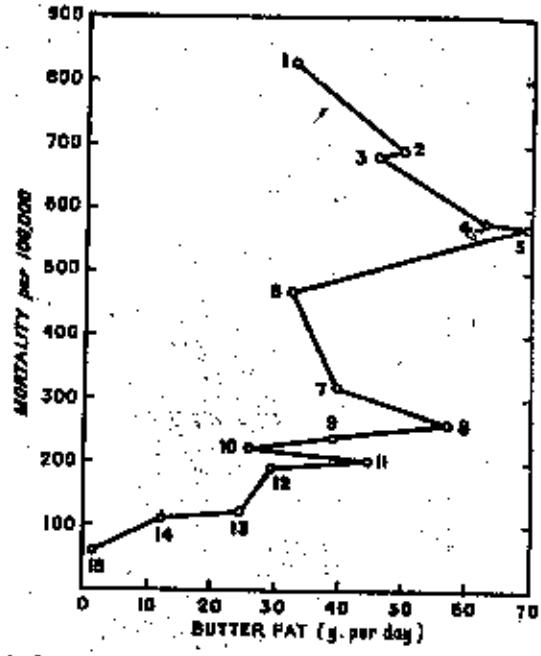


Fig. 4—Coronary mortality and butter-fat intake in various countries. For key, see fig. 1.

about 2500, would have a much higher coronary mortality if the fat intake were increased at the expense of, say, carbohydrate, without changing the caloric intake. I know of no evidence that this would be so. Nevertheless, I have related coronary mortality to the percentage of calories derived from fat (fig. 2). We see that there is almost no difference in the form of the curve from that in which total fat intake is used.

It has been suggested that the amount of animal fats, from milk and from meat, is more important than the amount of total fat in causing coronary thrombosis. The figures do not support this, since the relationship is less direct than with total fats (fig. 3). Those who believe that the culprit is butter fat, from butter, milk, and cheese, receive even less support (fig. 4). Nor is there support for the view that vegetable fat is protective, for there is no relationship, either positive or negative, between the intake of vegetable fat and coronary mortality (fig. 5).

Again, there is no relationship with the consumption of sources of hydrogenated fats—margarine, compound fats, and shortenings (fig. 6). The most obvious discrepancy here is in Norway, which has by far the highest consumption of these fats of all the countries we have examined. It has been suggested that this may be due to the high consumption of fish by the Norwegians (Sinclair 1956). The argument is that hydrogenated fats induce a deficiency of essential fatty acids (E.F.A.) and that this may be made good by E.F.A. supplied by fish. It so happens that the greater proportion of the fish consumed in Norway is cod, with an extremely low content of fat and E.F.A. The total amount of fat from

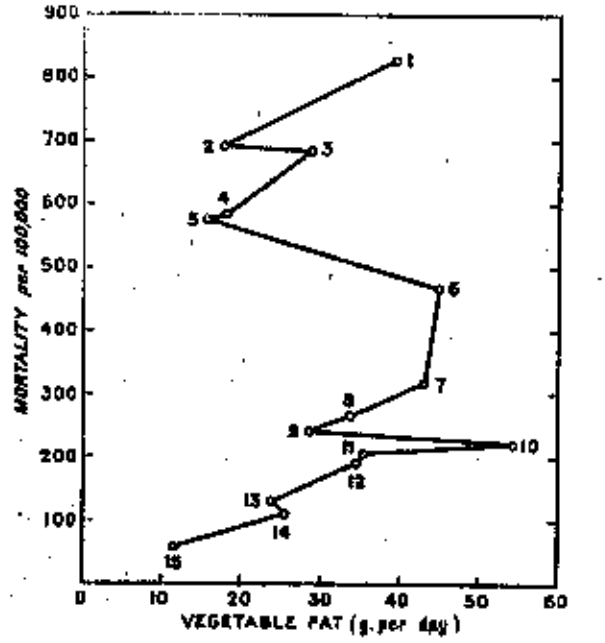


Fig. 5—Coronary mortality and vegetable-fat intake in various countries. For key, see fig. 1.

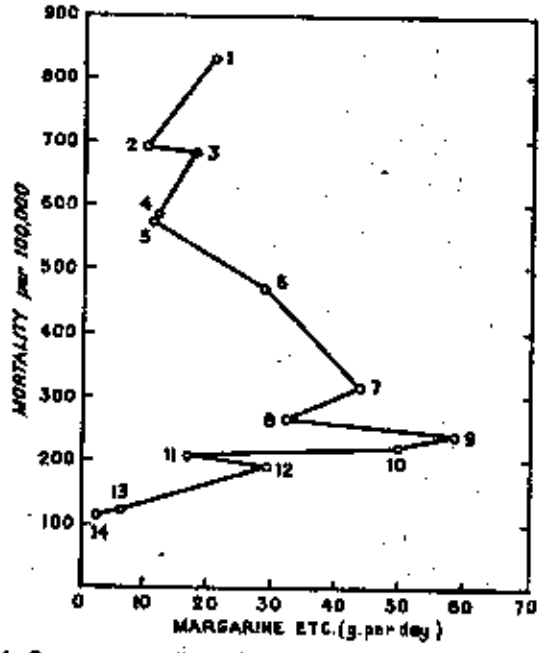


Fig. 6—Coronary mortality and intake of margarine, compound fats, and shortenings in various countries. For key, see fig. 1.

CORONARY MORTALITY AND INTAKE OF MARGARINE &c., AND FISH IN DIFFERENT COUNTRIES

Country	Coronary mortality	Intake (g. per head per day)	
		Margarine, &c.	Fish
Norway ..	239	57·4	110·9
Canada	659	17·4	16·7
Switzerland ..	205	16·3	5·7
New Zealand ..	576	10·6	19·4
W. Germany ..	190	18·3	18·5

fish averages only 3 g. a head a day. Nevertheless, let us examine the suggestion a little more closely. Let us first compare two countries with similar average consumption of sources of hydrogenated fats, Canada and Switzerland. The much lower coronary mortality in Switzerland could then be explained if there were a higher consumption of fish; in fact, the Swiss consume much less fish than the Canadians (see accompanying table). Again, let us look at two countries—New Zealand and Western Germany—with a similar fish consumption. Here, we might explain the much lower mortality in Western Germany if there were a lower intake of sources of hydrogenated fats, but in fact it is much higher.

It is true that the degree of hydrogenation of margarine and similar fats varies in different countries. Nevertheless, such figures as I have been able to obtain, while altering the relative position of some of the points in fig. 6, give no better relationship between the consumption of hydrogenated fats and deaths due to coronary thrombosis.

I thought it would be interesting, before leaving the comparison between different countries, to see how the consumption of the nutrients other than fat was related to coronary mortality. There seems to be no relationship with the intake of total protein (fig. 7) but some relationship, about as good as for total fat, with intake of animal protein (fig. 8). There is a better relationship with intake of sugar than with any other nutrient we have examined (fig. 9). The relationship with total caloric intake is about as good as that with the intake of total fat or of animal protein (fig. 10).

These international comparisons, then, do not support the view that total fat or animal fat is the direct and

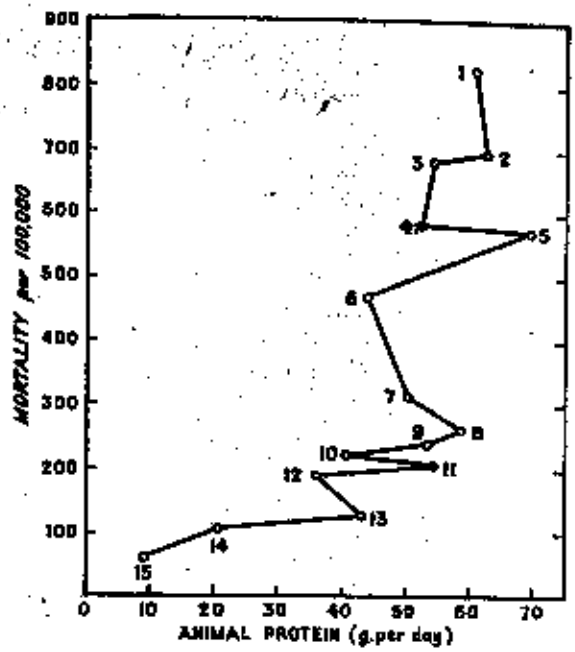


Fig. 8—Coronary mortality and animal protein intake in various countries. For key, see fig. 1.

single cause of coronary thrombosis. Neither do they support the view that hydrogenated fats are related at all to coronary thrombosis. The relationships which do emerge, especially that with sugar, suggest that coronary thrombosis is associated with higher living standards. This is supported by the relationship which exists between coronary mortality and national income per head (fig. 11).

Comparisons by Class

The latest figures of comparative rates of mortality from coronary disease in different occupations in this country were published for 1950 (Registrar-General 1951). The Registrar-General's classification is in five classes: V unskilled, IV semiskilled, III skilled, II intermediate, and I professional.

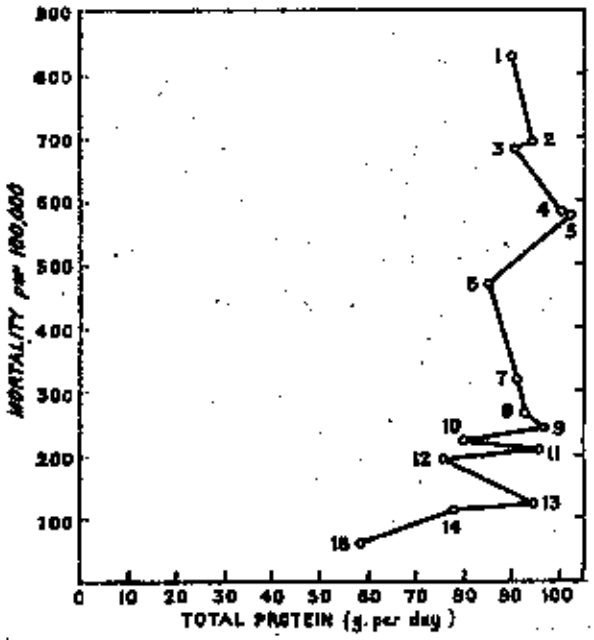


Fig. 7—Coronary mortality and total protein intake in various countries. For key, see fig. 1

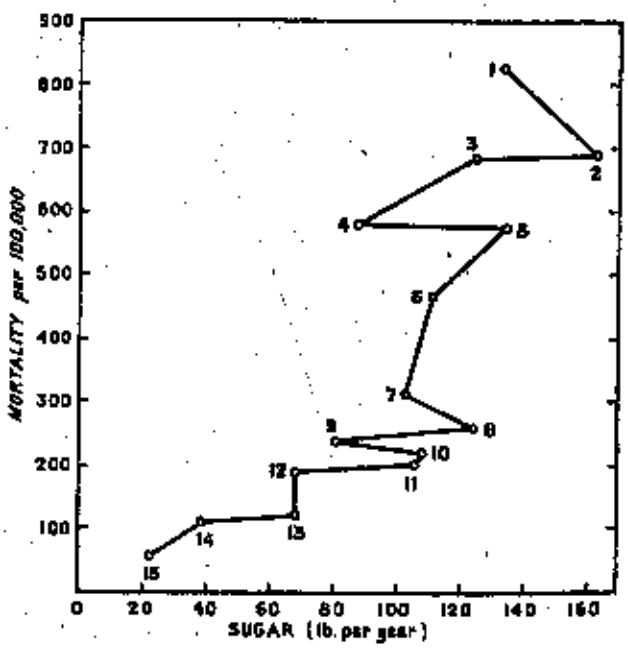


Fig. 9—Coronary mortality and sugar intake in various countries. For key, see fig. 1.

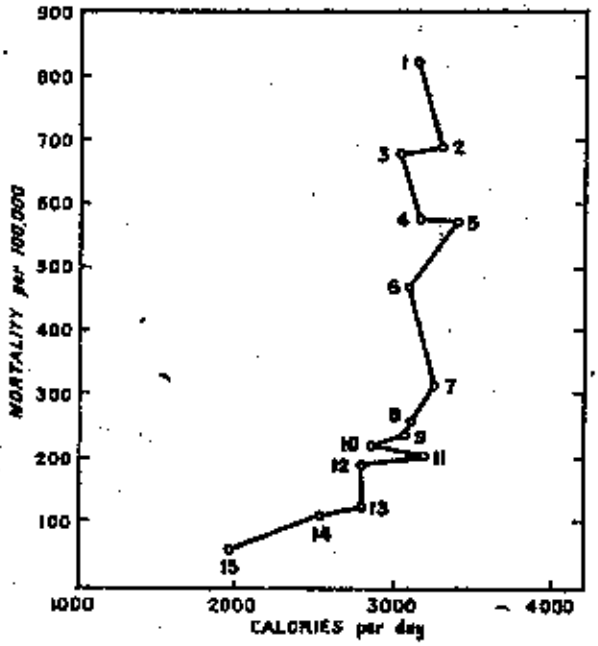


Fig. 10—Coronary mortality and caloric intake in various countries. For key, see fig. 1.

Figures published by the Ministry of Agriculture, Fisheries, and Food for different income classes for the same year, 1950, are not entirely satisfactory for comparing diets and coronary mortality. There is insufficient detail, for example, from which to calculate the intake of butter fat. Again, the lowest class, D, includes those living on pensions as well as those on low earned incomes. Since, however, general trends in food consumption were much the same in later years, I have chosen to use the figures for 1954. In that year, the data for food consumption are more complete, and class D is divided into those with and those without earners. In addition, the division is made into five "earning" classes (Ministry of Agriculture, Fisheries, and Food 1956). The classification of the Ministry is based on income: D1 up to £6 weekly (families with at least one earner), C £6–9, B £9–15, A2 £15–24, A1 over £24. It is clear, of course, that the two sorts of classification by occupation and by income will not be directly comparable. It is, however, reasonable to suggest that, on the whole, occupational class is also related to income. Thus, if there is a causal relationship between diet and coronary mortality, then obvious trends in mortality, shown when classification is made according to occupation, should show (at least to some extent) in trends in diet when classification is made according to income.

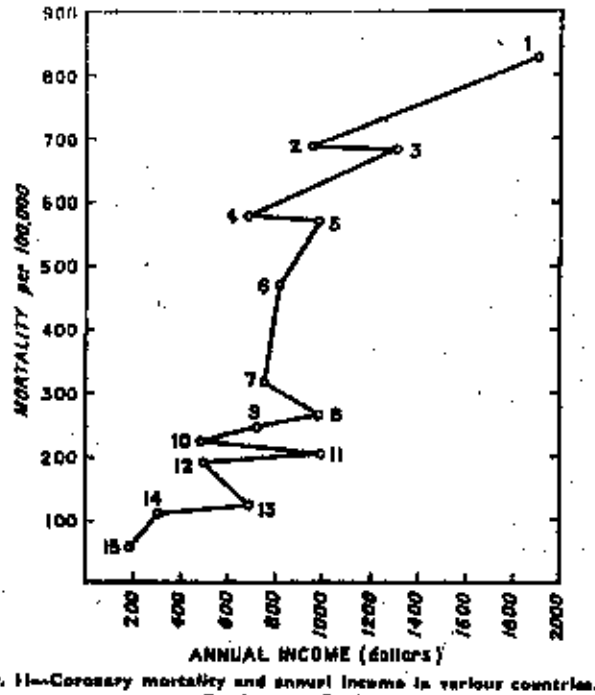


Fig. 11—Coronary mortality and annual income in various countries. For key, see fig. 1.

The differences in coronary mortality by occupation are considerable. The chance of a semiskilled worker dying of the disease is only about half that of a professional man (fig. 12).

The consumption of fat, and of butter fat, show the same sort of trends with increasing income, but the differences are small (figs. 13 and 14). On the other hand the consumption of margarine and other sources of hydrogenated fats falls with increasing income, the opposite of what would be expected if these fats were important in causing deaths by coronary thrombosis (fig. 15). Higher income was associated with a very small increase in the consumption of total protein, and a slightly greater increase in the consumption of animal protein (fig. 16). These associations are to some extent similar to those found by our comparisons between different countries, but there is no similar association between coronary mortality and the intake of sugar or of calories, since there is little or no difference by income-class (figs. 17 and 18).

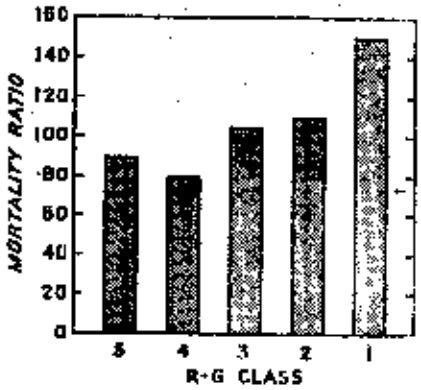


Fig. 12—Relative coronary mortality in various occupational classes in U.K., 1950; men aged 20–44.

Let me emphasise again that, since the two classifications of mortality and of diet are different, no significance can be attached to the exact values of the figures I have quoted. I believe nevertheless that it is legitimate to conclude that, for example, people in professional occupations, with a higher coronary mortality, earn more than people in unskilled or semiskilled occupations; because of this, they eat as a group somewhat more fat and animal protein and less margarine.

Historical Trends

I have given the figures for coronary deaths in this country only since 1928. The figures for the years before 1928, though based on a different classification, give no indication of any considerable fluctuation in mortality. I do not therefore wish to attach any great importance to dietary changes before that date, but I have thought it worth while to include such earlier figures for the diets as are available (Hollingsworth 1957).

First, let me draw attention to the almost continuous rise in certified deaths due to coronary thrombosis since 1928, interrupted only by a slight fall in 1940–42 and in 1953 (figures from the Registrar-General's Annual Statistical Reviews). By 1943 the mortality-rate was the same as before the war, and by 1950 it had doubled (fig. 19).

A superficial examination of the trends of consumption of total fats suggests that this is related to trends in coronary mortality. In fact, however, the fall in both

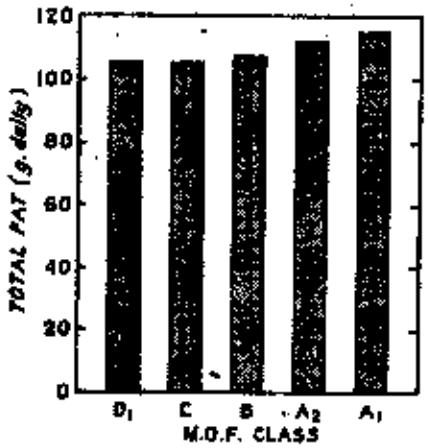


Fig. 13—Consumption of total fat in various income classes in U.K., 1954.

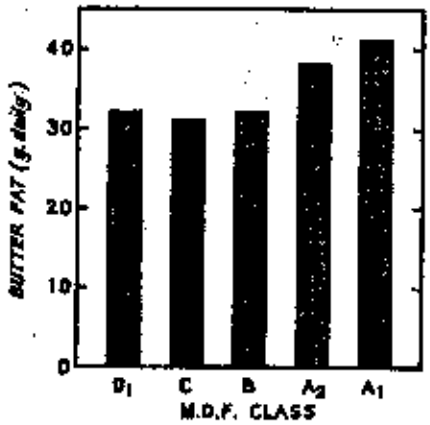


Fig. 14—Consumption of butter-fat in various income classes in U.K., 1954.

By far the best correlation I have found with trends in coronary mortality is in the number of radio and television licences (fig. 24). There is nearly as good a correlation with the number of registered motor-vehicles.

Discussion

Validity of Statistics

Coronary mortality.—For a variety of reasons, I have used, when they are available, figures for men aged 55–64. Mortality from coronary diseases is two to three times greater in men than in women. Deaths in men over 64 are less certainly due to single causes (Morris 1955). Finally, death due to coronary disease is much more common between 55 and 64 years than in any other decade. But no significant differences in trends emerge if the figures for total male mortality are used.

In comparing figures for different countries, I have used all the data which are available. I have, that is, included every country for which figures have been published both for coronary mortality and for food consumption.

The publication from which figures for coronary mortality have been taken says:

"It should be borne in mind . . . that the procedures followed for determining the underlying cause of deaths are still not absolutely identical in the various countries and that therefore the data cannot be considered strictly comparable between different countries" (World Health Organisation 1956).

Nevertheless, for the majority of the countries I have compared, the diagnosis is likely to be sufficiently similar not to invalidate the general trends which have emerged from the comparisons I have quoted. The least accurate figures are probably those for Japan, but the death-rate there is so

at the beginning of the war was followed by a rise in mortality several years before the consumption of fat had reached its full pre-war level (fig. 19). The lack of relationship with intake of animal fats is more obvious, since this had not returned to the pre-war level by 1954 (fig. 20). There was no relationship between coronary mortality and consumption of vegetable fat (fig. 21).

Consumption of sources of hardened fats is also not related to coronary mortality (fig. 22). At the beginning of the late war, consumption increased considerably while coronary mortality fell. The subsequent fairly steady rise in mortality was accompanied by fluctuating intakes of these fats. Finally, whilst as I have said the figures of coronary mortality before 1928 are probably less accurate, there is no evidence that the high intake of margarine and other hydrogenated fats around 1920 was associated with a high coronary mortality.

Sugar intake rose before the recent war, with a rise in coronary mortality. But from 1940 to 1950, sugar intake was lower than before the war, although coronary mortality had doubled during that time (fig. 23).

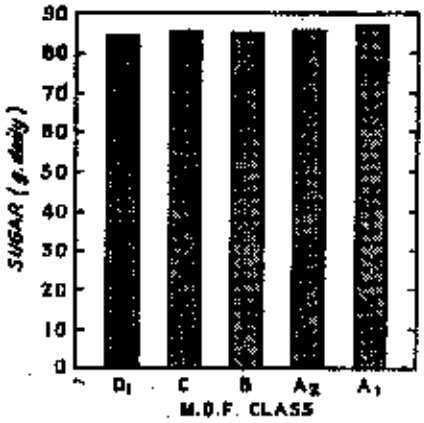


Fig. 17—Consumption of sugar in various income classes in U.K., 1954.

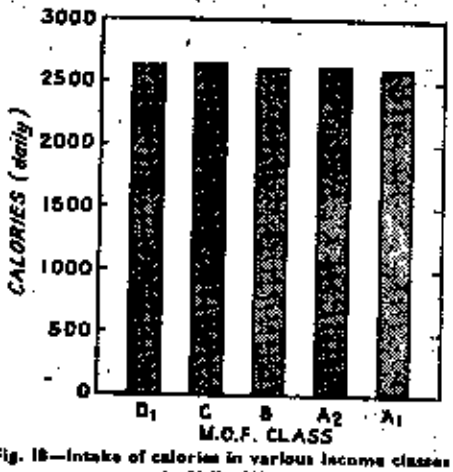


Fig. 18—Intake of calories in various income classes in U.K., 1954.

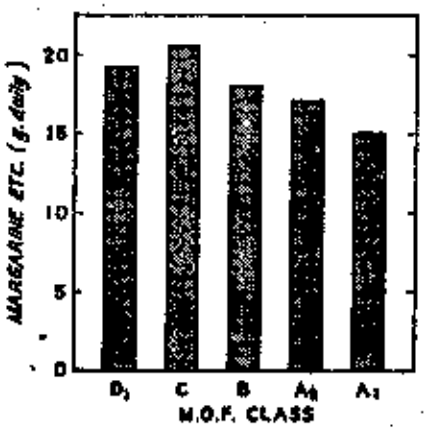


Fig. 15—Consumption of margarine, compound fats, and shortenings in various income classes in U.K., 1954.

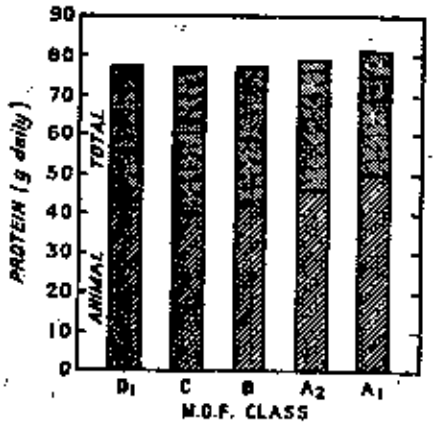


Fig. 16—Consumption of protein in various income classes in U.K., 1954.

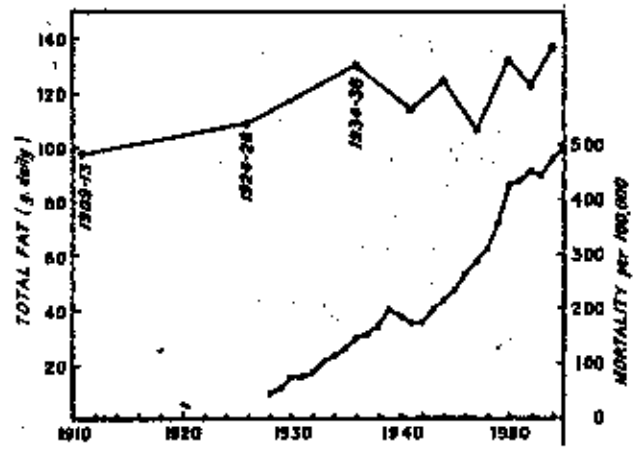


Fig. 19—Trends in coronary mortality and total fat intake in U.K.

much lower than that of any of the other countries studied that corrected figures would again not be likely to invalidate the general trends.

It is not very likely that there are significant differences in diagnosis of mortality in different classes in England and Wales. But it is possible that figures for the trends since 1928 are less reliable, in so far as there may well have been changes in the past 30 years in the tendency of doctors towards or against diagnosis of the disease. Again, however, it is generally agreed that there has in fact been a considerable rise in the disease, and the possible differences in diagnosis are unlikely to alter significantly any of the conclusions we have drawn.

Food consumption.—The same sorts of limitations apply to the published figures for food consumption. Similarly, we may take it that the wide differences in food consumption to which we have drawn attention

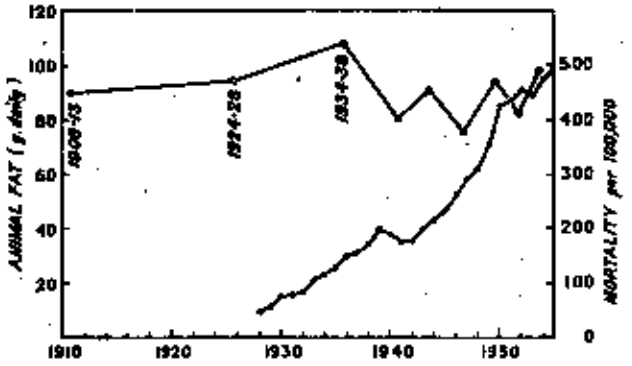


Fig. 20—Trends in coronary mortality and animal fat intake in U.K.

in making our comparisons were sufficient to leave our main conclusions unaffected, even if the absolute figures for food consumption are likely to include some degree of inaccuracy.

One point, however, is important. The figures for food consumption are averages for whole populations, or large sections of populations. There is almost no information about the distribution of food consumption between these large numbers of people. Let me illustrate this by a hypothetical example.

Consider two countries A and B with the same average daily consumption of sugar of 150 g. Let us suppose that in country A some people eat very little and some a great deal; we might find that 25% of them ate less than 120 g. and 25% ate more than 180 g. In country B, we may imagine a much more uniform distribution of consumption; here, only 5% of the people might be eating less than 120 g., and 5% more than 180 g. We shall see later that a plausible hypothesis is that a dietary factor may come into play only when a certain threshold of consumption is exceeded. If such a dietary factor were sugar, and the threshold were a daily intake of 180 g., then country A would have a much higher incidence of coronary disease than country B, even though the average daily intake of sugar was 150 g. in both.

Negative conclusions.—The hypothesis which we have set out to test by the comparisons we have made is that there is a single or major dietary factor causing coronary thrombosis. We have seen that any evidence which is suggestive from one set of figures is not confirmed from another set. Thus, whilst total fat consumption is in general higher in those countries with higher coronary mortality, it was lower for several years in this country whilst mortality continued to rise. We may legitimately conclude that the hypothesis is not supported by the available data, either for fat or for a particular sort of fat or for any other single nutrient.

One of the dietary hypotheses which has been put forward is that the disease is fundamentally caused by a single nutrient deficiency, that of essential fatty acids (Pfeifer and Holman 1955), but that other nutrients are involved because they modify the need for E.F.A. (Sinclair 1956). Thus it is said that the requirements for E.F.A. are increased by increase in total fat, decrease in vitamins B_6 and E, and increase in "unnatural"

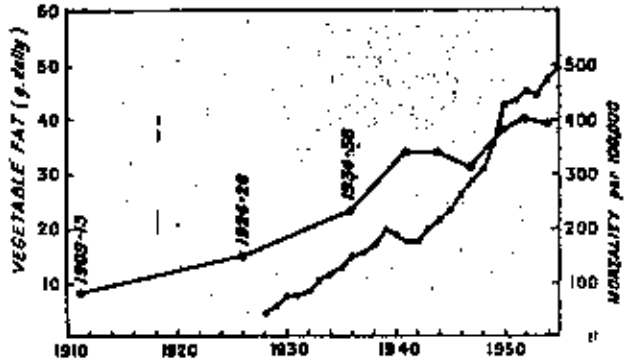


Fig. 21—Trends in coronary mortality and vegetable fat intake in U.K.

fats produced during hydrogenation. For example, the consumption of bread made of improved flour of low extraction will increase the requirements for E.F.A. and thus increase the chances of deficiency, because low extraction flour contains less vitamins B_6 and E, and because the process of improvement further reduces vitamin E. Consider then the situation in this country during 1940–50. Intake of total fat was smaller, and of hydrogenated fats greater, than before the war. Since there was an increase in the extraction-rate of flour, and no change in the method or degree of improvement the intake of vitamins B_6 and E, as well as of E.F.A., from this source was greater. The higher intake of

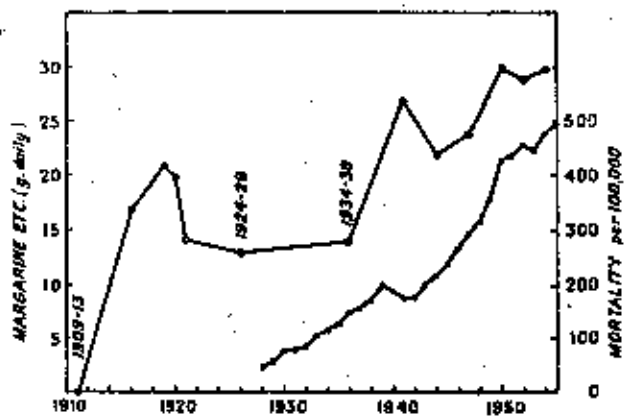


Fig. 22—Trends in coronary mortality and intake of margarine, compound fats, and shortenings in U.K.

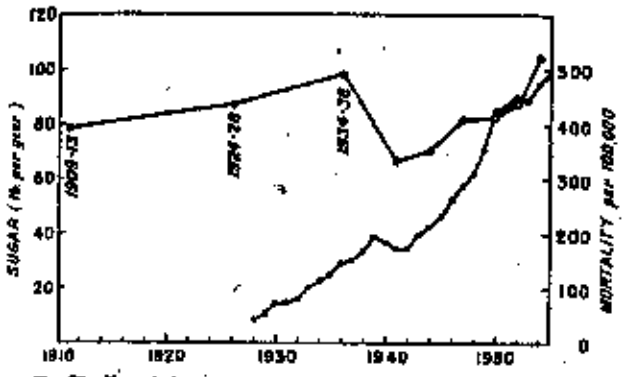


Fig. 23—Trends in coronary mortality and sugar intake in U.K.

hydrogenated fats also gave directly more vitamin E and more E.F.A. Thus, during the decade, deficiency of E.F.A. became less likely because of (1) increased E.F.A. intake, (2) decreased total fat intake, (3) increased vitamin B_6, (4) increased vitamin E. Deficiency of E.F.A. was more likely because of only one factor, the increase in "unnatural" fatty acids. If E.F.A. deficiency was commoner in 1950 than in 1940, these acids must therefore be presumed to be extremely potent "anti-E.F.A." substances. We should then expect that they would outweigh much of the effect of related dietary factors, and result in a close association between the amount of sources of hydrogenated fats consumed and the incidence of coronary disease. We have however seen, from our international comparisons, that high consumption of margarine, compound fats, and shortenings is not necessarily associated with increase in coronary

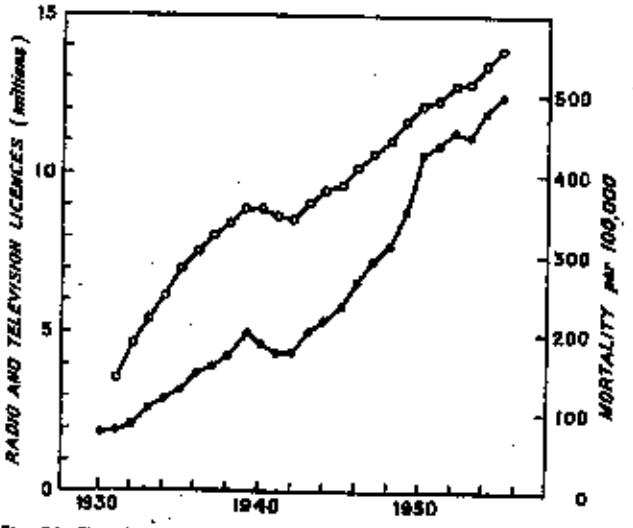


Fig. 24—Trends in coronary mortality and number of radio and television licences in U.K.

mortality; from our comparison of classes in this country, we have seen that high consumption of these fats is associated with lower coronary mortality.

It should not be necessary to say that these negative conclusions in no way detract from the important experimental observations such as those made on the effects of dietary components on the amount of cholesterol or of particular lipoproteins in the blood. Two points, however, are worth emphasising. One is that it has not been demonstrated that there is any certain relationship between these changes induced by relatively short-term dietary experiments, and the liability to coronary catastrophe in those normally consuming particular diets. Secondly, whilst most of the experiments have been concerned with the effect of changes in dietary fat, it has also been shown that increase or decrease in calories, mainly or entirely through carbohydrate, can increase or decrease some of the lipoproteins in the blood (Nichols et al. 1956, 1957). This observation may be relevant to a consideration of general, rather than specific, dietary factors—a consideration to which I shall return.

Positive Suggestions

It seems that the hypothesis of a unitary dietary cause cannot be sustained, but it is possible to extend its validity by introducing two subsidiary hypotheses, of lag and of threshold.

Hypothesis of lag.—It is reasonable to suppose that the development of coronary thrombosis, by the process commonly (though not universally) accepted, requires a fairly lengthy period. That is to say, it may well take several years for a constant high intake of, say, fat to produce atheromatous plaques in the coronary arteries sufficient to give thrombus formation of an intensity to lead to cardiac ischæmia and death. We may then suppose that the continued higher intake of fat in Britain up to 1940 led to a rise in coronary mortality in the next decade or more, even though fat consumption fell during that time. Yet this additional hypothesis is still not sufficient. We have seen that there was a fall in coronary mortality rates in Britain in the early part of the war; an even greater and more protracted fall was seen during the war in Norway. This implies that, if significant dietary changes can produce a significant change in coronary mortality, they may do so almost immediately.

Hypothesis of threshold.—Just as the well-known nutritional deficiencies will occur only with nutrient intakes below a certain threshold, so we may suppose that increasing fat intake might lead to coronary thrombosis only above a certain threshold. The shape of the curve relating fat intake with coronary deaths in different countries supports this. It suggests that, on the whole, a daily intake below 120 g. or so is associated with a low incidence of the disease and an intake above this figure with a high incidence. If to this we add that there may well be, as we have seen, differences in distribution of intake, so that the same average intakes in different countries may nevertheless mean quite different proportions consuming more than the threshold, we can go some way to explaining the considerable differences in mortality in different countries associated with similar high average intakes. But this would imply that, for example in this country, a higher proportion of general practitioners than of consultants consume fat in quantities above the threshold, since twice as many general practitioners die of coronary thrombosis. It is possible, though unlikely, that this is so, but the onus of proof lies with those who believe in a unitary dietary theory of the disease. They would have to explain, too, why farmers have a low incidence of coronary thrombosis (Registrar-General 1951), when one would have supposed that their intake of fats, especially of animal fats, would have been higher than the average during the decade or more of rationing. Finally, they would have to show that those countries with an average intake of, say, total fat of about 130 g. have a varying distribution of intake sufficient to account for their considerable differences in mortality.

Multiple causes.—A consideration of all the data I have quoted, and of the known differences in mortality in different occupations, suggests that such a unitary dietary theory, however modified by considerations of lag, of threshold, and of individual variation in dietary intake, is less plausible than a theory of multiple causes. I find it difficult to imagine that there are significant differences in diets between bus drivers and bus conductors, between general practitioners and consultants, between dock labourers and building labourers, yet the former of each pair has a much higher mortality from

coronary disease than the latter (Registrar-General 1951, Morris et al. 1952).

It seems more likely that several factors are concerned in producing coronary thrombosis and that we should do better to attempt to discover what they are, and their relative importance, than to insist on a single cause, whether dietary or not. One of these which is relevant to the data I have presented is diminished physical activity. The countries with higher incidence of coronary disease are those with, in general, greater leisure. There is for example quite a reasonable relationship between the number of motor-cars and the incidence of the disease in different countries, as there is between the rise in both in Britain in the past 30 years. Again, it is in the more sedentary occupations that mortality is usually higher.

Decreased physical activity may affect coronary disease in two ways. First, it may result in a person having a less well developed coronary supply than one who is more active, that is, fewer and narrower vessels for supplying the heart. This might mean that a thrombus forms more readily in such a person; it would certainly mean that a thrombus is more likely to cause fatal ischæmia. Second, diminished physical activity is likely to be associated with overweight, so that the work of the heart is greater. Recently, it has been shown that, whilst there is in general a direct relationship between physical activity and caloric intake, the relationship breaks down when physical activity is very low (Mayer et al. 1956). That is, persons who lead sedentary lives tend to have the same caloric intake as those with a small but nevertheless greater physical activity. Such sedentary persons are consequently of greater weight.

We now can see a possible general dietary factor rather than a specific one. In comparing countries, we saw that coronary mortality was related to better living standards, which shows in dietary terms as well as in terms of income, motor-cars, and radio licences. The diets generally contain more calories, as well as more protein, fat, and sugar. Thus, their populations are on the whole eating more and also tending to expend less physical effort. When we compared classes in Britain, we saw that the total caloric intake was much the same, but it is professional people (who, we may imagine, do less physically) who have the highest incidence of coronary thrombosis. I mentioned earlier that changes in caloric intake have been shown experimentally to change the amount of some lipoprotein fractions in the blood. This supports the view that a possible factor in coronary thrombosis may be the relationship between physical activity and the general level of diet, expressed in calories rather than in any single nutrient.

Predisposing and Precipitating Causes

The suggestion of multiple causes of coronary thrombosis makes it possible to suggest an explanation for the puzzling temporary fall in coronary deaths during the war, reported in Britain and in Norway. It seems likely that changes in the coronary arteries are produced over a long period, but that there are also more rapidly acting factors which may precipitate thrombus formation. The predisposing causes would be concerned in the long-term trends of incidence of the disease; the precipitating causes would be concerned in the short-term changes in incidence such as those in the early 1940s.

Profitable Investigations

What remains to be done is to isolate the possible causes and to evaluate their relative importance. I have mentioned the possibilities of a specific dietary factor, general dietary factors, and physical activity. Others which have been suggested include smoking and nervous strain. A study of much smaller groups than whole populations or broad occupational classes will help to reduce the number of variables. Ideally, one would like to relate, in individuals, the chances of developing coronary thrombosis, and the history as regards diet, exercise, smoking, and so on. This would involve a prospective study, such as has been carried out so revealingly in regard to the association between smoking and lung cancer. In the meantime, however, statistical studies such as we have here been considering are also of value, to the extent that they may, if used cautiously, yield useful hints as to possible causes. In particular, it would be useful if more information were available from different regions in single countries, where some of the possible causes are known to be similar and attention may be given to those which differ. Ultimately, when enough information is available, it should be possible by multifactorial analysis to decide which are the factors, and what is their relative importance.

Summary

A consideration of some of the more readily available data on the incidence of coronary deaths and on food consumption makes it difficult to support any theory which supposes a single or major dietary cause of coronary thrombosis.

It is suggested that relative over-consumption of food, associated with reduced physical exercise, may be one of several causes of the disease.

These causes are likely to be divisible into predisposing causes and precipitating causes. Their identification requires more study of groups where some of the possible causes are relatively constant, so that differences in incidence of coronary disease may be related to the other possible causes.

I am very grateful to Mr. T. B. Morgan and Mrs. M. Willoughby of my department for help in compiling these figures.

REFERENCES

Food and Agriculture Organisation (1955) *Food Consumption: Food Balance Sheets.*
Hollingsworth, D. (1957) The Englishman's Food (Drummond and Wilbraham). London (in the press).
Mayer, J., Roy, P., Mitra, K. P. (1956) *Amer. J. clin. Med.* 4, 169.
Ministry of Agriculture, Fisheries, and Food (1956) Domestic Food Consumption and Expenditure: 1954. H.M. Stationery Office.
Morris, J. N. (1956) *Med. Offr.* 94, 251.
— Heady, J. A., Barley, R. G. (1952) *Brit. med. J.* i, 503.
Nichols, A. V., Gofman, J. W., Dobbin, V. (1956) *Lancet*, ii, 1211.
— Dobbin, V., Gofman, J. W. (1957) *Geriatrics*, 12, 7.
Pfeiffer, J. J., Holmann, R. T. (1955) *Arch. Biochem.* 55, 520.
Registrar-General (1951) Decennial Supplement, England and Wales. Part 1. Occupational Mortality. H.M. Stationery Office.
Sinclair, H. M. (1956) *Lancet*, i, 381.
World Health Organisation (1955) Annual Epidemiological and Vital Statistics, 1952.
— (1956) *Epidem. vit. Stat. Rep.* 9, no. 10.

Proc. Nutr. Soc. 23:149-162. 1964.

Reproduced by permission of the copyright owner

Patterns and trends in carbohydrate consumption and their relation to disease

By JOHN YUDKIN, *Nutrition Department, Queen Elizabeth College, University of London*

It is generally held that, for the greater part of the million or more years of his existence, man was a hunter and forager; his diet was largely the bodies of animals he killed or found as carrion, with relatively small amounts of leaves, fruits and roots. Being omnivorous, he could of course sustain himself if necessary with a lower proportion of meat, and a higher proportion of vegetable foods, but by and large his diet was relatively poor in carbohydrate.

With the discovery and development of agriculture some 8 thousand or 10 thousand years ago, the ease of production of carbohydrate-rich foods such as cereals resulted in his diet with few exceptions becoming predominantly carbohydrate, with little fat and with small and often minimal amounts of protein. The only exceptions are

small pockets of people mostly in parts of Africa and in the Arctic Circle, who have retained a hunting economy until recently, or in some instances up to today; their diets are still rich in protein, moderately rich in fat, and relatively low in carbohydrate.

When circumstances allow—usually economic circumstances—there is a considerable increase in the protein and fat intake. This is seen both in the wealthier members of the poorer countries, and also in the average diet of the wealthier countries as compared with the poorer. It is commonly believed that this is accompanied by a fall in carbohydrate intake with increasing wealth. In fact, however, the absolute amount of carbohydrate consumed is very much the same in most countries, except in the poorest.

Diet in relation to income

The effect of increasing national income on the intake of protein, fat and carbohydrate is shown in Fig. 1, for the thirty-four countries for which relevant infor-

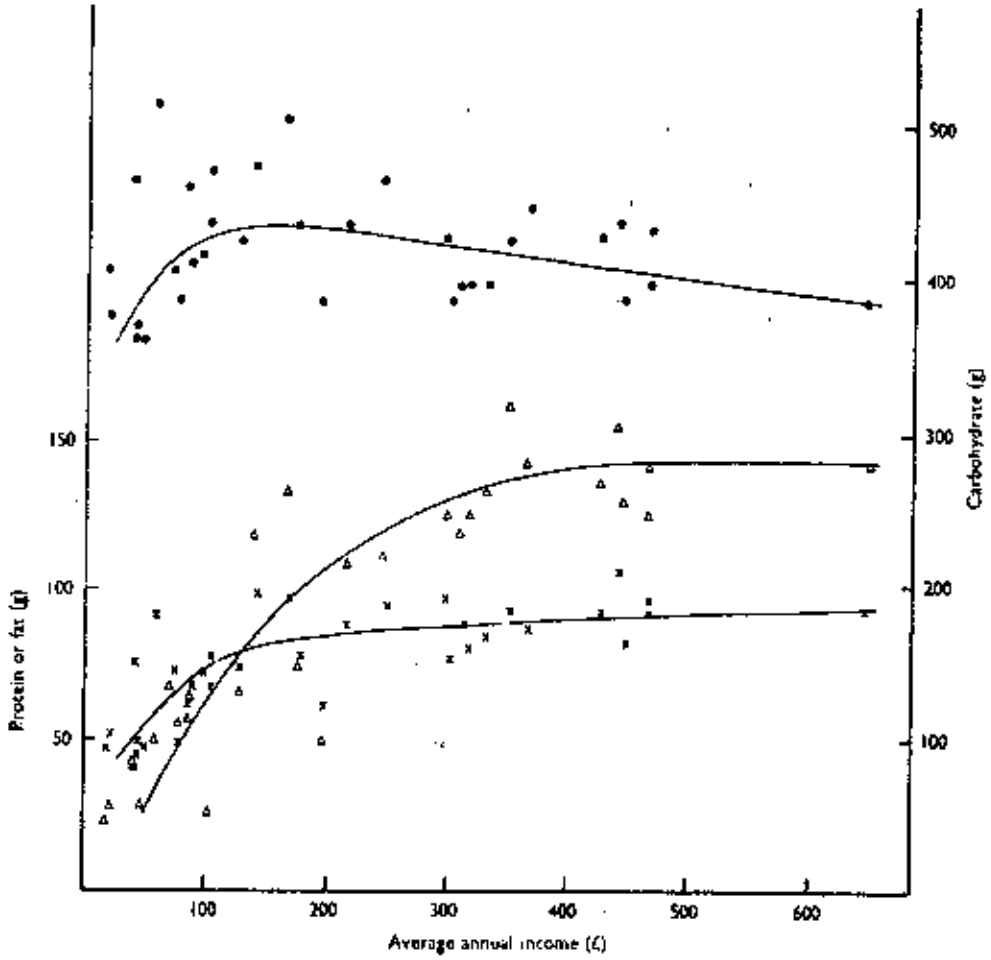


Fig. 1. Consumption of protein, fat and carbohydrate in thirty-four countries related to average national income. Sources: FAO (1963); Unilever (1964; information kindly supplied by Economics and Statistics Division, Unilever Ltd, London). ●, carbohydrate; △, fat; ×, protein.

mation was available. As income rises, there is a very steep rise in fat intake, and a less steep rise in protein intake; there seems to be no lower limit to fat intake, but there is of course a lower limit to protein intake below which life cannot be sustained. Moreover, fat intake is higher than protein intake in the wealthiest countries, as well as being lower in the poorest countries.

Carbohydrate intake is lowest in the poorest countries, where the diet is meagre in all its constituents. With decreasing poverty, carbohydrate consumption somewhat rises, but it falls off very little with increasing affluence.

Superficially, it may seem surprising that the wealthiest countries consume almost as much carbohydrate as most of the poorer countries, since the intake of such carbohydrate-rich foods as cereals is much lower. The reason however is that increasing wealth is accompanied by increasing intake of sugar, which almost exactly makes up for the decreasing intake of starch.

Table 1. *Effect of average national income on intake of calories, protein, fat, total carbohydrate and sugar*

Annual income (£)			Daily intake					
Range	Average	No. of countries	Calories (kcal)	Fat (g)	Protein (g)	Carbohydrate (g)	Sugar (g)	Sugar (% of carbohydrate)
Up to 50	38	6	2060	34	54	394	35	9
51 – 100	82	6	2500	61	68	437	59	13·5
101 – 200	148	7	2700	73	78	456	86	19
210 – 350	292	7	2970	118	86	419	102	24
More than 350	460	8	3200	140	92	422	139	33

Values for groups of countries not weighted for population.
Sources: FAO (1963); Unilever (1964; information kindly supplied by Economics and Statistics Division, Unilever Ltd, London); Viton & Pignalosa (1961).

Table 1 shows the intake of protein, fat and carbohydrate as in Fig. 1, but with the countries grouped according to income, and with sugar given as a separate item. From the poorest to the wealthiest group, average caloric intake increases by about 50%, protein by about 80% and fat by 300%. Total carbohydrate intake changes very little; the highest is in the relatively poor countries with average annual incomes between £100 and £200, but it is only 15% more than in the poorest. But, although the intake of carbohydrate is relatively constant, there is hidden in it a considerable increase in sugar with increasing income, which like the increase in fat is of the order of 300%. There is in fact a striking similarity not only in the relative increase in intake of fat and sugar, but in their absolute amounts. This is shown in Fig. 2, in which the diagonal represents an exact one-to-one relationship between the intakes of fat and of sugar.

Though the precise one-to-one relationship is fortuitous, the general relationship is not. Many of our sugar-containing foods—cakes, biscuits, puddings, chocolates, ice-cream—contain both sugar and fat, so that their levels of intake within a total diet tend to be interdependent. I shall have more to say on this point later.

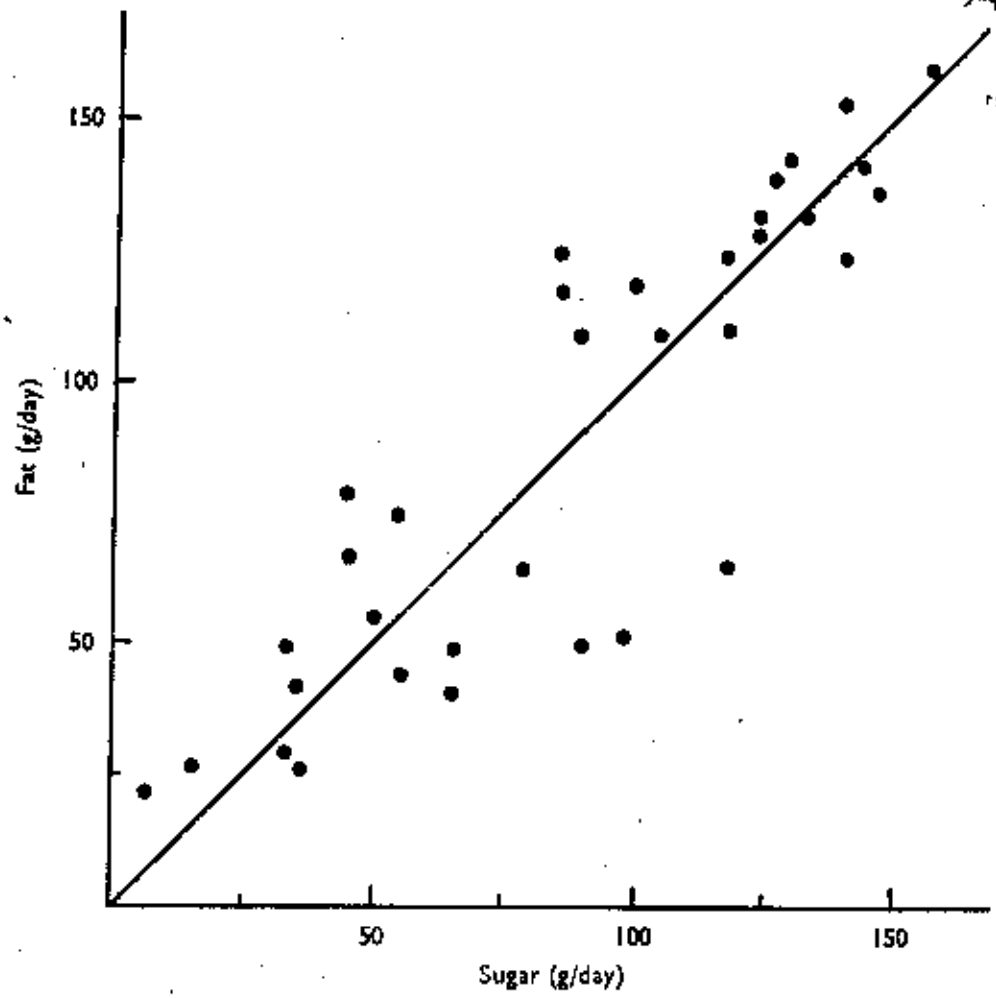


Fig. 2. Relationship between fat intake and sugar intake in thirty-four countries. Sources: FAO (1963); Viton & Pignalosa (1961).

In the poorest countries, sugar amounts to less than 10% of the total carbohydrate intake and contributes 145 kcal daily, or 7% of the low intake of calories; in the wealthiest countries, sugar amounts to 33% of the total carbohydrate intake and contributes 540 kcal daily, or 17% of the much higher intake of calories. In the United Kingdom, we obtain about 570 kcal daily from sugar, which is very little less than the 610 kcal we obtain from the carbohydrate in our bread.

Increase in sugar consumption

Total world production of sugar is increasing more rapidly than that of most other food commodities (Fig. 3). The increase is far greater than the growth of population—the average consumption has increased nearly threefold since the beginning of the century (Table 2). The proportionate increase is, in general, much greater for the poorer regions (Table 3). The exception is in the Far East, where,

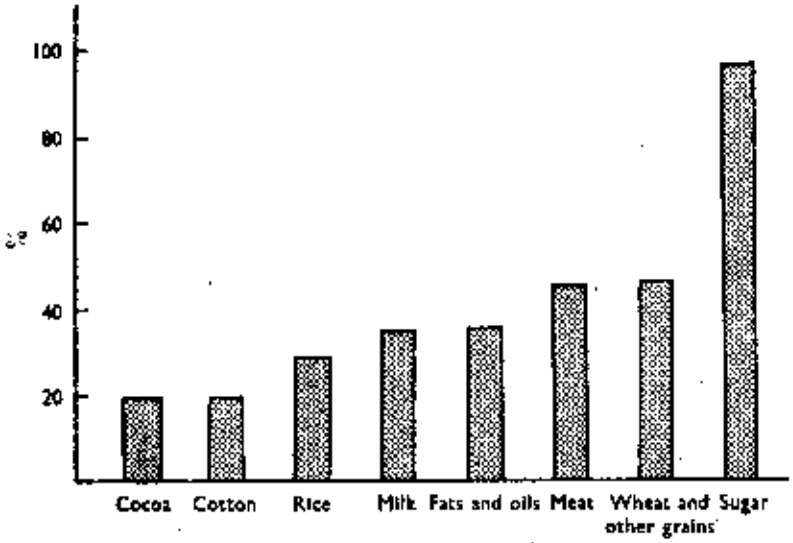


Fig. 3. Percentage increase in 1958 over prewar production of some agricultural commodities. Source: Viton & Pignalosa (1961).

Table 2. *Yearly world sugar consumption*

Year	Consumption kg/head	Consumption Index
1899	5·5	100
1909	7·5	135
1924	10·2	185
1929	12·4	225
1938	12·0	218
1949	11·8	215
1957	15·5	282

Source: Viton & Pignalosa (1961).

Table 3. *Regional increase in yearly sugar consumption (kg/head)*

Region	Consumption Prewar	Consumption 1957	Increase (%)
Far East*	4·7	6·6	40
Near East	4·9	12·4	145
Africa	5·0	10·0	100
Eastern Europe + USSR	12·9	25·8	100
Central America	16·6	28·3	70
South America	16·8	29·1	73
Western Europe	25·2	32·4	27
Oceania	43·3	45·4	5
North America	46·5	46·1	−1

Source: Viton & Pignalosa (1961).

*Excluding China.

however, consumption had fallen considerably during and after the war. By 1951 it had fallen to only two-thirds of its prewar amount, but since then it has increased faster than in any other region.

The rate of increase in sugar consumption in some individual countries is shown in Fig. 4. Only in the United States and perhaps in Australia does it look as if consumption has not changed, or perhaps even fallen off slightly, compared with

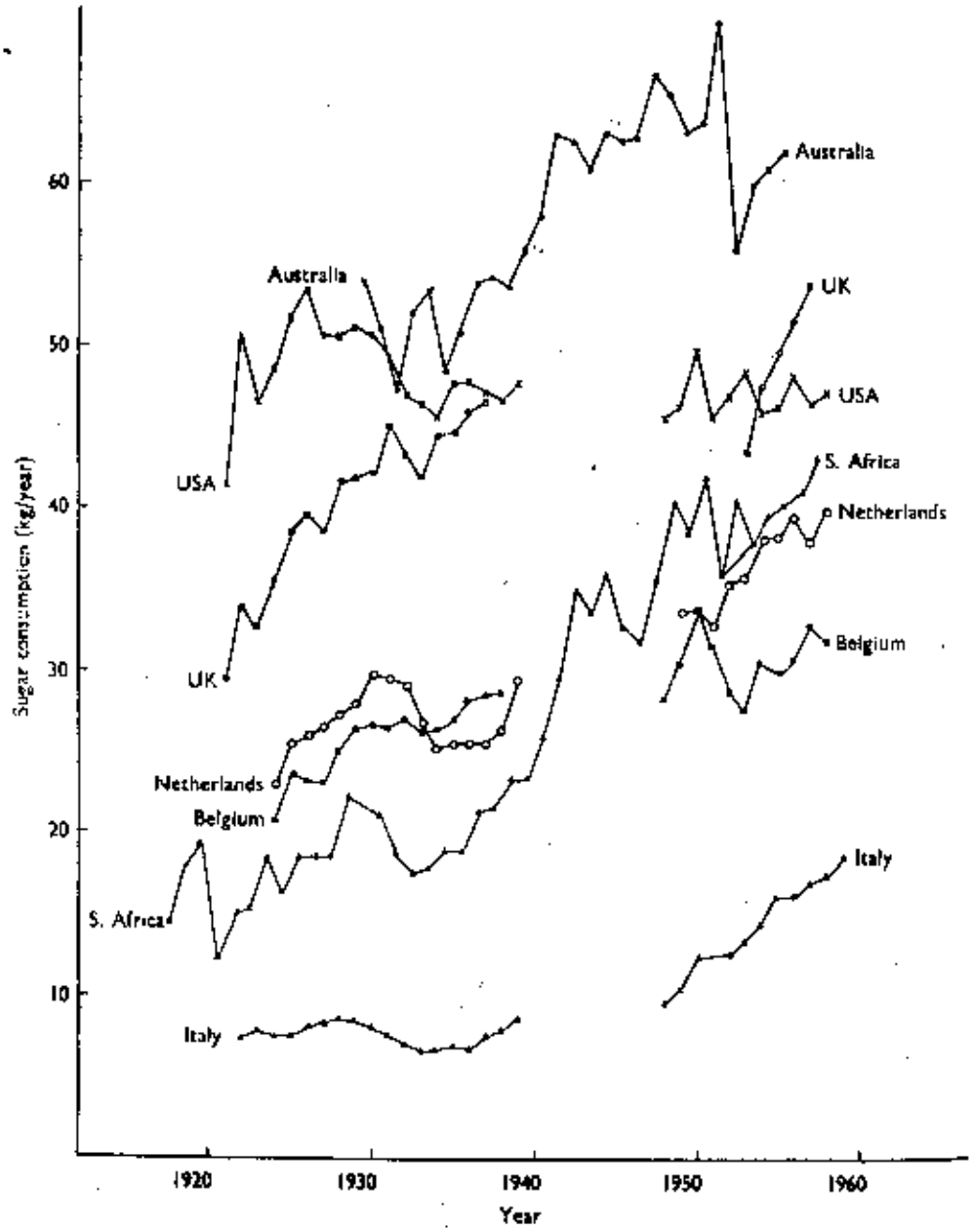


Fig. 4. Changing sugar consumption in some selected countries. Source: Viton & Pignaloza (1961).

Table 4. *Sugar used in manufacture as a percentage of total sugar consumption*

Country	Year	Sugar used in manufacture
South Africa	1956–7	20
West Germany	1956–7	40
France	1956–7	42
UK	1958	49
USA	1957	51
Australia	1955–6	55

Source: Viton & Pignaloza (1961).

prewar. On the other hand, the increase is proportionately greatest in Italy, which had (and has) the lowest consumption in this group.

Forms of sugar consumed

Sugar is purchased either directly as such (as household sugar) or in the form of manufactured sugar-containing foods (industrial sugar). In the latter form it occurs in such foods as cakes, biscuits and confectionery. The proportion bought as industrial sugar increases with increasing income. This can be seen when one compares different countries (Table 4). In some countries, the proportion of manufactured sugar has increased also with time, as they became wealthier (Fig. 5).

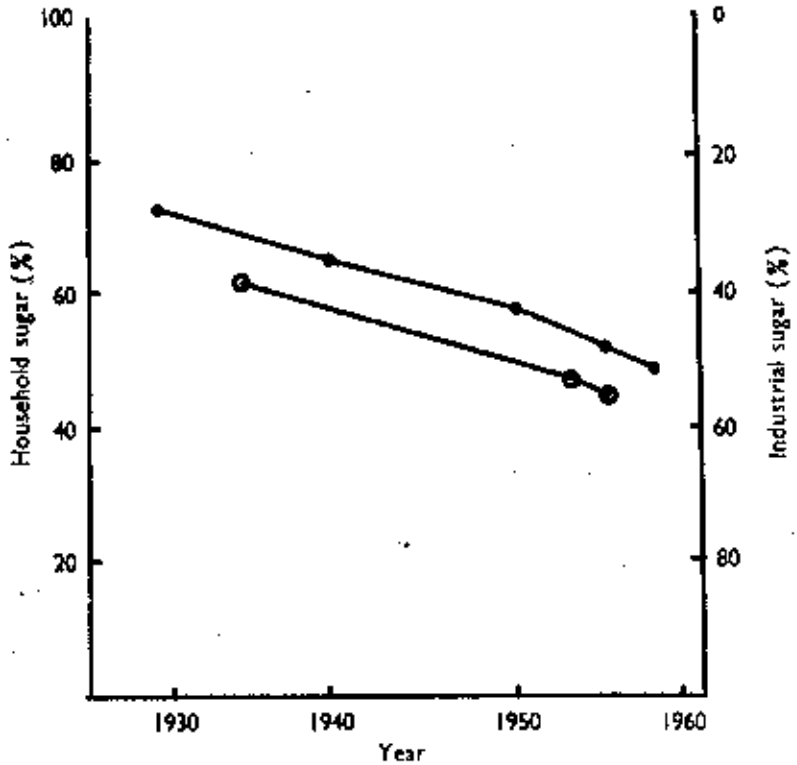


Fig. 5. Fall in proportion of sugar used in households in USA and Australia. Source: Viton & Pignaloza (1961). ●——●, USA; o——o, Australia.

In individual countries the amount of sugar consumed in manufactured foods is proportional to income. This can also be demonstrated indirectly by examining the cost of a unit amount of sugar, since clearly sugar-containing manufactured foods cost more than the sugar they contain. Both in Ireland and in the rural and urban areas of the USA, the wealthier families pay twice as much for a kg of sugar as the poorer families, no doubt because they are buying more, and more expensive, manufactured sugar-containing foods (Figs. 6, 7, 8).

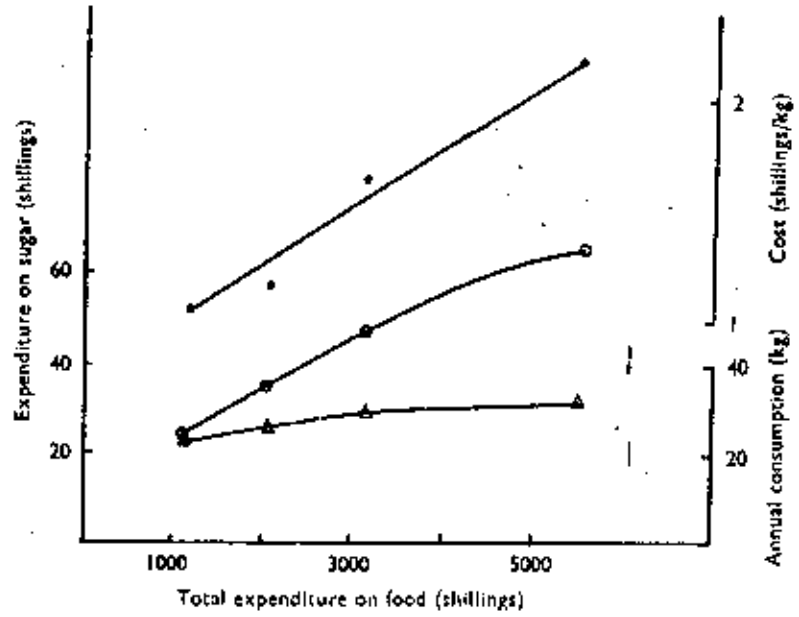


Fig. 6. Average annual sugar consumption and cost, related to total annual expenditure on food per head: Ireland 1951–2. Source: Viton & Pignaloss (1961). ●, cost; o, expenditure; △, consumption.

These manufactured foods contain in addition to sugar other calorigenic constituents, such as flour, fat and chocolate. I believe that this has a bearing on the consumption of other foods. For we sometimes forget that, unlike with other consumer goods, there are fairly narrow limits to the amounts of food we can consume (Yudkin, 1964). The poorest people may buy virtually no clothes at all; the wealthiest may buy 2 dozen shirts and three or four suits in a year. The poorest may live in a mud hut; the wealthiest may have a large town house, a country home and, nowadays, a cottage abroad. But there is a lower limit to the amount of food, below which we cannot live; and an upper limit of not more than perhaps twice this level, above which the most gluttonous cannot reach. Thus, a significant increase or decrease in the amount consumed of one food might well produce a decrease or increase in one or more of the other foods, though it is not easy to predict where this reciprocal change might occur (Yudkin, 1964).

The average calorie intake in the wealthiest countries does not seem to have changed significantly in the last three or four decades. In fact, one might perhaps

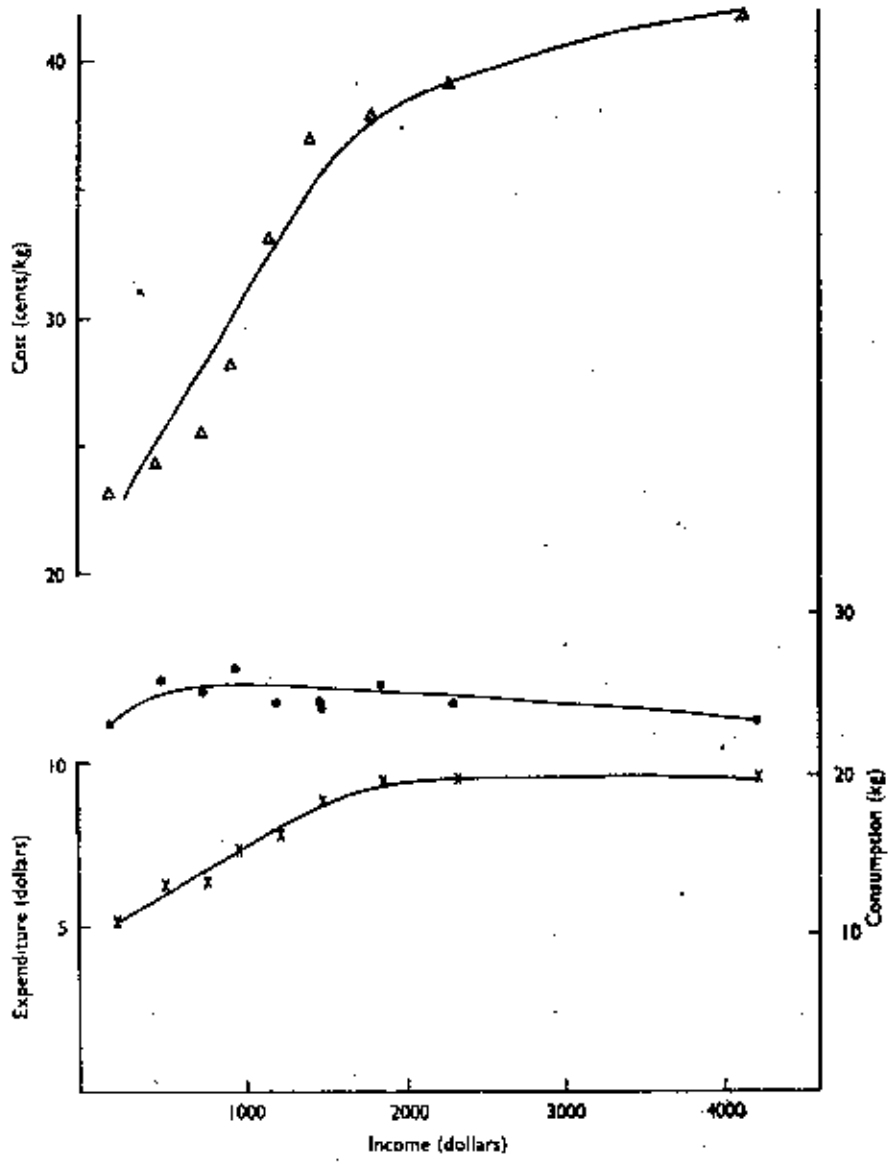


Fig. 7. Annual sugar consumption per head and cost, related to income: USA urban families 1955. Source: Viton & Pignalosa (1961). △, cost; ●, consumption; ×, expenditure.

have expected something of a fall because of the increase in mechanization at work and in the home, and the general increase in sedentariness.

Interaction of consumption levels

If then the wealthiest groups in the wealthiest countries are buying more sugar-containing manufactured foods, deriving calories both from the sugar and from the other constituents, then one must expect an increase in the prevalence of obesity, or

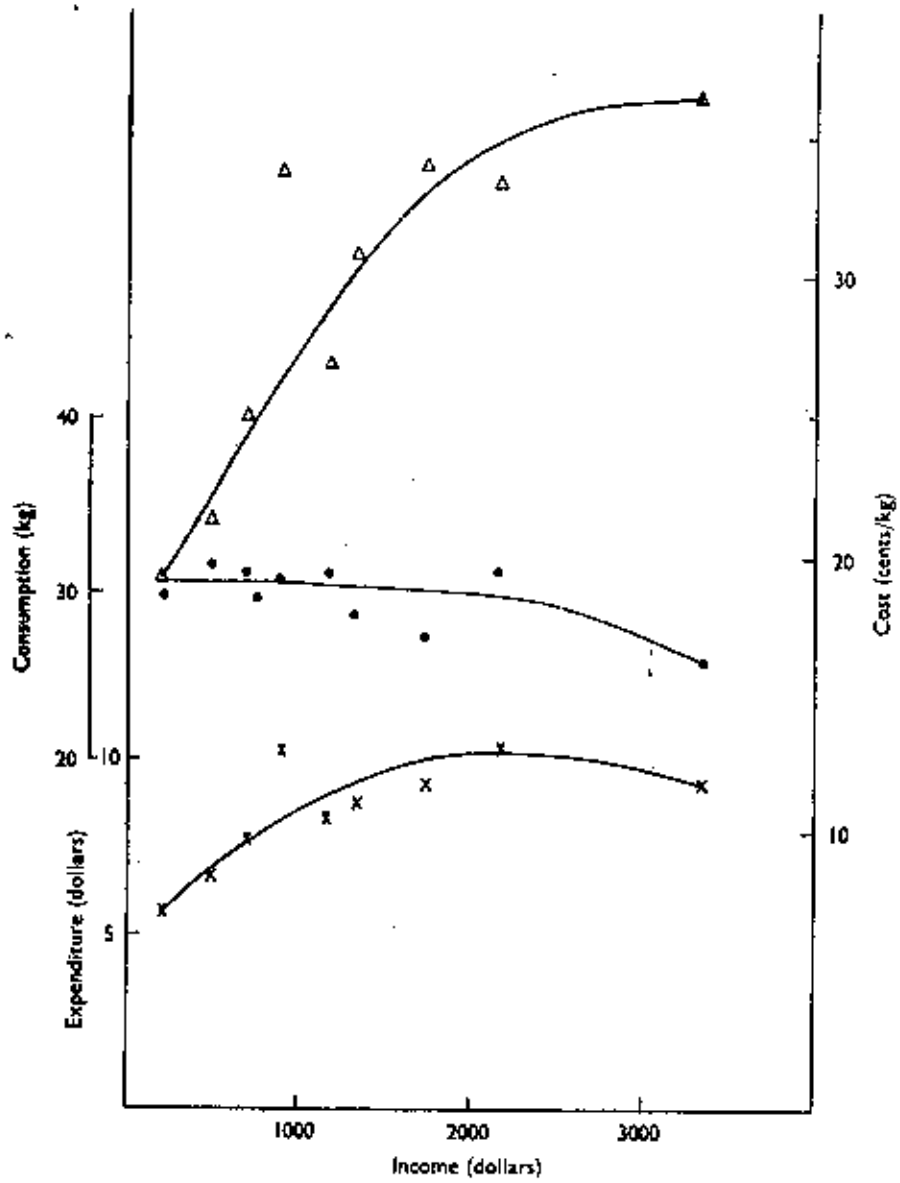


Fig. 8. Annual sugar consumption per head and cost, related to income: USA rural non-farm families 1955. Source: Viton & Pignalosa (1961). △, cost; ●, consumption; ×, expenditure.

a reduced consumption of other foods, or both. It is said that there has been a threefold increase in the proportion of obese persons in the United States since the beginning of the century. The proportion of overweight people amongst the wealthiest section of the population, though high, is, however, lower than in the poorer sections (Hundley, 1955). We might then expect to find that especially in the wealthier section there is a decrease in the consumption of other foods, to make up for the increase in manufactured sugar-containing foods, though the decrease might not be easily detectable if it were over a wide range of foods.

I believe that we can in fact detect two examples of decrease, both in what are usually regarded as nutritionally desirable foods. McKenzie and I have calculated that in this country there has been a fall in the amount of meat consumed by the wealthiest members of the population (Table 5). The figures for meat consumption

Table 5. *Weekly UK meat consumption (oz/head) related to income, and percentage of population in each income class*

	Consumption			Population	
Income class	1936	1960	Change (%)	1936	1960
Lower	32	35	+10	50	52
Middle	43	36	−16	40	38
Upper	49	38	−22	10	10

Sources: Orr (1936); Ministry of Agriculture, Fisheries and Food: National Food Survey Committee (1962).

per head imply that there has been an overall fall of 6% since before the war, whereas there has in fact been a rise of some 5%. It is likely, however, that Table 5 gives a reasonably accurate indication of the relative consumption per head of different groups in 1936 and in 1960; if we now adjust the figures to introduce an overall 5% increase without altering the relative group values, we still find that there has been a fall in meat consumption per head of 7% in the middle-income classes, and of 14% in the upper. We have similarly found evidence of a fall in consumption of fruit by the wealthiest section in America (Table 6).

Table 6. *Weekly US fruit consumption (lb/head) related to income, and percentage of population in each income class*

	Consumption			Population	
Income class	1942	1956	Change (%)	1942	1956
Lowest	2·5	3·0	+20	30	30
Highest	5·6	4·7	−16	19	20

Sources: United States Department of Agriculture (1944, 1956).

We believe that these two examples make it no longer possible to accept the frequently expressed belief that increasing income is invariably accompanied by increasing consumption of meat, fruit and other nutritionally desirable foods. Thus, Baines, Hollingsworth & Leitch (1963), speaking of the changes of the diets in the United Kingdom since before the Second World War, say 'The levelling effect of the war and post-war years was in all important respects upwards'. In fact, as we have seen, in at least some foods the levelling has been downwards from the high levels of consumption of people with the highest incomes, as well as upwards from the low levels of people with the lowest incomes.

Carbohydrate consumption and disease

I have shown that the main patterns and trends relate to sugar in particular rather than to carbohydrate in general. I believe that the trends have already had a detectable effect on the incidence of disease, in both the poorer and the wealthier countries.

In the poorer countries there has been increasing anxiety expressed concerning the competition between sugar-containing foods and the much-needed protein-rich foods (Burgess & Dean, 1962; Yudkin & McKenzie, 1964). Especially in the rapidly growing towns of some of these countries, there is a tendency for the poorest people to buy, especially for their children, sweet foods and fizzy drinks when they might be buying milk. Even the addition of sugar to cereal foods for infants, or the purchase of some proprietory infant foods containing sugar, can reduce the intake of protein to levels which cause kwashiorkor.

It has been reported that the quarter of the world's population with the lowest food consumption had even less to eat in 1954 than prewar; the fall was greatest for milk and meat (Blau, 1956). During the same period, its sugar consumption had increased by more than 50%.

The situation has improved only little since 1954. I have made calculations for 1958 for the nine low-income countries, Brazil, Ceylon, Chile, Egypt, Greece, India, Pakistan, Philippines and Yugoslavia, with a total population of some 650 millions. Compared with the prewar period, the average calorie intake in 1958 had increased by 9%, but meat consumption had fallen by 9% and animal protein intake by 7%. At the same time, sugar consumption had increased by 105%.

In the wealthier countries, the intake of highly palatable sweetened food and drinks, of which the sugar alone contributes over 500 kcal/head a day, is undoubtedly a potent contribution to the high prevalence of obesity, both in adults and in children. And, as we all know, obesity is a contributory factor in a long list of diseases, diseases of the joints, gall-bladder, kidneys and heart, all adding up to an increase in mortality. For a middle-aged man or woman weighing 25% above the average, this means a decreased expectation of life of some 4 years.

In addition, as I have said elsewhere, I believe that as well as contributing excessive calories, sugar may specifically be a factor in the causation of other diseases. Amongst these, I include dental caries, diabetes mellitus, dyspepsia and peptic ulceration, and myocardial infarction.

Cohen (1963) has produced evidence that the increased prevalence of diabetes and of myocardial infarction amongst immigrant Yemenis, as their period of residence in Israel increased, is most closely related to their consumption of sugar. This is supported by his experimental studies and, for myocardial infarction, by the investigations of Albrink (1962).

The most interesting of these recent contributions are, to my mind, those that underline the association between diabetes and myocardial infarction. It has long been known that atherosclerosis and myocardial infarction are common complications of diabetes. More recently, it has been shown that individuals with a history of ischaemic heart disease frequently have an impaired glucose tolerance. Cohen &

Teitelbaum (1964) have shown that this impairment can be brought about in rats by diets rich in sugar. Moreover, K. J. Kingsbury (1964, personal communication) finds that patients who have arterial disease show a diminished glucose tolerance more frequently than an elevated serum cholesterol.

The close relationship which I have shown to exist between sugar consumption and fat consumption removes the difficulty to which Himsworth (1949) drew attention, when he summarized his views on the role of diet in the aetiology of diabetes.

At first, he points out, he believed that the prevalence of diabetes was somehow associated with the amount, or the proportion, of total dietary carbohydrate. Like most workers up to the present time, he did not distinguish between forms of dietary carbohydrate. Later, however, he concluded that the evidence from epidemiology indicated that 'the dietary factor which parallels these changes [in mortality and prevalence of diabetes] most closely is the consumption of fat, and this correlation is surprisingly consistent'. He continues, 'We are thus left with the paradox that, though the consumption of fat has no deleterious influence on sugar tolerance, and fat diets actually reduce the susceptibility of animals to diabetogenic agents, the incidence of human diabetes is correlated with the amount of fat consumed'.

We can say that the paradox is now resolved, and that the association of diabetes with the dietary level of fat is quite fortuitous. It is simply indicative of a relationship with the dietary level of sugar, for the intakes of fat and of sugar are so closely allied.

I believe that the widely publicized relationship between fat intake and myocardial infarction is similarly fortuitous, and that the intake of fat is only a coincidental index of the intake of sugar. The aetiology of myocardial infarction is almost certainly multifactorial; the important dietary factor is likely to prove to be the level of sugar. Perhaps I may be permitted to recall that in 1957 I showed that the incidence of deaths due to myocardial infarction in different countries was better related to the consumption of sugar than to the consumption of total fat, or of any particular sort of fat (Yudkin, 1957).

Today's symposium on carbohydrate is a reflection of the increasing interest which nutritionists are beginning to show in this very neglected field. For my part I believe that we should pay much more attention to the sugar moiety of our dietary carbohydrate, especially since its rate of increase has been far and away greater than that of any other dietary component.

Summary

1. The diet of early man contained little carbohydrate. With the discovery of agriculture, the amount of carbohydrate increased, and the amounts of protein and fat decreased.

2. The ease and cheapness of production of carbohydrate-rich foods tend to keep diets low in protein and fat, except when economic circumstances allow.

3. When national diets are compared with national incomes, one finds that increasing incomes are associated with moderate increases in calories and in protein, considerable increases in fat, and little change in carbohydrate.

4. Higher national incomes are associated also with considerable increases in the sugar moiety of the carbohydrate. In the wealthiest countries, sugar contributes nearly 20% of the total calories. The average intake of sugar in each country is very similar to its intake of fat.

5. Increasing prosperity, both between countries and within a country, leads to an increasing proportion of sugar being bought in manufactured foods, rather than as household sugar. It is suggested that the effect of this is that wealthier countries, and the wealthier section of a national population, tend to have a higher intake of calories from the accompanying flour, chocolate, fat and other ingredients of these manufactured foods.

6. There is now evidence that one of the effects of this contribution of calories from sugar-containing foods is to reduce the consumption of nutritionally desirable foods, such as fruit and meat.

7. In the poorer countries, the consumption of sugar and sugar-containing foods may be responsible for malnutrition, especially for protein deficiency in children.

8. In the wealthier countries, there is evidence that sugar and sugar-containing foods contribute to several diseases, including obesity, dental caries, diabetes mellitus and myocardial infarction.

9. It is suggested that the known association of the prevalence of diabetes and of myocardial infarction with the level of fat intake is fortuitous and secondary. It is more likely that the primary association is with levels of sugar intake, which I have shown are, in turn, closely related to levels of fat intake.

REFERENCES

Albrink, M. J. (1962). *Arch. intern. Med.* **109**, 345.
Baines, A. H. J., Hollingsworth, D. F. & Leitch, I. (1963). *Nutr. Abstr. Rev.* **33**, 653.
Blau, G. (1956). *Commod. Policy Stud.* no. 10.
Burgess, A. & Dean, R. F. A. (1962). *Food Habits and Malnutrition.* London: Tavistock Publications.
Cohen, A. M. (1963). *Amer. Heart J.* **65**, 291.
Cohen, A. M. & Teitelbaum, A. (1964). *Amer. J. Physiol.* **206**, 105.
FAO (1963). *Food Balance Sheets.*
Himsworth, H. P. (1949). *Lancet*, i, 465.
Hundley, J. M. (1955). In *Weight Control.* Ames, Iowa: Iowa State College Press.
Ministry of Agriculture, Fisheries and Food: National Food Survey Committee (1962). *Domestic Food Consumption and Expenditure: 1960.* London: H.M. Stationery Office.
Orr, J. B. (1936). *Food, Health and Income.* London: MacMillan.
United States Department of Agriculture (1944). *Misc. Publ. U.S. Dep. Agric.* no. 550.
United States Department of Agriculture (1956). *Food Consumption of Households in the United States.* Washington, DC: United States Department of Agriculture.
Viton, A. & Pignalosa, F. (1961). *F.A.O. Commod. Bull. Ser.* no. 31.
Yudkin, J. (1957). *Lancet*, ii, 155.
Yudkin, J. (1963). *Lancet*, i, 1335.
Yudkin, J. (1964). *Progress*, **50**, 63.
Yudkin, J. & McKenzie, J. C. (editors). (1964). *Changing Food Habits.* London: MacGibbon and Kee.

CLINICAL NUTRITION

EDITOR: JEAN MAYER, PH.D., D.SC.*

JOHN YUDKIN, M.D., PH.D., F.R.I.C.

Queen Elizabeth College
University of London, London

Reproduced by permission of the copyright owner

Further reproduction prohibited without consent of copyright owner

Sugar and Coronary Thrombosis

Extensive research has failed to establish conclusively that a high intake of fat is the major cause of coronary thrombosis. However, there is no known exception to the generalization that the incidence of coronary thrombosis in populations is proportional to their sugar consumption. This hypothesis deserves research equally as intensive as that done on fat intake and blood cholesterol.

Editor's Comment

Whereas most United States and Scandinavian workers in the field of atherosclerosis have incriminated saturated fat as the chief nutritional agent in elevating blood cholesterol, Professor Yudkin and co-workers have steadfastly maintained that a high consumption of sugar is also a factor, perhaps the chief factor, in setting up conditions favorable to the development of coronary thrombosis. Here is a summary by Professor Yudkin of the arguments buttressing his position.—J.M.

During the past 200 years in Great Britain, sugar consumption has increased twenty-fivefold from an annual average of 5 lb in 1760 to about 25 lb in 1860 and 120 lb in 1960. We now eat in a week the amount of sugar our forebears of 200 years ago ate during six months. Epidemiologic studies have shown that increasing wealth, both in time and between populations, is accompanied by an increase in the incidence of coronary thrombosis and by changes in eating habits and diet in

*Professor of Nutrition, Department of Nutrition, Harvard University School of Public Health, and Consultant in Nutrition, Children's Hospital Medical Center, Boston, Massachusetts.

JOHN YUDKIN

Dr. Yudkin is professor of nutrition and dietetics, Queen Elizabeth College, University of London, London.

which the chief feature seems to be an increase in fat and more particularly an increase in sugar. Available evidence has been considered to point especially to a close relationship between coronary thrombosis and fat consumption. There are, however, several exceptions both in the trend and between various populations.

Recent Yemeni immigrants to Israel have little coronary thrombosis but those who have been in the country 20 years or so become prone to the disease. The major change in their diet is a considerable increase in sugar; the increase in fat is small.

Two relevant examples in comparing populations are from East Africa and from the island of St. Helena. The Masai and Samburu tribes of East Africa have almost no coronary disease although they take a great deal of fatty meat and milk. Their immunity to coronary disease despite this high fat intake is thought to be due to their high physical activity. If this were so, one would also expect to find a low incidence of the disease in St. Helena, where the islanders are also physically active; in addition, they eat much less fat than the East African tribes. Yet in fact the incidence of the disease is high among the islanders. They have a high sugar consumption while the East Africans have a low sugar consumption.

As far as I know, there is *no* exception to the generalization that the incidence of coronary thrombosis in populations is proportional to their sugar consumption. However, since the disease takes 20 or 30 years to develop, it will be related to long-term levels of sugar consumption rather than to recent changes.

Although these epidemiologic studies comparing populations clearly point to sugar rather than to any other dietary factor, they may show nothing more than a coincidental relationship. More direct evidence can be gained by studies *within* populations to determine differences between individuals in whom coronary thrombosis does and does not develop. Such studies have shown, for example, that the disease is more likely to develop in the sedentary person and the cigarette smoker than in the physically active person and the nonsmoker. Studies in America have shown that the diet of men in whom the disease develops does not differ from the average in terms of protein, fat or type of fat, or total carbohydrate. Sugar consumption was not separately measured although it was noted that the patients with coronary disease took more cups of coffee. In our studies we found that coronary patients took more cups of tea; they also took more sugar in each cup, as well as more sugar-containing foods. Moreover, statistical analysis showed that irrespective of the number of cups of tea, it was the amount of sugar consumed that was associated with the disease. The group with coronary thrombosis included some who drank little tea and some who drank a lot of tea, but all consumed a great deal of sugar.

Sucrose may produce local effects different from those produced by starch.

One of the criticisms of our theory that a high consumption of sucrose is a causative factor in coronary thrombosis arises from the

common but erroneous belief that all carbohydrates are metabolically equivalent. Diets of affluence that are associated with coronary thrombosis are generally those in which some of the starch has been replaced by sugar. Since sugar (sucrose) is rapidly hydrolyzed and absorbed as the monosaccharides glucose and fructose, and since the fructose is rapidly converted into glucose, it is argued that it cannot produce effects different from those of starch which is also absorbed as glucose. After our epidemiologic studies, therefore, we carried out tests comparing laboratory rats that received diets in which the carbohydrate moiety was either starch or sucrose.

The results confirm and extend earlier observations that there are indeed considerable metabolic differences between these groups of animals. For example, rats receiving the diet with sucrose grow more slowly; we found that this is not because they eat less but rather because they use their food less efficiently. They have different proportions of subcutaneous fat and liver fat, a different distribution of the fatty acids in the fat, and a difference, sometimes considerable, in the activity of several of the enzymes concerned in metabolism of carbohydrate and fat.

The mechanism by which sucrose produces these effects is not known, but there are two relevant differences between sucrose and starch that may provide a clue. First, the products of hydrolysis are absorbed more rapidly from the small disaccharide molecule sucrose than from the large polysaccharide molecule starch. Second, the products of hydrolysis are equal parts of glucose and fructose from sucrose and only glucose from starch. Thus the effects of including sucrose in the diet may be due to one or more of the following mechanisms: (1) a greater and more frequent fluctuation in the level of blood glucose, (2) changes in alimentary flora because the sucrose may not pass so far along the digestive tract as starch before it is hydrolyzed and absorbed, (3) metabolic changes induced by the presence of fructose in the bloodstream which may be different from those induced by glucose.

A question still to be answered is whether a reduction in sugar in our diets will reduce our chances of having coronary disease.

Probably more than one of these mechanisms is at work, producing different effects. Some of the changes in enzymes that we have found seem to be due to the fructose moiety of the sucrose molecule in that they are produced in even greater degree when fructose rather than glucose is fed. Other effects seem to be due to rapid fluctuations in blood sugar; for example, we have found an impairment of the insulin system in animals which have received sucrose in the diet. We might add that as well as metabolic effects sucrose may also produce different local effects from those produced by starch: on the teeth because it is more readily fermentable than starch, and on the mucous membrane of the stomach because of its much higher osmotic pressure.

But much still remains to be done in order to identify the mechanisms by which sucrose can produce the disease processes leading to coronary thrombosis. The most practical question still to be answered is whether a reduction in sugar in our diets will reduce our chances of having the disease. The only experiments of this sort conducted so far are those in which patients who have survived

an attack of coronary thrombosis have received diets in which saturated fat has been reduced and polyunsaturated fats increased. So far, negative results have been reported in some of these experiments, and positive results in one or two. In the latter, the changes in the diet have involved not only the amounts and type of fat but also a reduction of sugar.

Not everyone will necessarily agree with our opinion that the evidence incriminating sugar in coronary thrombosis is now more convincing than that incriminating fat. But most will agree that the tremendous research effort over a dozen years or more, by thousands of workers in dozens of countries, has failed to establish conclusively that the major cause of coronary thrombosis is a high intake of fat. If for no other reason than this, the hypothesis that a high intake of sugar is a major cause deserves to be tested by equally intensive research. The prize is the possibility of substantially reducing the toll of a disease that kills over 100,000 people each year in Great Britain alone.

Adapted from a presentation in *New Scientist*.

REFERENCES

1. AL-NAGDY, S., MILLER, D. S., QURESHI, R. U. and YUDKIN, J.: Metabolic differences between starch and sucrose. Nature 209:81-82, 1966.
2. BETT, D. G. G., MORLAND, J. and YUDKIN, J.: Sugar consumption in acne vulgaris and seborrhoeic dermatitis. Brit Med J 3:153-155, 1967.
3. YUDKIN, J.: Dietary fat and dietary sugar in relation to ischaemic heart disease and diabetes. Lancet 2:4-5, 1964.
4. ____: Evolutionary and historical changes in dietary carbohydrates. Amer J Clin Nutr 20:108-115, 1967.
5. ____: Patterns and trends in carbohydrate consumption and their relation to disease. Proc Nutr Soc 23:149-162, 1964.
6. YUDKIN, J. and KRAUSS, R.: Dietary starch, dietary sucrose and hepatic pyruvate kinase in rat. Nature 215:75, 1967.
7. YUDKIN, J. and MORLAND, J.: Sugar intake and myocardial infarction. Amer J Clin Nutr 20:503-506, 1967.
8. YUDKIN, J. and RODDY, J.: Levels of dietary sucrose in patients with occlusive atherosclerotic disease. Lancet 2:6-8, 1964.

Am. Heart Jour. 80:844-846, 1970.

Reproduced by permission
of the copyright owner

Sucrose, insulin, and coronary heart disease

The most attractive feature of the theory that dietary fat is a major factor in causing coronary heart disease (CHD) is that it is conceptually so simple. Many of the physicians and scientists who are not themselves involved in studying the disease have no difficulty in accepting what I might call the *Reader's Digest* view of the disease process. If you eat too much food containing fat and cholesterol, you increase the level of cholesterol in the blood. Some of this becomes deposited on the inside walls

of the arteries, giving rise to atherosclerosis. In due course, so much is deposited on the coronary arteries that they become partially or completely blocked, both with this deposit and with blood clot forming on it. As a result, you develop angina pectoris or coronary thrombosis.

One could make out a good case for the suggestion that it is the very simplicity and attractiveness of this concept, rather than any other single cause, that has been responsible for the continuing uncertainty and confusion about the etiology of this disease. For facts continue to accumulate that are not readily absorbed into this concept. In particular, it is becoming more and more evident that atherosclerosis and CHD involve far-reaching biochemical changes in the organism, and not merely a deposition of lipid material onto the intimal surface of arteries.

However, though the situation is still far from clear, recent research has begun to fit together some of the observations that have up till now seemed [illegible] and even irrelevant.

In summarizing this recent work, I am fully [illegible] of the fact that there will be some who [illegible] accept several of the bases of which I believe [illegible] etiologic theory is beginning to be built. [illegible] are those, for example, who deny that there [illegible] increase in the prevalence of the disease, [illegible] they would remove one foundation upon [illegible] build—namely, that we must look to the [illegible] of affluence to account for the increase in prevalence being associated with populations that have had an increase in living standards. [illegible], therefore, in the following outline are a [illegible] of assumptions that readers will either [illegible] or reject according to their own evaluation of existing evidence.

With this proviso, let me state the situation as I see it. A general theory of the etiology of CHD should meet two requirements in particular. First, it should produce evidence that some of the characteristics of affluence are indeed likely etiologic agents in the disease. Second, the theory should be capable of uniting these possible etiologic agents into a single and plausible pathogenesis.

Two characteristics of affluence are heavy cigarette smoking and greatly reduced physical activity, and there are, of course, a large number of publications pointing to these factors as causes of the [illegible].

In regard to diet, its most characteristic feature in affluent countries is a high consumption of sugar (sucrose). That dietary sucrose may be a cause of CHD is supported by a number of different kinds of evidence. These may be listed as evolutionary, historical, epidemiological, and experimental. From the evolutionary point of view, we [illegible] the diet of man and his immediate ancestors [illegible] several millions of years as a carnivore consumed not inconsiderable amounts of animal fat, and negligible amounts of vegetable fat; the amount of sucrose he consumed was also very small. Historically, there has been a rise of some twentyfold [illegible] amount of dietary sucrose in the last two [illegible] countries such as England and [illegible] rise in the prevalence of CHD. Epidemiologically, there is a good relationship between the average sugar consumption in different countries, and their prevalence of CHD. Moreover, carefully controlled studies have shown that individuals who develop CHD have been consuming more sugar than those who do not.

Experimentally, we have now a great array of facts concerning the effects of sucrose upon a variety of physiologic and biochemical characteristics in animals and in human volunteers. Some of these changes are in parameters that are known to be associated with CHD.

One of the most recent experimental findings in relation to dietary sucrose suggests that we may now be on our way to fulfilling the second requirement that we mentioned—that a theory of the etiology of CHD should bring together the known (or suspected) causative agents within a single possible mechanism of pathogenesis. It has been shown that one third of a group of men given a diet high in sucrose develop an increase in the immunoreactive insulin level in the blood.[4] In the same men this is accompanied by a considerable increase in body weight and an increase in platelet adhesiveness. We know that a raised insulin level is found in CHD and also in peripheral vascular disease. It is often found, too, in maturity onset diabetes, in obesity, in hypertension, and in cigarette smoking; all of these are associated with an increased proneness to CHD. On the other hand, physical activity and the lowering of overweight result in a decrease in insulin levels; both are associated with decreased proneness to CHD. Insulin injected into rats causes an increased deposition of lipid into the aortic wall.[5]

That only a proportion of men in our study showed "sucrose-induced hyperinsulinism" suggests that only some people are susceptible to the effects of sucrose in producing atherosclerotic disease. This hypothesis is supported by our findings in a study of groups of healthy and atherosclerotic men.[1] We still do not know, however, whether the same individuals that are susceptible or immune to the effects of sucrose in producing atherosclerosis are also those that are susceptible or immune to the effects of other atherogenic agents.

It would be ridiculously premature to say that in hyperinsulinism we now have the solution to the pathogenesis of CHD. But for the first time we can begin, however tentatively, to visualize, if not the ultimate mechanism of pathogenesis, at least something that may lie within the final common path in pathogenesis. A disturbance in insulin production or in insulin activity would account for far more than changes in the arterial wall. It would be involved in a large number of enzymatic disturbances as well as disturbances in other hormones than insulin. It becomes easier, for example, to understand why patients with occlusive arterial disease usually have abnormalities in glucose tolerance.

Finally, there remains the question of the much-publicized changes in blood lipids in atherosclerosis. In our experiment, sucrose ingestion caused a rise in triglycerides in all the men, although as we saw its effect on insulin, platelet adhesiveness and body

weight occurred in only one third of them. This dissociation of the effects of sucrose on lipid levels and on insulin levels suggests that they are produced by different mechanisms (see also reference 7). Such a dissociation is not difficult to imagine, since it is known that some of the effects of sucrose are due to its content of fructose, and others to its rapidity of absorption.[8,9]

If, then, atherosclerosis is mediated through an increase in insulin, and if an increase in lipids is produced by a separate and unrelated mechanism, we would be able to explain why the association between atherosclerosis and increased lipids has been frustratingly incomplete and uncertain. But we are now perhaps extrapolating too far from the data we have; let us be contented for now by concluding that the discovery of "sucrose-induced hyperinsulinism" opens up a new approach to the problem of atherogenesis.

John Yudkin
Department of Nutrition
Queen Elizabeth College
University of London
London, England

REFERENCES

1. Hammond, E. C., and Horn, D.: Smoking and death rates. Report on forty-four months of follow-up of 187,783 men, J.A.M.A. **166**:1159, 1294, 1958.
2. Morris, J. N., Heady, J. A., Raffle, P. A. B., Roberts, C. G., and Parks, J. W.: Coronary heart disease and physical activity of work, Lancet **2**:1053, 1953.
3. Yudkin, J.: Evolutionary and historical changes in dietary carbohydrates, Amer. J. Clin. Nutr. **20**:108, 1967.
4. Szanto, S., and Yudkin, J.: The effect of dietary sucrose on blood lipids, insulin, platelet adhesiveness and body weight in human volunteers, Postgrad. Med. J. **45**:602, 1969.
5. Stout, R. W.: Insulin stimulated lipogeitesis in arterial tissue in relation to diabetes and atheroma, Lancet **2**:702, 1968.
6. Yudkin, J., Szanto, S., and Kakkar, V. V.: Sugar intake, insulin and platelet adhesiveness in men with and without peripheral vascular disease, Postgrad. Med. J. **45**:609, 1969.
7. Nikkila, E. A., Miettinen, T. A., Vesenne, M. R., and Pelkonen, R.: Plasma insulin in coronary heart disease, Lancet **2**:508, 1965.
8. Aitken, J., Robinson, D., and Yudkin, J.: Effects of dietary carbohydrates on some liver enzymes, Proc. Nutr. Soc. **26**:30, 1967.
9. Akinyanju, P. A., and Yudkin, J.: Effects of dietary carbohydrates on the serum lipids of the rat, Proc. Nutr. Soc. **26**:31, 1967.

Proc. Nutr. Soc. 29:2A-3A. 1970.
2A

The relationship between sucrose intake, plasma insulin and platelet adhesiveness in men with and without occlusive atherosclerosis.

By JOHN YUDKIN and STEPHEN SZANTO, *Department of Nutrition, Queen Elizabeth College, London, W8* and V. V. KAKKAR, *Department of Surgery, King's College Hospital Medical School, London, SE5*

We have previously reported an experiment in which six men, out of nineteen given a diet rich in sucrose, were shown to develop increased platelet adhesiveness and hyperinsulinism (Szanto & Yudkin, 1969). From this study, and from studies of other workers, we suggested that hyperinsulinism is an early manifestation of atherosclerosis, that only a portion of the population will show sucrose-induced hyperinsulinism, and that only these will be susceptible to the effect of dietary sucrose in producing occlusive atherosclerotic disease, including both ischaemic heart disease and peripheral arterial disease.

If this is correct, individuals with these diseases should have relatively high levels of insulin, and these should be related to their usual sucrose intake. On the other hand, individuals with no atherosclerosis should have lower levels of insulin, and these should not be related to their sugar intake. The same considerations should apply to the relationship between platelet adhesiveness and sugar intake.

Table 1. *Correlation between sugar intake, insulin level and platelet adhesiveness in twenty-seven men with PVD and twenty-seven 'healthy' men, before and during glucose tolerance test*

(Tau values, and *P* values for two-tailed test)

Time of insulin assay	Sugar intake and insulin		Sugar intake and platelet adhesiveness	
	Tau	*P*	Tau	*P*
	(A) Men with PVD			
Fasting	0·30	<0·05	0·28	<0·05
30 min	0·52	<0·001		
120 min	0·34	<0·02		
	(B) 'Healthy' men			
Fasting	−0·03	NS	0·18	NS
30 min	−0·19	NS		
120 min	−0·06	NS		

NS, not significant.

We have tested this prediction in two groups of subjects. The first was a group of twenty-seven male patients with peripheral vascular disease (PVD), the second was a group of twenty-seven apparently healthy men attending the medical centre of the Institute of Directors, from whom we excluded men with signs of diabetes mellitus, or of hypertension, or of ischaemic heart disease as revealed by the electrocardiograph. The sucrose intake of the two groups was almost the same, with an average of about 120 g/d. Both the platelet adhesiveness and insulin level were higher in the patients. Also in the patients, sugar intake was significantly correlated with platelet adhesiveness and with insulin levels (measured before and after oral glucose). There was no such correlation in the group of 'healthy' individuals. (See Table 1).

REFERENCE

Szanto, S. & Yudkin, J. (1969). *Proc. Nutr. Soc.* 28, 11A.

Reportof Council on Foods and Nutr., J. A. M. A. 120:763-765. 1942.

Council on Foods and Nutrition

RECENT RESTRICTIONS ON THE SALE OF SUGAR IN THE UNITED STATES HAVE FOCUSED ATTENTION ON SOME OF THE NUTRITIONAL PROBLEMS ASSOCIATED WITH THIS COMMODITY. SOME RESTRICTION OF THE CONSUMPTION OF SUGAR MAY BE DESIRABLE FROM THE STANDPOINT OF PUBLIC HEALTH. THE CONSUMPTION OF SUGAR AND OF OTHER RELATIVELY PURE CARBOHYDRATES HAS BECOME SO GREAT DURING RECENT YEARS THAT IT PRESENTS A SERIOUS OBSTACLE TO THE IMPROVED NUTRITION OF THE GENERAL PUBLIC. THE COUNCIL DECIDED TO SUMMARIZE ITS VIEWS ON THIS SUBJECT AND HAS AUTHORIZED PUBLICATION OF THE FOLLOWING REPORT.

FRANKLIN C. BING, Secretary.

SOME NUTRITIONAL ASPECTS OF SUGAR, CANDY AND SWEETENED CARBONATED BEVERAGES

Sugar, like flour, is a highly processed food which is considered to be a staple item in the modern diet. The Council in 1939 authorized the publication by Cowgill [1] of a report entitled "The Need for the Addition of Vitamin B_1 to Staple American Foods." On the basis of the material reviewed in that report, the Council [2] adopted certain policies relative to restoration to processed foods of nutritive values lost in preparation. These policies later formed the basis for recommendations by the Food and Nutrition Board of the National Research Council, which in turn led to action taken jointly by the cereal industry and the government to make available and to promote the preferential use of the white flour and white bread which now are designated "enriched."

Accompanying tabulations (tables 1 and 2) contain comparative estimates of the thiamine provided by sample diets selected from the literature. These and other data indicate that modern diets have provided only from a third to a half of the amount of thiamine contained in earlier diets.

The report of Cowgill brought out certain facts which bear importantly on the subject of the present report on the significance of sugar in nutritional economy. Whereas cereal grains consumed in the form of undermilled flour, rich in thiamine, formerly contributed as much as 50 or 55 per cent of the calories of the average diet, the contribution of flour to the average diet of recent times has been only about 25 per cent. The place represented by the difference between the 50 or 55 per cent of all calories formerly contributed by flour, and the 25 per cent later contributed, has been taken by other foods and mainly by sugar.

LARGE CONTRIBUTION OF CALORIES AS SUGAR

The per capita annual gross consumption of sugar in the United States increased steadily from about 10 pounds (4.5 Kg.) in 1821 to 108 pounds (49 Kg.) in 1931. Since then this gross consumption has shown little change. The latest available and more accurate estimate, which corrects for industrial non-food uses of sugar, and that exported as part of processed fruit, condensed milk and other foods, places the present annual per capita consumption at about 85 pounds (38.6 Kg.). Even this amount represents 420 calories daily, or from 13 to 17 per cent of the 2,500 to 3,000 calories of an adult average diet.

Sugar as consumed in recent years, whether it originates from sugar cane or sugar beets, is for the most part highly refined sucrose. What vitamins or minerals may have been present in the cane or in the beets are almost completely removed in the processing of crystalline sugar. The finished product is practically chemically pure sucrose. Consumption of molasses, other syrups and brown sugar, compared to that of white sugar, is relatively small. Some inorganic salts can be found in them, but because of the nature of their preparation their content of thiamine is disappointing. Furthermore, not included in reports of the consumption of sugar are considerable amounts of corn starch and corn sugar (dextrose). These products are highly purified and as free from vitamins and minerals as crystalline sucrose.

1. Cowgill, G. R.: The Need for the Addition of Vitamin B_1 to Staple American Foods, J. A. M. A. 113:2146 (Dec. 9) 1939.
2. Council on Foods and Nutrition, Annual Meeting, J. A. M. A. 113:680 (Aug. 19) 1939.

These circumstances reveal that the increased use of sugar since the middle of the nineteenth century has carried responsibility of almost the same order of magnitude as the roller milling of wheat for the very great lowering of the thiamine content of the average diet. There also undoubtedly has occurred a comparable loss of nicotinic acid (niacin) in diets, although this is more difficult to estimate. Other foods than the cereal grains either contain little thiamine or are not consumed in large enough amount to contribute importantly to the daily dietary allowances of thiamine; therefore, if the average diet is to be made to provide allowances of thiamine (and niacin?) comparable to those provided by former diets, a point of attack almost as important as roller milled white flour is sugar.

INTERFERENCE WITH A SATISFACTORY SUPPLY OF THIAMINE AND OTHER VITAMINS

What could or should be done about sugar? It is technically possible to augment certain of the nutritive qualities of sugar, as was done with flour, by making restorative additions of desired nutrients. Or the aim could be at assuring a lower per capita consumption of sugar so that the contribution of sugar calories, which carry insignificant amounts of vitamins and minerals, would be curtailed to what it was before sugar became abundantly available. Before exploring either of these possibilities, other reasons for concern about the recent level of consumption of sugar may be considered.

Biochemical investigation has revealed that the oxidative activities of tissue cells, whereby energy is released from foods, depend on the presence in the cells of respiratory enzyme systems. It also has been shown that the vitamins thiamine, niacin and riboflavin represent indispensable constituents of the major enzyme systems concerned with oxidation of carbohydrate (dextrose). When deficiency exists in the supply of thiamine the oxidation of sugar is impeded to such a degree that products of its incomplete oxidation can readily be demonstrated in the blood. If the tissues possess ample reserves of vitamins no harm is done by ingesting carbohydrate, but, since sugar makes no contribution to such reserves, the vitamins required must come from other foods. It follows that when the vitamin poor constituents of a diet sufficiently outweigh the vitamin providing constituents, a situation is created from which deficiency disease will logically result. Such a situation can readily develop.

TABLE 1.—*Analysis of English Diets for Adult Men* *

	Calories	Protein, Gm.	Calcium, Gm.	Iron, Mg.	Vitamin A, International Units	Ascorbic Acid, Mg.	Thiamine, Mg.
Middle class diet today	3,810	110	0.6	12.3	5,170	70	1.2
Poverty diet today	3,000	78	0.3	8.4	300	15	0.06†
Middle (artisan) class diet, 1840	2,130	185	0.2	45	1,220	0	1.77
Navy ration, 1811	2,750	110	0.7	18	2,600	0	3.16
St. Bartholomew's Hospital, 1580	2,600	80	1.9	13	5,100	10	1.89
Meat-eating classes, fifteenth century	3,650	250	1.3	80	7,000	7	3.30
Peasant diet, fifteenth century	3,300	140	1.2	21	1,700	10-20	4.20
Recommended by Food and Nutrition Board ‡	3,000	70	0.8	12	5,000	75	1.8

* From J. C. Drummond's "The Englishman's Food," London, J. Cape, 1940.

† A diet containing as little thiamine as this (0.06 mg. per thousand calories) provokes symptoms of severe athiaminosis. See Williams, Mason, Smith and Wilder.[4]

‡ For moderate activity.

Experiments were performed by Williams and his associates[3] with diets constructed to simulate the hypothetic average American diet. That is to say, the calories from unenriched white flour contributed 30 per cent of all the calories and those from sugar and other vitamin free foods 10 and 5 per cent, respectively. These diets, fed for several months, provoked the symptoms and chemical disturbances of athiaminosis.

3. Williams, R. D.; Mason, H. L.; Smith, B. F., and Wilder, R. M.: Induced Thiamine (Vitamin B_1) Deficiency and the Thiamine Requirement of Man: Further Observations, Arch. Int. Med. 69: 721 (May) 1942.

The "average" American diet thus appears to be inadequate except when its foods, other than its content of flour and sugar, are very wisely chosen. The situation is one in which the necessity for discriminating selection of foods is greater than can be viewed with equanimity. Unless all flour and all bakers' bread becomes enriched, provision of thiamine and niacin will continue to be less than enough to provide an adequate factor

TABLE 2.—*Low Income American Diets of 1926 and 1851 (for Adult Men)*

Working Man's Diet of 1926 *	Gm.	Low Income Diet of 1851 †	Gm.
Bread and cereals	300	Flour	338
Sugar and sweets	61.5	Sugar	57
Fats	53	Butter	27
Lean meat	295	Meat	192
Milk	278	Milk	60
Potatoes	210	Potatoes	400
Other vegetables and fruits	35.6		

* Calculated from data in appendix O, Cummings, R. O.: The American and His Food, ed. 2, Chicago, University of Chicago Press, 1931. Such a diet provides approximately 2,740 calories, 92 Gm. of protein, 0.61 Gm. of calcium, 13 mg. of iron, 1,771 international units of vitamin A, 62.5 mg. of ascorbic acid and 1.4 mg. of thiamine.

† Calculated from a fifth of the values given in Weekly Food Budget for a Family of Five, Philadelphia, 1851, appendix F, Cummings, R. O.: The American and His Food. Such a diet provides approximately 2,340 calories, 92 Gm. of protein, 0.95 Gm. of calcium, 24 mg. of iron, 1,924 international units of vitamin A, 45 mg. of ascorbic acid and 2.53 mg. of thiamine. The bread winner of the family probably received 2,700 calories. If he did, values for all nutrients should be increased by about 20 per cent, thus raising the allowance of thiamine to 3.0 mg.

‡ No allowance made for loss of thiamine in handling and cooking foods. Such losses are believed to lower the thiamine content of the foods as ingested by as much as 90 per cent.

of safety, and even with flour and bread enriched the large amount of sugar probably interferes with a content in the average diet of satisfactory amounts of thiamine and several other vitamins and minerals.

USE OF SUGAR WITH OTHER FOODS ENCOURAGED

The suggestion of enriching sugar as is done with flour, by adding the vitamins essential for the oxidation of carbohydrate, is not regarded with favor. Critics of enrichment of flour have objected that many vitamins are removed from flour in its milling, and only thiamine and niacin are restored. The apprehension has been expressed that such imbalanced restoration may be harmful. In the case of flour this criticism can successfully be answered. The other B vitamins of wheat appear to be more evenly distributed through the grain and thus are lost much less in milling. However, in the case of sugar only traces of vitamins survive the process of refining.

The suggestion has been offered that sugar could be made more nutritious by combining it with the minerals and vitamins of milk.[4] This could be accomplished by enriching it with deproteinized whey, which would provide the vitamins of the entire B complex and also minerals. Also if sugar could be always used only as a means of making highly nutritious foods like milk or whey more appetizing, much less odium would attach to it than does. Some candies contain appreciable amounts of powdered milk. Other candies carry nuts. Some cake is rich in egg and milk. Malted milk and chocolate flavored skimmed milk drinks provide considerable nutritive value from the milk or skimmed milk which they contain. When sugar is consumed it would be well if it was taken in the form of mixtures such as these that bear important nutritive values. But even here one is essentially "diluting with calories" the food which is sweetened.

Accurate information is not available as to what proportion of the total use of sugar is for use with other foods. However, much sugar is consumed in candies, some prepared desserts and sweetened beverages, which carry nothing of nutritional significance except sugar. Estimates of the consumption of candy show that it may be as great as 16 pounds (7.3 Kg.) per person each year. Much of this candy is consumed by children and adolescents. Figures are available on sales of sweetened beverages.[5] They indicate that manufacturers of such beverages

4. Wilder, R. M.: Nutrition in the United States: A Program for the Present Emergency and the Future, Ann. Int. Med. 14: 2189 (June) 1941.

5. N. W. Ayer & Son, Inc.: Personal communication to the Council

produced over eighteen billion 6 ounce bottles of soft drinks in 1939. It is also admitted that since 1939 the consumption of soft drinks has increased by from 20 to 30 per cent. From such data it appears that the per capita consumption of soft drinks must be in the neighborhood of more than three bottles a week per capita. According to data published by the Foodstuffs Division of the U. S. Department of Commerce[6] the amount of sugar contained in "beverages, nonalcoholic" during 1939 constituted only 2.4 per cent of that used for all purposes. However, this figure does not include sugar added to beverages at the point of sale; and, on the basis of the data already mentioned concerning sales of sweetened beverages, the amount of sugar thus used figures out at approximately 12 per cent of the total. It seems obvious that, regardless of the method used to estimate the amount of sugar consumed as soft drinks, one obtains a result that is definitely undesirable from the standpoint of the nation's nutritional welfare.

WAYS AND MEANS OF LOWERING CONSUMPTION

The alternate approach to the nutritional problem created by sugar would be through effort directed at lowering its consumption. Because of war conditions this now is being done. Sugar rationing and its benefits to health will probably remain in effect for the duration of the war. The resulting benefit will be greater if, in rationing, priority is given to use of sugar as a sweetening agent for more nutritious foods. This has been recommended. The Food and Nutrition Board of the National Research Council has taken the position (1) that even a substantial curtailment of sugar is likely not to injure the nutrition of the American people, while other available foods can replace calories eliminated by sugar restriction; (2) that the use of sugar where small percentages make the more nutritious foods, such as bread and dairy products, more appetizing deserves high priority and should not be restricted; (3) that the use of sugar for preserving or flavoring other foods as in canning should be favored, and (4) that very considerable reduction of sugar in products which carry no other nutrients in considerable amounts, such as candy and soft drinks, would certainly not be deleterious to the nutrition of the public. The Council on Foods and Nutrition concurs in these views, which regrettably have not as yet been adopted in official rulings on the allocation of sugar.

Physicians presumably will continue to advise against the use of sugar between meals. Such advice should logically apply to the consumption of sweetened beverages as well as to the use of candy. Likewise action may be taken, as has been done with alcoholic drinks, to control the advertising of products, like candy and soft drinks, which tend to be used excessively by many persons to the detriment of health. There is merit also to the suggestion of Roberts[7] that attempts be made through school boards to place a zone around school buildings in which the sale of candy and soft drinks would be prohibited.

Current views as to the exact cause of dental caries are numerous and divergent. Published summaries of findings and conclusions relating to the causes and control of this disease[8] reveal that many investigators attribute harmful effects to the excessive consumption of highly refined carbohydrates. Without question, overuse of sugars and starches will lessen the ingestion of foods which are needed for the maintenance of normal nutrition. Faulty nutrition is not desirable from the standpoint of the teeth or of the other body tissues. It is therefore to be expected that dentists as well as physicians will continue to advise against excessive use of sugar in the diet.

EXCESSIVE USE OF SUGAR BY CHILDREN AND WAR WORKERS DEPLORED

The figures given for the per capita annual consumption of sugar, and those for sales of carbonated beverages, are average figures. People, however, do not eat averages, and diets vary from those containing very little to those containing very much of any item, be it butter, bread or milk. A disproportionate amount of candy and soft drinks is consumed by children, and the advertising of candy and soft drinks usually makes a play for sales to children. The Council has received numerous inquiries from teachers and others asking for guidance in the problem presented by sales of sweetened carbonated beverages in the schools. The anxiety expressed in such letters has mostly been on the score of the caffeine content of some of these beverages. The use of caffeine by children is not considered wise, but equally undesirable, as the writers of some of the letters have sensed, is the fact that the use of sweetened drinks and candies displaces the use of other foods. The sweetened drinks tend to replace milk, and candies the solid foods—meats, vegetables, fruits and grain products—that make up a nourishing meal.

It is obvious that a school lunch suffers gross deterioration when the beverage chosen in place of milk is a solution of sugar in flavored water. It also is generally conceded that excessive sugar eating between meals, or sugar eaten in smaller amounts within an hour of the regular mealtime, impairs the appetite for food at meals.

Another place where a disproportionate consumption of sugar in the form of candies and sweetened carbonated beverages may lead to incalculable harm is the industrial plant engaged in war production. The Committee on Nutrition in Industry[9] has reported that, in the great majority of these plants, food dispensaries, where they exist, are operated on the "we give the men what they want" policy, and that candy, pies, cakes and soft drinks are apt to constitute too great a proportion of their stock. The same report contains the following quotation from Haggard and Greenberg,[10] who emphasized that food taken between meals is an integral part of the diet as a whole. They stated:

"The energy content of the food given at two between meal feedings (in industrial plants) might amount to 300 to 400 calories and thus constitute 15 per cent of the total intake of food. It was further evident that the diets of many of the employees were actually deficient in vitamins and minerals, or verged on such deficiency. Supplementary feedings with foods containing only carbohydrate further exaggerated these deficiencies. This feature of supplementary feedings constitutes the only valid criticism against eating 'between meals,' which otherwise serves to alleviate much tiredness and decreased productiveness in factory workers."

To obviate such criticism of between meal feedings the food selected, including beverages, should make satisfactory provision of minerals and vitamins as well as of calories.

Between-meal consumption of sugar in the armed forces is less disadvantageous than it is in industry, because the rations in the army and the navy are closely supervised and provide a liberal supply of vitamins. However, even with soldiers and sailors supplied with satisfactory rations, the tendency to say in effect "Let's give the boys what they want. It isn't going to hurt them," if allowed to go to the extent of permitting indiscriminate use of soft drinks and candy, will undermine efficiency. Indiscriminate and uncontrolled supply of poor food for between-meal eating cannot be condoned with impunity anywhere.

COUNCIL OPINION

In view of the several considerations here recounted, it is the opinion of the Council that the present restrictions in the use of sugar will help improve the nutritive quality of American diets. From the health point of view it is desirable especially to have restriction of such use of sugar as is represented by consumption of sweetened carbonated beverages and forms of candy which are of low nutritional value. The Council believes it would be in the interest of the public health for all practical means to be taken to limit consumption of sugar in any form in which it fails to be combined with significant proportions of other foods of high nutritive quality.

6. Sugar and Molasses Trade of the United States in 1939. Prepared in the Foodstuffs Division of the U. S. Department of Commerce, February 1940.

7. Roberts, Lydia J.: Cutting Down on Candy, Hygeia 2: 411 (July) 1924.

8. American Dental Association: Dental Caries, ed. 2, 1941.

9. Committee on Nutrition in Industry, National Research Council: The Food and Nutrition of Industrial Workers in Wartime, National Research Council Reprint and Circular Series, No. 111, April 1942.

10. Haggard, H. H., and Greenberg, L. A.: The Selection of Foods for Between Meal Feeding in Industry, J. Am. Dietet. A. 17: 753 (Oct.) 1941.

Joint FAO/WHO Food Standards Programme
CODEX ALIMENTARIUS COMMISSION
CAC/RS 4-1969

RECOMMENDED INTERNATIONAL STANDARD FOR WHITE SUGAR

FOOD AND AGRICULTURE ORGANIZATION
OF THE UNITED NATIONS
WORLD HEALTH ORGANIZATION

Food and Agriculture Organization of The United Nations.
(World Health Organization) CAC/RS 4-1969. pp1-10.

INTRODUCTION

The FAO/WHO Codex Alimentarius Commission was established to implement the Joint FAO/WHO Food Standards Programme. Membership of the Commission comprises those Member Nations and Associate Members of FAO and/or WHO which have notified the Organizations of their wish to be considered as Members. By 1 May 1970, 75 countries had become Members of the Commission. Other countries which participate in the work of the Commission or of its subsidiary bodies in an observer capacity are expected to become Members in the near future.

The purpose of the Joint FAO/WHO Food Standards Programme is to protect the health of consumers and to ensure fair practices in the food trade; to promote coordination of all food standards work undertaken by international governmental and non-governmental organizations; to determine priorities and initiate and guide the preparation of draft standards through and with the aid of appropriate organizations to finalize standards and after acceptance by governments, publish them in a Codex Alimentarius either as regional or world-wide standards.

The Commission has held seven session, the most recent being in April 1970. At the sixth session, the Commission adopted as a recommended standard to be sent to all Member Nations and Associate Members of FAO and/or WHO a world-wide Standard for White Sugar (1). Members of the Commission are requested to notify the Secretariat of the Codex Alimentarius Commission - Joint FAO/WHO Food Standards Programme, FAO, Rome - of their acceptance of the recommended standard in accordance with the acceptance procedure for Codex Commodity Standards contained in paragraph 4 of the General Principles of the Codex Alimentarius. An extract from the General Principles containing this procedure is contained in the Appendix to this publication. The full text of the General Principles of the Codex Alimentarius is contained in the Second Edition of the Commission's Procedural Manual (Ref. No. CX 8/7 - 1969). Member Nations and Associate

(1) A complete list of standards for sugars adopted by the Codex Alimentarius Commission and sent to governments for acceptance is given on page 11.

Members of FAO and/or WHO which are not Members of the Commission are also invited to notify the Secretariat if they wish to accept the recommended standard. The recommended standard will be published in the Codex Alimentarius as a world-wide Codex standard when the Commission determines that it is appropriate to do so in the light of acceptances received.

The methods of analysis and sampling referred to in this standard are not necessarily intended to replace routine methods of analysis and sampling, but are for acceptance and use as international referee methods of analysis and sampling by governments for the settlement of international disputes.

CAC/RS 4-1969

RECOMMENDED INTERNATIONAL STANDARD FOR WHITE SUGAR

1. SCOPE

This standard applies to white sugar except that paragraph 3.1.4 (Loss on drying) does not apply to white sugar in lump or cube form or to crystal candy sugar (crystal korizato) or to rock sugar (korizato).

2. DESCRIPTION

White sugar is purified and crystallized sucrose (saccharose).

3. ESSENTIAL COMPOSITION AND QUALITY FACTORS

3.1 Specification A

3.1.1	Polarization	not less than 99.7°S
3.1.2	Invert sugar content	not more than 0.04% m/m
3.1.3	Conductivity ash	not more than 0.04% m/m
3.1.4	Loss on drying (3 hours at 105°C)	not more than 0.1% m/m
3.1.5	Colour	not more than 60 ICUMSA units

3.2 Specification B

3.2.1	Polarization	not less than 99.5°S
3.2.2	Invert sugar content	not more than 0.1% m/m
3.2.3	Conductivity ash	not more than 0.1% m/m
3.2.4	Loss on drying (3 hours at 105°C)	not more than 0.1% m/m
3.2.5	Colour	not more than 150 ICUMSA units

4. FOOD ADDITIVES

4.1 Sulphur dioxide

Specification A	not more than 20 mg/kg
Specification B	not more than 70 mg/kg

5. CONTAMINANTS

Specifications A and B

5.1 Arsenic (As)	not more than 1 mg/kg
5.2 Copper (Cu)	not more than 2 mg/kg
5.3 Lead (Pb)	not more than 2 mg/kg (temporarily endorsed)

6. HYGIENE

It is recommended that the products covered by the provisions of this Standard be prepared in accordance with the appropriate sections of the General Principles of Food Hygiene recommended by the Codex Alimentarius Commission (Ref. Nº CAC/RCP 1-1969).

7. LABELLING

In addition to Sections 1, 2, 4 and 6.1 of the General Standard for the Labelling of Prepackaged Foods (Ref. Nº CAC/RS 1-1965), the following specific provisions apply:

7.1 The Name of the Food

7.1.1 All products designated as white sugar without qualification must conform to Specifications 'A' of Sections 3 and 4, and the provisions of Sections 5 and 6 of this Standard, and products not conforming may not be so designated.

7.1.2 Products conforming to Specifications 'B' of Sections 3 and 4 but not conforming to Specifications 'A' of these Sections may be designated plantation white sugar, mill white sugar or any equivalent name accepted for such sugar in the country in which it is sold, or any designation which is not misleading and which does not include the word white.

7.1.3 All products designated plantation white sugar or mill white sugar must conform to Specification 'B' of Sections 3 and 4 and the provisions of Sections 5 and 6, and products not conforming may not be so designated.

7.1.4 Where the designations plantation white sugar or mill white sugar or any other equivalent name incorporating the word white is used, the word white shall not be given undue prominence in relation to the words plantation or mill or to any other word in such name.

7.2 Net Contents

The net contents shall be declared by weight in either the metric ("Système International" units) or avoirdupois or both systems of measurement, as required by the country in which the product is sold.

7.3 Name and Address

The name and address of the manufacturer, packer, distributor, importer, exporter or vendor of the product shall be declared.

7.4 Country of Origin

7.4.1 The country of origin of the product shal be declared if its omission would mislead or deceive the consumer.

7.4.2 When the product undergoes processing in a second country which changes its nature, the country in which the processing is performed shall be considered to be the country of origin for the purposes of labelling.

8. METHODS OF ANALYSIS AND SAMPLING

The methods of analysis and sampling referred to hereunder are international referee methods.

8.1 Determination of polarization

According to the FAO/WHO Codex Alimentarius Method (FAO/WHO Codex Alimentarius Methods of Analysis for Sugars, CAC/RM 7-1969, Determination of polarization). Results are expressed in degrees S.

8.2 Determination of invert sugar content

8.2.1 For products with invert sugar content between 0.05 and 0.3% m/m

According to the Berlin Institute Method (ICUMSA Methods of Sugar Analysis, 1964, p. 85). Results are expressed as % m/m invert sugar.

8.2.2 For products with invert sugar content above 0.05% m/m

According to the Lane and Eynon Volumetric Method (ICUMSA Methods of Sugar Analysis, 1964, p. 13). Results are expressed as % m/m invert sugar.

8.3 Determination of conductivity ash

According to the FAO/WHO Codex Alimentarius Method (FAO/WHO Codex Alimentarius Methods of Analysis for Sugars, CAC/RM 8-1969, Determination of conductivity ash). Results are expressed as % m/m conductivity ash.

8.4 Determination of loss on drying

According to the FAO/WHO Codex Alimentarius Method (FAO/WHO Codex Alimentarius Methods of Analysis for Sugars, CAC/RM 3-1969, Determination of loss on drying at 105°C for 3 hours). Results are expressed as % m/m loss on drying.

8.5 Determination of colour

According to the FAO/WHO Codex Alimentarius Method (FAO/WHO Codex Alimentarius Methods of Analysis for Sugars, CAC/RM 6-1969, Determination of colour). Results are expressed in ICUMSA units.

8.6 Determination of sulphur dioxide

According to the FAO/WHO Codex Alimentarius Method (FAO/WHO Codex Alimentarius Methods of Analysis for Sugars, CAC/RM 5-1969, Determination of sulphur dioxide (according to [illegible] and Oldfield method)). Results are expressed as mg SO_2/kg.

8.7 Determination of arsenic

According to the colorimetric (silver diethyldithiocarbamate) method of the Association of Official Analytical Chemists (Official Methods - AOAC, 1965, 24.011 - 24.014, 24.016 - 24.017, 24.006 - 24.008). Results are expressed as mg arsenic/kg.

8.8 Determination of copper

According to the ICUMSA method (ICUMSA Methods of Sugar Analysis, 1964, p.106, b. Copper). Results are expressed as mg copper/kg.

8.9 Determination of lead

According to the ICUMSA 'wet ashing' method (ICUMSA Methods of Sugar Analysis, 1964, p.48. c. 'Wet-ashing' Procedure for low-grade Products). Results are expressed as mg lead/kg.

- - - - -

SELECTED BIBLIOGRAPHY

- International Commission for Uniform Methods of Sugar Analysis (ICUMSA), 23 Avenue d'Iena, Paris 16ème, France:

  - Report of the Proceedings of the Twelfth Session held at the National Bureau of Standards and at the Shoreham Hotel, Washington, D.C., U.S.A., from 2 to 6 June 1956.

  - Report of the Proceedings of the Thirteenth Session held at the University of Hamburg and at the Atlantic Hotel, Hamburg, Germany, from 26 to 31 August 1962.

  - Report of the Proceedings of the Fourteenth Session held at the A/S De Danske Sukkerfabrikker and at Ingeniørhuset, Copenhagen, Denmark, from 22 to 27 May 1966.

  - ICUMSA Methods of Sugar Analysis, edited by H.C.S. De Whalley, Elsevier Publishing Co. Amsterdam, London, New York, 1964.

2. Official Methods of Analysis of the Association of Official Agricultural Chemists (10th ed. 1965), A.O.A.C., P.O.B. 540, Benjamin Franklin Station, Washington 4, D.C.

3. Standard Analytical Methods of the Member Companies of Corn Industries Research Foundation Inc. prepared by the Analytical Procedures Subcommittee of the Technical Advisory Committee, 2nd Ed. - 1001, Connecticut Ave., Washington, D.C. 200036.

4. United States Pharmacopoeia; 17th Revn. (New York), 1965, Mack Publishing Co., Easton, PA.

- - - - -

Nutr. Rev. 28:228-229, 1970.

SUGAR AND CORONARY HEART DISEASE

Cigarette smokers use more sugared hot beverages than nonsmokers, an observation which confounds a superficial association between sugar and coronary heart disease.

Among the nutritional diversions of the last decade has been the hypothesis, promulgated mainly by J. Yudkin (Yudkin and J. Morland, *Am. J. Clin. Nutrition* **20**, *503* (*1967*)), that dietary sugar is a major factor in the contemporary epidemic of coronary heart disease (CHD). Historically, and even today, nutrition has been susceptible to anecdotal or tenuously founded claims in regard to special (either beneficial or deleterious) properties of particular foods or nutrients—claims easily raised and publicized but rather more difficult to test in a rigorous manner. When coupled with CHD, a disease with a strong nutritional component but with the additional complex involvement of other host and environmental factors, one is faced with a very formidable combination indeed, for there is the continuing temptation to look for simplistic, single-factor solutions which will at once explain away the many apparent mysteries of atherosclerosis and its clinical complications.

The basis for the sugar-CHD link derived from the association between the parallel secular trends both in sugar consumption and in mortality from CHD over the past 50 years and was supported by comparisons of sugar use among survivors of myocardial infarction and controls (Yudkin and J. Roddy, *Lancet* **2**, *6* (*1964*)). Recently, two more carefully designed population studies have cast some doubt on the concept of a direct link and have suggested that cigarette smoking may be the operative intermediary factor. It should be pointed out that there still are inconsistencies in the epidemiologic evidence linking smoking to CHD (C. C. Seltzer, *J. Am. Med. Assn.* **203**, *193* (*1968*); *Arch. Environ. Health* **20**, *418* (*1970*)).

One of these two newer reports (P. C. Elwood *et al.*, *Lancet* 1, *1014* (*1970*)) is based on two community surveys. Among women, 2,834 of 3,149 subjects 20 through 64 years of age in a defined population group were questioned about age, body weight, sugar consumption, the symptoms of angina pectoris, and cigarette smoking habits. Smokers used more sugar per day than nonsmokers and women with angina pectoris used about 10 g. per day more sugar than those without. The latter difference was not statistically significant.

nor was sugar usage consistently related to the amount of cigarette smoking. As a check on the reliability of the estimates of sugar consumption, 100 women were resurveyed two years later and the simple correlation coefficient between the two estimates was 0.8 ($p < 0.001$).

The male community survey included 344 men (79 per cent of an age and occupation stratified sample who had been examined in 1958). Electrocardiograms as well as the same questions used in the female sample were collected. Neither on the basis of angina pectoris nor on the basis of the electrocardiographic patterns was there any demonstrable relationship between consumption of sugar and CHD. Here too, sugar consumption per day was greater among pack-a-day smokers (103 g.) than nonsmokers (83 g.). Thus both of these studies suggested a relationship between the use of sugar and of smoking habits, a confounding factor in relating sugar to CHD.

A companion article by A. E. Bennett, R. Doll, and R. W. Howell (*Lancet* 1, *1011 (1970)*) lends further support to this observation. This report is actually of three separate surveys. Among male hospitalized patients aged 35 through 64, mean daily sugar use in hot beverages ranged from 34.6 g. among nonsmokers to 53.4 among smokers of 15 or more cigarettes per day. Data on 2,483 males 40 through 54 years of age (representing a 90 per cent response of a random sample of industrial workers) related smoking habits to a questionnaire-derived estimate of sugar consumption. Total daily sugar use was higher among cigarette smokers than nonsmokers, the difference being accounted for by sugar consumption in hot drinks. Lastly, a random sample of 453 males 26 through 65 years of age residing in a defined area also yielded data showing that heavy cigarette smokers used more hot beverages (8.1 cups per day compared with 5.2 in light smokers and 4.8 in nonsmokers) as well as more sugar per cup (9.1 g. among heavy smokers, 7.8 g. among light smokers). Considering all of these studies together, there was noted a significant association ($p < 0.05$) between cigarette smoking and both hot beverage use and sugar consumption.

Various speculations about this association were raised by Bennett *et al.* Are both smoking and tea or coffee drinking related to some underlying psychological need or are they merely socializing habits shared by chance? Could oral-pharyngeal dryness in smokers lead to a greater use of beverages? Does the greater use of sugar per cup of hot beverage among smokers suggest an altered taste threshold for sweetness? Whatever the mechanisms involved, it now appears that the apparent association between CHD and sugar is neither as straightforward nor as clear as originally hypothesized.

Further reproduction
prohibited without consent
of copyright owner

Reproduced by permission
of the copyright owner

Fd Cosmet. Toxicol. Vol. 9, pp. 439–444. Pergamon Press 1971. Printed in Great Britain

ARTICLES OF GENERAL INTEREST

SWEET MYSTERY OF LIFE

Whether a high intake of sugar in the form of sucrose, or possibly lactose, has any effect upon the development of atherosclerosis and the coronary heart disease which is the clinical outcome of advanced atherosclerosis is a vexed question, which has occupied many columns of the medical journals for some years. It has been observed that there is a tendency for coronary heart disease to increase in frequency as more and more refined sucrose takes the place of starch in the diet, and it has been suggested that a person's consumption of sucrose may be a more important factor in determining his liability to myocardial infarction than is his consumption of fats (*Cited in F.C.T.* 1966, **4**, 445).

Observations in animals

A high sucrose content in the diet has been shown to be associated with pathological renal and hepatic changes which shorten the lifespan of rats (Dalderup & Visser, *Nature, Lond.* 1969, **222**, 1050). Weanling rats were fed a diet in which sucrose replaced 15% of the calories normally provided by starch. Up to the age of 1 yr there was no obvious difference between these animals and the controls fed starch, but after another 3 months males on the sucrose-enriched diet showed a higher death rate than females on either diet or than male controls. The average lifespan (in days) of males given sucrose was 486 compared with 566 in controls, and of females 582 compared with 607. At death, all rats showed a yellowish, swollen liver with fatty changes but intact structure, and enlarged kidneys with pitted surfaces, sometimes showing cysts, and a friable texture. Microscopically, a severe glomerulonephritis was evident. Dalderup & Visser (*loc. cit.*) concluded that these pathological developments were accelerated by the high level of sucrose in the diet.

Further studies of the effect on the tissue content of substituting sugars for starch in the diet of male rats have been reported by Ahrens *et al.* (*Experientia* 1970, **26**, 37). The animals studied in this case were weanlings and received 50% of their calories as fat and 12% as carbohydrate for 30 days. Carbohydrate was either entirely starch or was a mixture of starch with lactose (3·1%) and sucrose, glucose and fructose (each 1·55%), but both diets had a calorific value of 5·07 kcal./g. A further variable was introduced by giving half of each group treadmill exercise daily for 15 min, the other half remaining sedentary. Neither diet nor exercise had any significant effect on gain in body weight or body composition, and the calorie intake (about 40 kcal./day) and serum cholesterol level were similar in all groups. However, the increase in tissue cholesterol showed no correlation with weight gain in rats fed the starch-only diet, but was highly dependent on weight gain in those given mixed carbohydrates. Tissue cholesterol was not influenced by the mild exercise performed by some of the rats.

There is some evidence that trivalent chromium (Cr) may be an essential trace metal for carbohydrate metabolism (*Cited in F.C.T.* 1969, **7**, 270), and it has been suggested that

atherosclerosis and some forms of diabetes mellitus may result partly from Cr deficiency (*Nutrition Reviews* 1969, **27**, 185). Since the refining of sucrose involves a reduction in its Cr content, the findings of Schroeder (*J. Nutr.* 1969, **97**, 237) in rats fed crude or refined sugar may be relevant to the possible atherosclerotic influence of sucrose. After 5 months, male rats fed a low-Cr diet plus brown or raw sugar (containing 0·12 and 0·06 ppm Cr respectively) had lower serum cholesterol levels than others fed refined white sugar (containing 0·02 ppm Cr). During the next 5–6 months the mean serum cholesterol level in rats fed white sugar rose from 74 to 104 mg/100 ml. No such rise appeared in other rats fed brown sugar, or white sugar with a Cr (5 ppm) supplement. Serum glucose levels in both sexes were lower in the groups fed brown than in those fed white sugar. The addition of Cr supplements had only slight effects on serum glucose in young rats fed white sugar and none in older males, although it slightly lowered the levels in older females. Schroeder (*loc. cit.*) considers that abnormalities of cholesterol metabolism in man may depend on a Cr deficiency induced by the consumption of refined sugar.

Human studies

In the final analysis, human studies must prove a more reliable guide to the factors involved in atherosclerosis. Macdonald *et al.* (*Br. J. Nutr.* 1970, **24**, 413) have reported that triglycerides and phospholipids in the serum of young men given sucrose vary with the mode of consumption. Groups maintained on a low-sucrose diet for 18 days were given in addition either 7 g sucrose/kg/day in two doses ('gorging'), or the same total amount 'nibbled' in six lots daily, in a cross-over trial. Serum triglyceride levels rose significantly on days 3 and 7 of gorging, and on days 14 and 18 of nibbling, while serum phospholipids felt significantly throughout the nibbling but not the gorging period. Both regimens produced a significant fall in serum albumin. The method of sucrose ingestion did not affect total serum-cholesterol levels, however. Nibbling, but not gorging, improved glucose tolerance in these young men, a finding which suggests that a different pattern of insulin release may account for the effect of sucrose on triglycerides.

Mann *et al.* (*Lancet* 1970, **i**, 870) carried out a study of 51 middle-aged healthy office workers, of whom one third cut sucrose completely out of their diet and replaced it with alternative foods, another third substituted half their dietary starch with other foods, and the rest continued with their accustomed diet, for 5 months. In the low-sucrose group, serum triglyceride levels fell by a mean of 22%, remaining low until the diet was discontinued and correlating with a loss in weight. Those men with initially high serum triglyceride levels showed a greater reduction than their companions with lower initial levels. Serum cholesterol levels in the group fell by an average of 4%. Subjects in the reduced-starch group showed no significant change in serum lipids, and did not lose weight except in the first 2 wk. They found their regimen difficult to maintain, however.

It has been claimed that there is no correlation between sucrose consumption and serum levels of cholesterol, β-lipoprotein, uric acid or other clinically significant biological values (Howell & Wilson, *Br. med. J.* 1969, **4**, 559). Moreover, a study carried out at Chandigarh, North India, where the intake of sucrose is about 1 oz daily (compared with about 3 oz in the UK) has revealed an incidence of coronary heart disease similar to that in some parts of the USA (Walker, *Lancet* 1969, **ii**, 1071). Malmros (*ibid* 1970, **i**, 94) has pointed out that the consumption of even large amounts of sucrose does not substantially increase serum cholesterol in man. Three volunteers on a diet providing 3000 kcal. and containing 450–500 g sucrose showed no increase in serum cholesterol, although serum triglycerides and the

pre-β-lipoprotein fraction rose. Artificially induced hypercholesterolaemia will induce atheromatosis, but the evidence suggests that, compared with that of cholesterol and natural saturated fats, the atherogenic potential of sucrose is mild. Yudkin (*ibid* 1970, I, 418) has retorted that some people may take less than 20 g sucrose daily, and some more than 400 g, but that it is unlikely that many take some 40% of their calories almost entirely as saturated or as polyunsaturated fat. Although no relationship has been established between levels of sucrose consumption and of serum cholesterol, an increased consumption of saturated fat does not invariably raise serum cholesterol levels either. However, a moderate increase in sucrose consumption can raise serum levels of cholesterol, phospholipid and triglycerides, and in some subjects may also raise plasma insulin levels, alter glucose tolerance and increase platelet adhesiveness.

Effect on blood platelets

The effect of sucrose on platelet adhesiveness cannot be discounted as a factor in myocardial infarction. Szanto & Yudkin (*Nature, Lond.* 1970, **225**, 4671 studied electrophoretic mobility of platelets as a more precise measurement than adhesiveness, in three subjects who developed hyperinsulinism when given sucrose and in three who did not. All subjects substituted sucrose for starch in their diet, as far as was possible, for 2 wk. Initially both groups showed similar, and normal, platelet mobility. In the hyperinsulinaemic subjects, platelet mobility had changed at the end of the 2-wk test period and resembled that observed in atherosclerotic subjects; after a further 14 days on a normal diet, the platelets had partly, but not completely, reverted to their previous characteristics. Plasma taken from these subjects during the hyperinsulinaemic stage and added to platelet concentrates from the normal subjects caused a similar alteration in the mobility of the normal platelets. However, the addition of insulin itself to platelets did not alter their mobility. Increases in platelet adhesiveness averaging 35% have been observed in young men given an atherogenic diet of 3600 kcal. daily, containing 50% carbohydrate, 30% fat, 11% protein and 9% alcohol with cholesterol supplements (Akinyanju *et al. ibid* 1968, **218**, 975). One group received all carbohydrate as sucrose during the first week of the test, while the other group took cooked wheat starch instead, and during the second week the diets were interchanged. Both diets produced a similar rise in serum cholesterol, which persisted throughout the 2-wk test period. Serum triglycerides showed a small rise in concentration during the week on sucrose and a small fall in concentration during the week on starch, with somewhat larger changes when the diets were taken in the reverse order. All the subjects taking sucrose but none taking starch excreted sucrose in their urine. It may be concluded from this that contrary to widely-held belief, sucrose may not need to be completely hydrolysed before it can be absorbed from the gut.

A smoke-screen round the problem

A study by Paul *et al.* (*Lancet* 1968, ii, 1049) of the sucrose intake of 42 men who had developed myocardial infarction, and of 24 others who died of coronary heart disease indicated that those who consumed more sucrose developed more coronary disease; at the same time, however, they also drank more coffee and smoked more cigarettes. Another study covering a group of hospital patients, men employed by UKAEA, and a random sample of the population of a London borough has been reported by Bennett *et al.* (*ibid* 1970, i, 1011) and has indicated quite distinctly that cigarette smoking goes hand in hand with a high sucrose intake, mostly in beverages. The non-smokers consumed a mean of 24·6 g sucrose

daily, whereas smokers of 15 or more cigarettes/day consumed 53·4 g and the consumption of sucrose generally reflected the number of cigarettes smoked. Elwood *et al.* (*ibid* 1970, i, 1014) found no statistically significant difference between the sucrose intake of women in a Welsh mining valley who suffered from angina pectoris and the intake of those who did not. Among the men, those with electrocardiographic evidence of ischaemic heart disease took on average less sucrose than the others; but it was again found, in both sexes, that those who took most sucrose were also those who smoked most cigarettes. Elwood *et al.* (*loc. cit.*) therefore support the suggestion that there is an association between the consumption of sucrose and of tobacco which might give the appearance of an association between sucrose intake and ischaemic heart disease. However, Yudkin (*ibid* 1970, i, 1111) refuses to recognize any logic in the argument that because a high sugar consumption and heavy smoking both go with a tendency to develop ischaemic heart disease, and because heavy smoking tends to be accompanied by heavy sugar consumption, smoking, but not a high sugar intake, is a cause of the disease. The basis for such a conclusion certainly seems, at present, too confusing to be convincing.

Effect of sucrose on drug absorption

Leaving the highly unsatisfactory evidence that sucrose may or may not be a factor in atherosclerotic heart disease, we may look into a rather different aspect of its effects. Kato *et al.* (*Jap. J. Pharmac.* 1969, **19**, 331) have reported that when rabbits were given an intragastric dose of aminopyrine in water, the serum concentration of the drug rose more rapidly, and to nearly twice the peak value, than when the dose was given in a syrup composed (in g/litre) of sucrose (400), sorbitol (50), sodium cyclamate (4) and saccharin (0·5). The same phenomenon occurred when dipyrone (sulpyrine) was given, the concentration of its serum metabolite, *N*-methyl-4-aminophenazone, being higher and rising more quickly with an aqueous solution than a syrup. The explanation was found after the gastric emptying rates of 50% sucrose and 1% carboxymethylcellulose (CMC) were measured in rats, using phenol red indicator to demonstrate the entry of the solutions into the small intestine. CMC emptied more rapidly from the stomach than did sucrose. Moreover, pentobarbitone narcosis and strychnine convulsions were retarded when the drugs were given in a sucrose syrup, compared with the rate of reaction when they were given in CMC. The high osmotic pressure of the sucrose solution was probably an important factor in the rate of gastric emptying.

Here we have a significant piece of evidence showing that the action of drugs can be modified by the concurrent taking of sucrose. Whether this factor can be brought to bear on the controversy over the possible atherosclerotic potential of sugar remains to be seen.

FOOD AND DRUG Research LABORATORIES, INC.

WAVERLY DIVISION
Route 17
P.O. Box 107
Waverly, New York 14892
(607) 565-2531

FINAL REPORT

Submitted to: DHEW/Public Health Service
Food and Drug Administration CA-272
5600 Fishers Lane-Room 5C-13
Rockville, Maryland 20852

Date October 22, 1973

Laboratory No. 1765 b
Contract No. FDA 71-260

Sample: White crystalline material

Marking: FDA 71-76 (Pure cane sugar; sucrose)

Examination Requested: Teratologic evaluation of FDA 71-76 in mice.

Procedure: See Appendix I

Results: See Tables 1 through 4 and Appendix II

Conclusion:

On the basis of the data presented herein, the following conclusion appears to be warranted:

"The administration of up to 1600 mg/kg (body weight) of the test material to pregnant mice for 10 consecutive days had no clearly discernible effect on nidation or on maternal or fetal survival. The number of abnormalities seen in either soft or skeletal tissues of the test groups did not differ from the number occurring spontaneously in the sham-treated controls."

FOOD AND DRUG RESEARCH LABORATORIES, INC.

Kenneth Morgareidge

Kenneth Morgareidge, Ph.D.
Vice President

This report is submitted for the exclusive use of the person, partnership, or corporation to whom it is addressed, and neither the report nor the name of these Laboratories nor of any members of its staff, may be used in connection with the advertising or sale of any product or process without written authorization.

FOOD AND DRUG RESEARCH LABORATORIES, INC.

Groups: 301 through 306

Material: FDA 71-76

Date: September 21, 1973

Laboratory No.: 1765 b

Table 1

Fate Summary
(Mice)

Group	Material	Dose** mg/kg	Total		Surviving at Term	
			Mated	Pregnant	Total	Pregnant[1]
301	Sham	0.0	25	22	25	22
302	Aspirin*	150.0	25	25	25	25
303	FDA 71-76	16.0	25	22	25	22
304	FDA 71-76	74.3	25	22	25	22
305	FDA 71-76	345.0	25	24	25	24
306	FDA 71-76	1600.0	25	21	25	21

* Positive Control: 150.0 mg/kg

** Administered as a water solution (10 ml per kg of body weight)

1) Includes all dams examined at term

Group: 301 through 306

Material: FDA 71-76

Table 2
Reproduction Data
(Mice)

Date: September 21, 197[illegible]

Laboratory No. 1765 b

Group:	301	302	303	304	305	306
Dose (mg/kg):	Sham	Aspirin**	16.0	74.3	345.0	1600.0
Pregnancies						
Total No.	22	25	22	22	24	21
Died or Aborted (before Day 17)	0	0	0	0	0	0
To term (on Day 17)	22	25	22	22	24	21
Live Litters						
Total No.*	22	25	22	22	24	21
Implant Sites						
Total No.	243	258	232	247	276	223
Average/dam*	11.1	10.3	10.6	11.2	11.5	10.6
Resorptions						
Total No.*	7	9	8	10	10	4
Dams with 1 or more sites resorbed	6	7	5	5	7	2
Dams with all sites resorbed	--	--	--	--	--	--
Per cent partial resorptions	27.3	28.0	22.7	22.7	29.2	9.52
Per cent complete resorptions	--	--	--	--	--	--
Live Fetuses						
Total No.	234	249	221	234	264	217
Average/dam*	10.6	10.0	10.1	10.6	11.0	10.3
Sex ratio (M/F)	0.93	0.92	0.91	0.92	1.02	1.13
Dead Fetuses						
Total*	2	--	3	3	2	2
Dams with 1 or more dead	2	--	3	3	2	2
Dams with all dead	--	--	--	--	--	--
Per cent partial dead	9.09	--	13.6	13.6	8.33	9.52
Per cent all dead	--	--	--	--	--	--
Average Fetus Weight, g	0.92	0.85	0.94	0.97	0.95	0.96

* Includes only those dams examined at term.

** Positive Control: 150.0 mg/kg

FOOD AND DRUG RESEARCH LABORATORIES, INC.

Groups 301 through 306 | Laboratory No. 1765 b

Table 3

Material FDA 71-76 | Date September 21, 1973

Summary of Skeletal Findings*
(Mice)

Findings / Group No.:	301	302	303	304	305	306
Dose (mg/kg):	Sham	Aspirin**	16.0	74.3	345.0	1600.0
Live Fetuses Examined (at term)	161/22	175/25	154/22	162/22	183/24	152/21
Sternebrae						
Incomplete oss.	13/7	42/18	9/7	12/6	27/12	9/8
Scrambled						
Bipartite	6/5	10/8	6/4	3/3	5/4	2/2
Fused						
Extra						1/1
Missing	9/7	37/13	7/3	15/8	11/7	4/4
Other						
Ribs						
Incomplete oss.						
Fused/split						
Wavy	1/1	1/1				
Less than 12						
More than 13	44/15	41/19	35/17	45/17	28/15	48/17
Other						
Vertebrae						
Incomplete oss.	3/3	17/8	1/1		2/2	
Scrambled						
Fused						
Extra ctrs. oss.						
Scoliosis						
Tail defects						
Other						
Skull						
Incomplete closure						
Missing		1/1				
Craniostosis						
Other; facial bones, inc.		1/1	1/1		1/1	
Extremities						
Incomplete oss.	3/3	12/8	2/1		1/1	1/1
Missing						
Extra						
Miscellaneous						
Hyoid; missing	33/12	64/21	27/8	13/6	32/11	10/7
Hyoid; reduced	8/7	15/12	16/8	18/9	19/12	22/13

* Numerator=Number of fetuses affected; Denominator=Number of litters affected.

** Positive control: 150.0 mg/kg

FOOD AND DRUG RESEARCH LABORATORIES, INC.

Groups 301 through 306 Date September 21, 1973

Material FDA 71-76 Laboratory No. 1765 b

Table 3-a

Summary of Soft Tissue Abnormalities

(Mice)

Group	Material	Dose Level mg/kg	Dam	Number of Pups	Description
302	Aspirin*	150.0	A 5975	1	Hepatomegaly
			A 5990	2	Umbilical hernia
305	FDA 71-76	345.0	B 5065	1	Meningoencephalocele; ablepharia
306	FDA 71-76	1600.0	B 5091	1	Gastroschisis

* Positive Control: 150.0 mg/kg

FOOD AND DRUG RESEARCH LABORATORIES, INC.

Groups 301 through 306

Species Mice

Date September 21, 1973

Laboratory No. 1765 b

Table 4

Average Body Weights*

Group	Material	Dose Level	Day 0	Day 6	Day 11	Day 15	Day 17**
		mg/kg	g				
301	Sham	0.0	25.6	29.5	35.0	41.6	47.7 (22)
302	Aspirin***	150.0	25.0	28.6	32.7	40.5	43.8 (25)
303	FDA 71-76	16.0	24.9	28.8	33.4	40.5	46.8 (22)
304	FDA 71-76	74.3	26.4	29.8	34.4	42.0	47.4 (22)
305	FDA 71-76	345.0	27.8	31.5	38.1	45.0	51.0 (24)
306	FDA 71-76	1600.0	25.1	29.2	34.4	41.2	47.7 (21)

* Of pregnant dams
** Number of surviving dams in parentheses (c.f. Table 1)
*** Positive control: 150.0 mg/kg

Appendix I

Teratology Study in Mice

Virgin adult female albino CD-1 outbred mice were gang-housed in disposable plastic cages in temperature and humidity-controlled quarters with free access to food and fresh tap water. They were mated with young adult males, and observation of the vaginal sperm plug was considered Day 0 of gestation. Beginning on Day 6 and continuing daily through Day 15 of gestation, the females were dosed with the indicated dosages by oral intubation; the controls were sham treated.

Body weights were recorded on Days 0, 6, 11, 15, and 17 of gestation. All animals were observed daily for appearance and behavior with particular attention to food consumption and weight, in order to rule out any abnormalities which may have occurred as a result of anorexic effects in the pregnant female animal.

On Day 17 all dams were subjected to Caesarean section under surgical anesthesia, and the numbers of implantation sites, resorption sites, and live and dead fetuses were recorded. The body weights of the live pups were also recorded. The urogenital tract of each dam was examined in detail for anatomical normality.

All fetuses were examined grossly for the presence of external congenital abnormalities. One-third of the fetuses of each litter underwent detailed visceral examinations employing the Wilson technique. The remaining two-thirds were cleared in potassium hydroxide (KOH), stained with alizarin red S dye and examined for skeletal defects.

FOOD AND DRUG RESEARCH LABORATORIES, INC.

Group 301

Material Sham

Dose 0.0 mg/kg

Appendix II

Reproduction Data in Mice (Individual)

Date September 21, 1973

Laboratory No. 1765

Dam No.	Fate*	Implant Sites	Fetuses Alive	Fetuses Dead	Sex M	Sex F	Resorption Sites	Average Fetus Weight (g)	Remarks
S 5966	NP	0						----	
S 5967	P	12	12		6	6		1.00	
S 5968	P	12	12		6	6		0.92	
S 5969	P	13	12	1	5	7		0.92	
S 5970	NP	0						----	
S 5971	P	11	11		5	6		0.95	
S 5972	P	10	10		5	5		1.01	
S 5973	P	13	13		6	7		0.92	
S 5974	P	9	9		5	4		0.90	
S 5975	NP	0						----	
S 5976	P	12	11		4	7	1	0.78	
S 5977	P	13	12		6	6	1	0.79	
S 5978	P	10	10		6	4		0.88	
S 5979	P	11	11		5	6		0.88	
S 5980	P	13	13		6	7		0.88	
S 5981	P	10	9		4	5	1	0.98	
S 5982	P	11	9		3	6	2	0.92	
S 5983	P	8	8		4	4		0.95	
S 5984	P	10	9		5	4	1	0.86	
S 5985	P	9	9		4	5		0.93	
S 5986	P	12	12		7	5		0.96	
S 5987	P	12	12		6	6		0.88	
S 5988	P	9	9		4	5		0.90	
S 5989	P	10	9	1	3	6		0.89	
S 5990	P	13	12		8	4	1	1.07	

* P = Pregnant; NP = Not Pregnant

FOOD AND DRUG RESEARCH LABORATORIES, INC.

Group 302

Material Aspirin

Dose 150.0 mg/kg

Appendix II

Reproduction Data in Mice (Individual)

Date September 21, 1973

Laboratory No. 1765

Dam No.	Fate*	Implant Sites	Fetuses Alive	Fetuses Dead	Sex M	Sex F	Resorption Sites	Average Fetus Weight (g)	Remarks
A 5966	P	10	10		6	4		0.95	
A 5967	P	9	9		5	4		0.80	
A 5968	P	11	11		5	6		1.02	
A 5969	P	11	11		5	6		0.87	
A 5970	P	9	8		4	4	1	0.89	
A 5971	P	10	10		6	4		0.84	
A 5972	P	9	9		4	5		0.80	
A 5973	P	10	10		6	4		0.74	
A 5974	P	7	6		3	3	1	0.96	
A 5975	P	13	12		5	7	1	0.75	
A 5976	P	11	11		6	5		0.84	
A 5977	P	12	12		5	7		0.79	
A 5978	P	11	11		4	7		0.76	
A 5979	P	13	13		5	8		0.84	
A 5980	P	8	7		3	4	1	0.96	
A 5981	P	14	14		8	6		0.73	
A 5982	P	8	8		4	4		0.80	
A 5983	P	11	10		5	5	1	0.87	
A 5984	P	11	11		6	5		0.99	
A 5985	P	10	10		1	9		0.86	
A 5986	P	10	9		6	3	1	0.93	
A 5987	P	9	9		4	5		0.93	
A 5988	P	10	10		3	7		0.83	
A 5989	P	11	11		7	4		0.75	
A 5990	P	10	7		3	4	3	0.79	

* P = Pregnant; NP = Not Pregnant

FOOD AND DRUG RESEARCH LABORATORIES, INC.

Group 303

Material FDA 71-76

Dose 16.0 mg/kg

Appendix II

Reproduction Data in Mice (Individual)

Date September 21, 1973

Laboratory No. 1765 b

Dam No.	Fate*	Implant Sites	Fetuses Alive	Fetuses Dead	Sex M	Sex F	Resorption Sites	Average Fetus Weight (g)	Remarks
B 5001	P	12	12		6	6		0.94	
B 5002	NP	0						----	
B 5003	P	9	8	1	4	4		1.18	
B 5004	P	12	12		5	7		0.90	
B 5005	P	10	10		5	5		0.95	
B 5006	P	10	10		7	3		0.93	
B 5007	P	11	11		7	4		0.91	
B 5008	P	11	11		4	7		0.87	
B 5009	NP	0						----	
B 5010	P	11	9		5	4	2	0.95	
B 5011	P	7	7		3	4		0.85	
B 5012	P	11	9		4	5	2	0.92	
B 5013	P	12	11	1	5	6		0.84	
B 5014	P	10	10		4	6		0.89	
B 5015	P	13	11		5	6	2	0.88	
B 5016	P	14	14		4	10		0.80	
B 5017	P	14	14		8	6		0.99	
B 5018	P	8	8		3	5		0.91	
B 5019	NP	0						----	
B 5020	P	11	10		5	5	1	0.93	
B 5021	P	7	7		4	3		1.26	
B 5022	P	9	8		5	3	1	1.01	
B 5023	P	10	10		4	6		0.87	
B 5024	P	12	12		4	8		0.88	
B 5025	P	8	7	1	4	3		0.97	

* P = Pregnant; NP = Not Pregnant

FOOD AND DRUG RESEARCH LABORATORIES, INC.

Group 304

Material FDA 71-76

Dose 74.3 mg/kg

Appendix II

Reproduction Data in Mice (Individual)

Date September 21, 1973

Laboratory No. 1765 b

Dam No.	Fate*	Implant Sites	Fetuses Alive	Fetuses Dead	Sex M	Sex F	Resorption Sites	Average Fetus Weight (g)	Remarks
B 5031	NP	0						----	
B 5032	P	6	1		1	0	5	1.81	
B 5033	P	12	12		7	5		1.00	
B 5034	P	15	15		6	9		0.79	
B 5035	NP	0						----	
B 5036	P	10	9		5	4	1	1.09	
B 5037	P	10	8		3	5	2	0.87	
B 5038	P	12	12		4	8		1.29	
B 5039	P	8	8		6	2		0.89	
B 5040	P	16	16		6	10		0.82	
B 5041	P	9	8		3	5	1	0.81	
B 5042	P	14	14		6	8		0.81	
B 5043	NP	0						----	
B 5044	P	11	11		2	9		0.88	
B 5045	P	10	9	1	5	4		0.87	
B 5046	P	12	12		5	7		1.03	
B 5047	P	11	10	1	5	5		0.88	
B 5048	P	10	9	1	5	4		0.88	
B 5049	P	11	11		8	3		0.98	
B 5050	P	12	12		4	8		1.05	
B 5051	P	12	12		8	4		0.80	
B 5052	P	11	10		5	5	1	0.94	
B 5053	P	12	12		6	6		0.89	
B 5054	P	10	10		5	5		0.89	
B 5055	P	13	13		7	6		0.96	

* P = Pregnant; NP = Not Pregnant

FOOD AND DRUG RESEARCH LABORATORIES, INC.

Group 305

Material FDA 71-76

Dose 345.0 mg/kg

Appendix II

Reproduction Data in Mice (Individual)

Date September 21, 1973

Laboratory No. 1765 b

Dam No.	Fate*	Implant Sites	Fetuses Alive	Fetuses Dead	Sex M	Sex F	Resorption Sites	Average Fetus Weight (g)	Remarks
B 5061	P	12	12		7	5		0.94	
B 5062	P	8	8		4	4		1.00	
B 5063	P	6	6		3	3		1.00	
B 5064	P	11	10	1	5	5		0.94	
B 5065	P	10	10		7	3		1.00	
B 5066	NP	0						----	
B 5067	P	13	12		6	6	1	0.86	
B 5068	P	11	11		6	5		0.92	
B 5069	P	15	15		9	6		0.81	
B 5070	P	14	13	1	6	7		0.90	
B 5071	P	11	11		5	6		1.04	
B 5072	P	13	13		8	5		0.97	
B 5073	P	12	12		4	8		0.97	
B 5074	P	13	13		6	7		1.09	
B 5075	P	11	10		5	5	1	0.96	
B 5076	P	9	9		5	4		1.04	
B 5077	P	10	10		5	5		0.93	
B 5078	P	15	12		7	5	3	1.01	
B 5079	P	14	13		7	6	1	0.88	
B 5080	P	11	10		4	6	1	1.01	
B 5081	P	11	11		6	5		0.88	
B 5082	P	15	15		5	10		0.91	
B 5083	P	10	9		4	5	1	0.95	
B 5084	P	12	12		7	5		0.88	
B 5085	P	9	7		2	5	2	0.85	

* P = Pregnant; NP = Not Pregnant

FOOD AND DRUG Research LABORATORIES, INC.

WAVERLY DIVISION
Route 17
P.O. Box 107
Waverly, New York 14892
(607) 566-2931

FINAL REPORT

Submitted to: DHEW/Public Health Service
Food and Drug Administration CA-272
5600 Fishers Lane-Room 5C-13
Rockville, Maryland 20852

Date October 22, 1973

Laboratory No. 1766 b
Contract No. FDA 71-260

Sample: White crystalline material

Marking: FDA 71-76 (Pure cane sugar; sucrose)

Examination Requested: Teratologic evaluation of FDA 71-76 in rats

Procedure: See Appendix I

Results: See Tables 1 through 4 and Appendix II

Conclusion:

On the basis of the data presented herein, the following conclusion appears to be warranted:

"The administration of up to 1600 mg/kg (body weight) of the test material to pregnant rats for 10 consecutive days had no clearly discernible effect on nidation or on maternal or fetal survival. The number of abnormalities seen in either soft or skeletal tissues of the test groups did not differ from the number occurring spontaneously in the sham-treated controls."

FOOD AND DRUG RESEARCH LABORATORIES, INC

Kenneth Morgareidge

Kenneth Morgareidge, Ph.D.
Vice President

This report is submitted for the exclusive use of the person, partnership, or corporation to whom it is addressed, and neither the report nor the name of these Laboratories nor of any members of its staff, may be used in connection with the advertising or sale of any product or process without written authorization.

FOOD AND DRUG RESEAR LABORATORIES, INC.

Groups: 301 through 306

Material: FDA 71-76

Date: September 21, 1973

Laboratory No.: 1766 b

Table 1

Fate Summary
(Rats)

Group	Material	Dose ** mg/kg	Total		Surviving at Term	
			Mated	Pregnant	Total	Pregnant[1]
301	Sham	0.0	25	21	25	21
302	Aspirin*	250.0	25	23	25	23
303	FDA 71-76	16.0	25	25	25	25
304	FDA 71-76	74.3	25	24	25	24
305	FDA 71-76	345.0	25	22	25	22
306	FDA 71-76	1600.0	25	22	25	22

* Positive Control: 250.0 mg/kg

** Administered as a water solution (See Appendix I)

1) Includes all dams examined at term

FOOD AND DRUG RESEARCH LABORATORIES, INC.

Groups 301 through 306 | Laboratory No. 1766 b

Table 3

Material FDA 71-76 | Date September 21, 1973

Summary of Skeletal Findings*
(Rats)

Findings / Group No.:	301	302	303	304	305	306
Dose (mg/kg):	Sham	Aspirin**	16.0	74.3	345.0	1600.0
Live Fetuses Examined (at term)	154/21	146/23	200/25	183/24	174/22	163/22
Sternebrae						
Incomplete oss.	41/16	78/22	37/16	54/17	52/19	61/19
Scrambled	1/1	1/1				
Bipartite		21/11	3/3	1/1	3/3	4/4
Fused						
Extra						
Missing	8/6	138/23	2/2	6/5	10/6	19/8
Other						
Ribs						
Incomplete oss.		3/3				
Fused/split		9/6				
Wavy	24/9	84/22	11/6	25/12	22/7	13/5
Less than 12		2/2			1/1	
More than 13		112/22	1/1	4/3	2/2	
Other						
Vertebrae						
Incomplete oss.	19/10	127/23	17/10	22/11	13/7	8/6
Scrambled						
Fused						
Extra ctrs. oss.						
Scoliosis						
Tail defects						
Other						
Skull						
Incomplete closure	29/13	65/18	25/12	29/12	23/9	25/14
Missing		17/8				
Craniostosis						
Other						
Extremities						
Incomplete oss.		17/8				
Missing						
Extra						
Miscellaneous						
Hyoid; missing	17/9	91/22	16/8	5/5	11/7	12/6
Hyoid; reduced	11/6	8/8	28/11	26/11	13/5	14/8

* Numerator=Number of fetuses affected; Denominator=Number of litters affected.

** Positive control: 250.0 mg/kg

roup: 301 through 306

Material: FDA 71-76

Table 2
Reproduction Data
(Rats)

Date: September 21, 1973

Laboratory No. 1766 b

Group:	301	302	303	304	305	306
Dose (mg/kg):	Sham	Aspirin**	16.0	74.3	345.0	1600.0
Pregnancies						
Total No.	21	23	25	24	22	22
Died or Aborted (before Day 20)	0	0	0	0	0	0
To term (on Day 20)	21	23	25	24	22	22
Live Litters						
Total No.*	21	23	25	24	22	22
Implant Sites						
Total No.	225	246	289	266	248	236
Average/dam*	10.7	10.7	11.6	11.1	11.3	10.7
Resorptions						
Total No.*	6	38	1	2	1	5
Dams with 1 or more sites resorbed	5	8	1	2	1	3
Dams with all sites resorbed	--	--	--	--	--	--
Per cent partial resorptions	23.8	34.8	4.00	8.33	4.55	13.6
Per cent complete resorptions	--	--	--	--	--	--
Live Fetuses						
Total No.	218	203	288	264	247	231
Average/dam*	10.4	8.83	11.5	11.0	11.2	10.5
Sex ratio (M/F)	0.91	0.75	1.09	1.08	1.09	1.14
Dead Fetuses						
Total*	1	5	--	--	--	--
Dams with 1 or more dead	1	2	--	--	--	--
Dams with all dead	--	--	--	--	--	--
Per cent partial dead	4.76	8.70	--	--	--	--
Per cent all dead	--	--	--	--	--	--
Average Fetus Weight, g	3.71	2.36	3.77	3.73	3.84	3.77

* Includes only those dams examined at term.
** Positive Control: 250.0 mg/kg

FOOD AND DRUG RESEARCH LABORATORIES, INC.

Groups 301 through 306 Date September 21, 1973

Material FDA 71-76 Laboratory No. 1766 b

Table 3-a
Summary of Soft Tissue Abnormalities
(Rats)

Group	Material	Dose Level mg/kg	Dam	Number of Pups	Description
302	Aspirin*	250.0	A 6968	2	Encephalomyelocele; exophthalmos
			A 6970	2	Encephalomyelocele
				1	Gastroschisis
			A 6974	1	Meningoencephalocele
			A 6975	2	Encephalomyelocele
				1	Exophthalmos
			A 6976	1	Gastroschisis
			A 6979	1	Encephalomyelocele; gastroschisis
			A 6980	1	Encephalomyelocele; gastroschisis
			A 6983	2	Encephalomyelocele; gastroschisis
				1	Exophthalmos
			A 6986	1	Meningoencephalocele
			A 6987	1	Encephalomyelocele; gastroschisis; exophthalmos
				2	Meningoencephalocele
305	FDA 71-76	345.0	B 6071	1	Lateral hernia

* Positive Control: 250.0 mg/kg

FOOD AND DRUG RESEARCH LABORATORIES, INC.

Groups 301 through 306

Date September 21, 1973

Species Rats

Laboratory No. 1766 b

Table 4

Average Body Weights*

Group	Material	Dose Level	Day 0	Day 6	Day 11	Day 15	Day 20**
		mg/kg	g				
301	Sham	0.0	216	221	238	268	337 (21)
302	Aspirin***	250.0	212	234	244	262	312 (23)
303	FDA 71-76	16.0	215	235	248	270	312 (25)
304	FDA 71-76	74.3	207	226	243	266	320 (24)
305	FDA 71-76	345.0	211	229	250	271	334 (22)
306	FDA 71-76	1600.0	211	225	233	268	334 (22)

* Of pregnant dams
** Number of surviving dams in parentheses (c.f. Table 1)
*** Positive control: 250.0 mg/kg

Appendix I

Teratology Study in Rats

Virgin adult female albino rats (Wistar derived stock) were individually housed in mesh bottom cages in temperature and humidity-controlled quarters with free access to food and fresh tap water. They were mated with young adult males, and observation of the vaginal sperm plug was considered Day 0 of gestation. Beginning on Day 6 and continuing daily through Day 15 of gestation, the females were dosed with the indicated dosages by oral intubations. The controls were sham treated with the vehicle at a level equivalent to the group receiving the highest test dose. The test material was prepared and doses calculated according to the following table:

Dosage (mg/kg)	Dose (ml/kg)	Concentration (mg/ml)
≤ 250	1	≤ 250
251 - 500	2	125 - 250
501 - 750	3	133 - 250
751 - 1000	4	187 - 250
1001 - 1250	5	200 - 250
1251 - 1500	6	208 - 250
1501 - 1600	6.4	235 - 250

Body weights were recorded on Days 0, 6, 11, 15, and 20 of gestation. All animals were observed daily for appearance and behavior with particular attention to food consumption and weight, in order to rule out any abnormalities which may have occurred as a result of anorexic effects in the pregnant female animal.

On Day 20 all dams were subjected to Caesarean section under surgical anesthesia, and the numbers of implantation sites, resorption sites, and live and dead fetuses were recorded. The body weights of

the live pups were also recorded. The urogenital tract of each dam was examined in detail for anatomical normality.

All fetuses were examined grossly for the presence of external congenital abnormalities. One-third of the fetuses of each litter underwent detailed visceral examinations employing the Wilson technique. The remaining two-thirds were cleared in potassium hydroxide (KOH), stained with alizarin red S dye and examined for skeletal defects.

FOOD AND DRUG RESEARCH LABORATORIES, INC.

Group 301

Material Sham

Dose 0.0 mg/kg

Appendix II

Reproduction Data in Rats (Individual)

Date September 21, 1973

Laboratory No. 1766

Dam No.	Fate*	Implant Sites	Fetuses Alive	Fetuses Dead	Sex M	Sex F	Resorption Sites	Average Fetus Weight (g)	Remarks
S 6966	P	11	11		6	5		4.01	
S 6967	P	10	10		4	6		3.62	
S 6968	P	9	9		6	3		3.57	
S 6969	P	12	12		6	6		3.79	
S 6970	NP	0						----	
S 6971	P	10	10		6	4		4.40	
S 6972	NP	0						----	
S 6973	NP	0						----	
S 6974	P	11	11		8	3		3.69	
S 6975	P	11	11		4	7		3.79	
S 6976	P	10	9		2	7	1	3.46	
S 6977	P	7	7		3	4		4.07	
S 6978	P	8	7		5	2	1	3.69	
S 6979	NP	0						----	
S 6980	P	11	11		4	7		3.67	
S 6981	P	12	10	1	4	6	1	3.58	
S 6982	P	10	8		7	1	2	3.68	
S 6983	P	14	14		7	7		3.75	
S 6984	P	13	12		5	7	1	3.45	
S 6985	P	12	12		3	9		3.77	
S 6986	P	10	10		7	3		3.68	
S 6987	P	9	9		2	7		3.65	
S 6988	P	13	13		4	9		3.51	
S 6989	P	10	10		4	6		3.29	
S 6990	P	12	12		7	5		3.83	

* P = Pregnant; NP = Not Pregnant

FOOD AND DRUG RESEARCH LABORATORIES, INC.

Group 302

Material Aspirin

Dose 250.0 mg/kg

Appendix II

Reproduction Data in Rats (Individual)

Date September 21, 1973

Laboratory No. 1766

Dam No.	Fate*	Implant Sites	Fetuses Alive	Fetuses Dead	Sex M	Sex F	Resorption Sites	Average Fetus Weight (g)	Remarks
A 6966	P	11	11		3	8		2.19	
A 6967	P	11	10		5	5	1	2.62	
A 6968	P	8	8		5	3		2.03	
A 6969	P	11	11		5	6		2.74	
A 6970	P	9	8		3	5	1	2.09	
A 6971	P	10	10		3	7		2.71	
A 6972	P	8	8		4	4		2.48	
A 6973	P	8	8		3	5		2.58	
A 6974	P	10	9	1	2	7		2.12	
A 6975	P	12	12		1	11		2.41	
A 6976	P	13	9	4	6	3		2.17	
A 6977	P	11	3		1	2	8	1.86	
A 6978	P	11	11		6	5		2.96	
A 6979	P	11	4		1	3	7	1.79	
A 6980	P	15	15		6	9		2.42	
A 6981	NP	0						----	
A 6982	P	13	11		6	5	2	3.77	
A 6983	P	13	7		5	2	6	1.84	
A 6984	P	6	4		0	4	2	2.04	
A 6985	P	11	11		4	7		2.42	
A 6986	P	11	11		6	5		1.96	
A 6987	P	9	9		6	3		2.00	
A 6988	P	14	3		2	1	11	2.41	
A 6989	P	10	10		4	6		2.71	
A 6990	NP	0						----	

* P = Pregnant; NP = Not Pregnant

FOOD AND DRUG RESEARCH LABORATORIES, INC.

Group 303

Material FDA 71-76

Dose 16.0 mg/kg

Appendix II

Reproduction Data in Rats (Individual)

Date September 21, 1973

Laboratory No. 1766 b

Dam No.	Fate*	Implant Sites	Fetuses Alive	Fetuses Dead	Sex M	Sex F	Resorption Sites	Average Fetus Weight (g)	Remarks
B 6001	P	8	8		4	4		3.80	
B 6002	P	13	13		9	4		3.52	
B 6003	P	10	10		7	3		3.74	
B 6004	P	14	14		8	6		3.85	
B 6005	P	10	10		7	3		3.68	
B 6006	P	8	8		5	3		3.84	
B 6007	P	12	12		5	7		4.05	
B 6008	P	12	12		6	6		3.74	
B 6009	P	9	9		6	3		3.69	
B 6010	P	9	9		2	7		3.79	
B 6011	P	13	13		7	6		3.59	
B 6012	P	14	14		6	8		3.68	
B 6013	P	12	12		8	4		3.88	
B 6014	P	13	13		6	7		3.65	
B 6015	P	12	12		4	8		3.80	
B 6016	P	11	11		8	3		3.97	
B 6017	P	13	13		4	9		3.85	
B 6018	P	11	11		7	4		3.62	
B 6019	P	11	11		6	5		3.52	
B 6020	P	12	11		3	8	1	3.94	
B 6021	P	13	13		7	6		3.72	
B 6022	P	13	13		5	8		3.70	
B 6023	P	12	12		5	7		3.87	
B 6024	P	12	12		7	5		3.93	
B 6025	P	12	12		8	4		3.72	

* P = Pregnant; NP = Not Pregnant

FOOD AND DRUG RESEARCH LABORATORIES, INC.

Group 304

Material FDA 71-76

Dose 74.3 mg/kg

Appendix II

Reproduction Data in Rats (Individual)

Date September 21, 1973

Laboratory No. 1766 b

Dam No.	Fate*	Implant Sites	Fetuses Alive	Fetuses Dead	Sex M	Sex F	Resorption Sites	Average Fetus Weight (g)	Remarks
B 6031	P	12	12		6	6		4.02	
B 6032	P	13	12		4	8	1	3.26	
B 6033	P	12	12		8	4		3.72	
B 6034	P	10	10		4	6		4.10	
B 6035	P	11	11		8	3		3.76	
B 6036	NP	0						----	
B 6037	P	4	4		2	2		4.02	
B 6038	P	12	12		6	6		3.85	
B 6039	P	9	9		5	4		3.55	
B 6040	P	11	11		7	4		3.41	
B 6041	P	14	13		10	3	1	3.74	
B 6042	P	13	13		5	8		3.64	
B 6043	P	11	11		8	3		3.66	
B 6044	P	9	9		8	1		3.78	
B 6045	P	12	12		6	6		4.04	
B 6046	P	14	14		5	9		3.42	
B 6047	P	12	12		3	9		3.70	
B 6048	P	11	11		5	6		3.44	
B 6049	P	10	10		3	7		3.61	
B 6050	P	10	10		3	7		3.96	
B 6051	P	13	13		6	7		3.51	
B 6052	P	11	11		6	5		3.79	
B 6053	P	12	12		7	5		3.88	
B 6054	P	11	11		5	6		3.68	
B 6055	P	9	9		7	2		4.03	

* P = Pregnant; NP = Not Pregnant

FOOD AND DRUG RESEARCH LABORATORIES, INC.

Group 305

Material FDA 71-76

Dose 345.0 mg/kg

Appendix II

Reproduction Data in Rats (Individual)

Date September 21, 1973

Laboratory No. 1766 b

Dam No.	Fate*	Implant Sites	Fetuses Alive	Fetuses Dead	Sex M	Sex F	Resorption Sites	Average Fetus Weight (g)	Remarks
B 6061	P	12	11		6	5	1	3.71	
B 6062	P	11	11		4	7		3.84	
B 6063	P	14	14		9	5		3.91	
B 6064	P	9	9		5	4		3.67	
B 6065	P	7	7		4	3		4.19	
B 6066	P	11	11		8	3		4.00	
B 6067	P	10	10		6	4		3.85	
B 6068	P	10	10		4	6		3.49	
B 6069	P	10	10		5	5		3.59	
B 6070	P	11	11		5	6		3.64	
B 6071	P	11	11		4	7		3.35	
B 6072	P	12	12		9	3		4.06	
B 6073	P	11	11		5	6		4.17	
B 6074	NP	0						----	
B 6075	P	9	9		5	4		4.12	
B 6076	P	12	12		4	8		3.23	
B 6077	P	12	12		6	6		3.72	
B 6078	P	14	14		8	6		3.65	
B 6079	P	15	15		10	5		3.78	
B 6080	P	11	11		6	5		3.74	
B 6081	P	13	13		5	8		3.81	
B 6082	P	13	13		6	7		4.87	
B 6083	P	10	10		5	5		3.99	
B 6084	NP	0						----	
B 6085	NP	0						----	

* P = Pregnant; NP = Not Pregnant

FOOD AND DRUG RESEARCH LABORATORIES, INC.

Group 306

Material FDA 71-76

Dose 1600.0 mg/kg

Appendix II

Reproduction Data in Rats (Individual)

Date September 21, 1973

Laboratory No. 1766 b

Dam No.	Fate*	Implant Sites	Fetuses Alive	Fetuses Dead	Sex M	Sex F	Resorption Sites	Average Fetus Weight (g)	Remarks
B 6091	P	7	7		3	4		2.46	
B 6092	P	11	11		7	4		3.88	
B 6093	P	13	13		8	5		4.11	
B 6094	NP	0						----	
B 6095	P	11	11		4	7		3.94	
B 6096	P	10	10		7	3		3.64	
B 6097	P	11	11		7	4		3.80	
B 6098	NP	0						----	
B 6099	P	6	6		3	3		3.91	
B 6100	P	11	11		5	6		4.21	
B 6101	P	13	13		6	7		3.88	
B 6102	P	12	12		9	3		3.49	
B 6103	P	10	10		3	7		3.39	
B 6104	P	11	11		6	5		3.91	
B 6105	P	11	11		4	7		3.70	
B 6106	P	11	11		4	7		4.06	
B 6107	P	14	11		7	4	3	3.64	
B 6108	P	13	13		7	6		3.49	
B 6109	P	13	12		8	4	1	3.54	
B 6110	P	9	8		3	5	1	3.52	
B 6111	P	12	12		6	6		3.25	
B 6112	NP	0						----	
B 6113	P	8	8		7	1		3.98	
B 6114	P	9	9		5	4		4.43	
B 6115	P	10	10		4	6		4.63	

* P = Pregnant; NP = Not Pregnant

3G0030

LAW OFFICES
MARKEL, HILL & BYERLEY
COMMONWEALTH BUILDING
1625 K STREET, N.W.
WASHINGTON, D.C. 20006

MICHAEL F. MARKEL
WAYNE K. HILL
WALTER E. BYERLEY

(202) 628-5950

RECEIVED
JUL 10 9 32 AM '73

July 9, 1973

Mr. Alan T. Spiher, Jr.
Chief, GRAS Review Branch
Division of Food and Color Additives
Bureau of Foods
Food and Drug Administration
200 C Street, S.W.
Washington, D.C. 20204

Dear Mr. Spiher:

Acting as counsel for Hokkaido Sugar Co., Ltd., we submit herewith, in quadruplicate, its petition for affirmation that "sugar treated with the enzyme alpha-galactosidase" is generally recognized as safe (GRAS). We have discussed this petition with you informally in the past.

It should be noted that the petition submitted herewith by hand is in three Parts. Part I constitutes the petition itself and includes the required Environmental Impact Analysis Report. Part II includes all of the exhibits referred to in the petition except the safety evaluation studies. Part III consists solely of safety evaluation studies.

Your attention is directed to the fact that certain exhibits in Part II are regarded as confidential, and it is requested that you treat them accordingly.

We request your expeditious consideration of this petition and the publication of a notice concerning its filing.

Respectfully submitted,

MARKEL, HILL & BYERLEY

By Wayne K. Hill
Wayne K. Hill
Counsel for

HOKKAIDO SUGAR CO., LTD.

WKH:vr

3G0030

LAW OFFICES
MARKEL, HILL & BYERLEY
COMMONWEALTH BUILDING
1625 K STREET, N.W.
WASHINGTON, D. C. 20006

MICHAEL F. MARKEL
WAYNE K. HILL
WALTER E. BYERLEY

(202) 659-5500

September 25, 1973

Dr. W. B. Dockstader
GRAS Review Branch, BF-335
Bureau of Foods
Department of Health, Education, and Welfare
Public Health Service
Food and Drug Administration
200 C Street, S.W.
Washington, D.C. 20204

Subject: GRASP Petition No. 3G0030

Dear Dr. Dockstader:

Reference is made to the subject petition which was filed on July 13 and which was the subject of a published notice of filing in the Federal Register for August 9, 1973.

Since the formal filing of the subject petition, we have received additional data pertinent to our request for affirmation of the GRAS status of enzyme-treated beet sugar. While these data are, in part, cumulative, we feel they are pertinent and may be of interest in your further consideration of the subject petition. The data establish that there is no evidence of any enzyme contamination of the finished sucrose produced by the process covered in the subject petition.

We enclose herewith a memorandum dated August 1, 1973, signed by J. C. Linden, in which the results of assay of enzyme-treated sugar are given. Of particular significance is the fact that the enzyme is obtained from a mold source and the mold count of the enzyme-produced sugar was zero.

We also attach a description of the assay procedure.

Reference is also made to the statement in the August 9 notice of filing to the effect that final action on the subject petition will not be possible until completion of GRAS reviews of sucrose and certain other sugars. As you know, we are disturbed by this statement, and have urged that our enzyme-processed sugar be recognized as GRAS, at least on an interim basis, pending completion of the sucrose review. We continue to urge this solution rather than be subjected to an unnecessarily long delay. Any information you can give us on the progress of this petition will be appreciated. We, in turn will be happy to respond to any future inquiry concerning it.

Respectfully submitted,

MARKEL, HILL & BYERLEY

By Wayne K. Hill
Wayne K. Hill
Counsel for

HOKKAIDO SUGAR CO., LTD.

WKH/rc
Enclosures

RECEIVED
AUG 2 1973
T. J. ARMY

Loveland, Colorado August 1, 1973

TO: Milovan Bosnjak

FROM: J. C. Linden

SUBJECT: FDA Approval of Melibiase

Markel, Hill and Byerly, the lawyers concerned with the above, inquired about the assay of enzymatic and microbiological activity in the final product. Accordingly, granulated sugar was obtained from the Hokkaido Sugar Company, Ltd. and assayed following procedures submitted to them.

α-galactosidase activity per 10 g solids

less than 50 units

mesophiles per 10 g solids

	Bacteria	Mold	Yeast
mycophil pH 4	0	0	0
mycophil pH 6	14	0	0
nutrient	70	0	0
thermophilic	50	0	0

This bacteria count does not exceed accepted standards on the attached specifications. It should be noted that the enzyme is obtained from a mold source.

Mr. Byerly or Mr. Hill will be interested in receiving these results.

J. C. Linden
Senior Research Biochemist
Technical Services Laboratory

J. C. Linden
JCL/es
cc: Laslo Tot
Brooks Stein
T. J. Army

3G0030

A PETITION
FOR THE AFFIRMATION THAT SUGAR TREATED WITH THE ENZYME ALPHA-GALACTOSIDASE IS GENERALLY RECOGNIZED AS SAFE

PART I

Submitted on behalf of

Hokkaido Sugar Co., Ltd.
Tokyo, Japan

by Counsel

Markel, Hill & Byerley
1625 K Street, N. W.
Washington, D.C. 20006

Table of Contents - PART I

	Page
Subpart A - Monograph	
(1) Summary	1
(2) Description of the substances	3
(a) The end product	3
(i) Common Name	3
(ii) Chemical Name	3
(iii) Trade Name	3
(iv) Chemical Abstract Service Reg. No.	3
(v) Empirical Formula	3
(vi) Structural Formula	3
(vii) Specifications for food grade material	3
(viii) Quantitative Composition	3
(b) The enzyme	3
(i) Common Name	3
(ii) Chemical Name	3
(iii) Trade Name	3
(iv) Chemical Abstract Service Reg. No.	4
(v) Empirical Formula	4
(vi) Structural Formula	4
(vii) Specifications for food grade material	4
(viii) Quantitative Composition	4

Page

(3) Manufacturing Process---------- 5

(a) Sucrose---------- 5

(b) The Enzyme---------- 6

(4) Use---------- 8

(a) Sucrose---------- 8

(b) The Enzyme---------- 8

(5) Methods for detection of the substance in food---------- 10

(a) Sucrose---------- 10

(b) The Enzyme---------- 10

(6) Safety---------- 12

(a) Sucrose---------- 12

(b) The Enzyme---------- 12

(7) Label of the Enzyme---------- 13

Subpart B - Environmental Impact Analysis Report---------- 14

Subpart C - Exhibit List---------- 18

Part II - Non-Safety Exhibits---------- Separate Volume

Part III - Safety Evaluation Studies---------- Separate Volume

SUBPART A

MONOGRAPH

(1) Summary

Sucrose (table sugar) is a common food, used for hundreds of years throughout the world. Various processes, including the use of enzymes, have been used to produce sucrose over this period.

The purpose of this petition is to obtain affirmation that the use of an enzyme, alpha-galactosidase, in a recently discovered mold in the production of sucrose from beets does not change the GRAS status of the sucrose so obtained.

As a by-product of the production of sugar from sugar beets, a liquid known as molasses is produced. This molasses contains sucrose, which can be precipitated as a calcium saccharate out of the molasses by means of the Steffen process.

However, molasses also contains raffinose, a sugar which interferes with the crystallization of sucrose. As raffinose is precipitated as a calcium compound by the Steffen process, continued recycling of the molasses eventually raises the raffinose level to a point where it is impossible to crystallize further sucrose.

It has been discovered that the enzyme alpha-galactosidase, an endogenous enzyme contained in the mycelium of the micro-organism Mortierella vinacea var. raffinoseutilizer, hydrolyzes raffinose to sucrose and galactose. Sucrose can be crystallized

out of the solution while galactose remains in solution and eventually leaves the process in the molasses or in the Steffen waste which is sold as animal feed. Thus, the hydrolytic activity of alpha-galactosidase accomplishes two ends: (1) it directly increases the sucrose production from a given quantity of molasses, (2) it keeps the raffinose content to a level low enough to allow maximum crystallization of the sucrose.

After the molasses is subjected to the enzymatic action of alpha-galactosidase, it is screened to remove the remaining pellets of Mortierella vinacea var. raffinoseutilizer and sent directly into the Steffen process for extraction of sugar.

This screening procedure, and the subsequent processing, serves to remove all traces of both the microorganism and the enzyme from the end product, granulated sugar (sucrose). There are, therefore, no residues in the end product, sucrose. Moreover, even if residues did occur, both the microorganism and alpha-galactosidase are innocuous, as confirmed by the extensive data set forth in exhibits referred to in the safety portion of this petition and included in Part III of this petition.

All exhibits cited in the text of this petition, except the safety evaluation studies, are contained in Part II of this petition which is separately bound. The safety evaluation studies constitute a separately bound Part III to this petition.

All exhibits are tabbed for convenient reference, and a listing identifying all exhibits is contained in both Part I and Part II of this petition.

(2) Description of the Substances

(a) The end product:

(i) Common or usual name: Sugar.

(ii) Chemical name: Sucrose.

(iii) Trade name: Various.

(iv) Chemical Abstract Service Reg. No.: Not applicable.

(v) Empirical Formula: Not applicable.

(vi) Structural Formula: Not applicable.

(vii) Specifications for food grade material:

(Codex Alimentarius)

Polarization	99.7% min.
Invert Sugar	0.04% max., by wt.
Conductivity Ash	0.04% max., by wt.
Loss on Drying (3hrs. at 105°C)	0.1% max., by wt.
Color (ICUMSA units)	60 max.
Sulfur Dioxide	20 mg/kg max.
Arsenic (As)	1 mg/kg max.
Lead (Pb)	2 mg/kg max.
Copper (Cu)	2 mg/kg max.

(viii) Quantitative Composition:

Sucrose	99.75%
Water	0.02%
Oligosaccharides	0.1%
Mineral Ash	0.05%
Calcium Salts of Organic Acids	0.08%

(b) The enzyme:

(i) Common or usual name: Alpha-Galactosidase or Melibiase.

(ii) Chemical Name: alpha-D-galactoside galactohydrolase, E.C. 3.2.1.22.

(iii) Trade name: MELIBIA-D.

(iv) Chemical Abstract Service Reg. No.: Not applicable.

(v) Empirical Formula: Not applicable.

(vi) Structural Formula: Not applicable.

(vii) Specifications for food grade material:
(See Exhibit 1 and 2);

Pellet size:	20 to 50 mesh
Moisture:	8% to 10%
Activity:	$1.5(10^6)$ to $2.0(10^6)$ u/gm.
Heavy Metals:	0.63 to 1.4 ppm.

(viii) Quantitative Composition: Not applicable.

(3) Manufacturing Process

(a) Sucrose:

As is shown in Exhibit 3, the enzyme process is inserted into the sugar-making process at the beginning of the Steffen process.

Exhibit 4, items 1 through 4, describes in detail the enzyme process. The process consists of a continuous flow of molasses into a multi-chamber enzyme reactor (See Schematic in Exhibit 5). The Brix, pH, and temperature of the molasses are carefully controlled to provide optimum conditions for hydrolysis of the raffinose by the enzyme. From 240 to 300 tons of molasses per day can be handled in the reactor.

Each chamber of the reactor is separated from the preceding chamber by a wall, at the top of which is a screen. At the bottom of each chamber is a stirrer to keep the enzyme in suspension. As each chamber fills with molasses, the molasses flows over the wall, through the screen and into the next chamber.

Each day, 200 to 310 lbs of enzyme pellets (depending upon the amount of molasses processed and the efficiency of hydrolysis) is added to a chamber of the reactor.

Upon exit from the reactor, the molasses is screened a final time through a finer (100 mesh) screen to remove the spent enzyme (approximately 300 lbs daily) which is analyzed for

activity and weighed daily to determine efficiency of the reactor. All of the enzyme screened from the reactor effluent stream is discarded.

The molasses then enters the Steffen house for processing (Exhibit 3 and Exhibit 4, items 5 through 9). In the Steffen house saccharate cake is separated from the Steffen waste and sent into the sugar factory for further processing (Exhibit 3 and Exhibit 4, items 10 through 12). The Steffen waste has its calcium precipitated and discarded, while the clear filtrate is evaporated, added to dried beet pulp, and the mixture sold as animal feed.

Thus, the total amount of enzyme used daily is 200 to 310 pounds in 240 to 300 tons of Steffen molasses, a level of approximately .05%.

(b) The enzyme

Alpha-galactosidase is an endogenous enzyme produced by the microorganism Mortierella vinacea var. raffinoseutilizer (ATCC No. 20034). The organism is produced by a pure-culture process, described more fully in the patent for Method of Reducing Raffinose Content of Beet Molasses, attached as Exhibit 6. The enzyme is characterized by its activity. See Exhibit 1 for the range of values of twelve different batches. Three different reports of analysis of the enzyme for moisture and heavy metal content are presented as Exhibit 2.

Morphologically, M. vinacea var. raffinoseutilizer is identical to the more common M. vinacea Dixon-Stewart (type strain). See Exhibit 6 for physical description. However, the variety raffinoseutilizer can easily be distinguished from the Dixon-Stewart strain by its production of alpha-galactosidase on selected media, and by the influence of the nitrogen source in the medium to the sugar utilization of the organism. See Exhibit 7.

(4) Use

(a) Sucrose: The sucrose produced by this process is suitable for all uses of sucrose.

(b) The enzyme: At present, the only known use for this enzyme, and the only use relevant to this petition, is in the reduction of raffinose in sugar beet molasses.

The hydrolysis of raffinose in sugar beet molasses was first carried out under laboratory conditions. Results of this experiment are reported in Exhibit 8.

Use of the process in actual production has been accomplished, and the results are reported in Exhibit 9.

Finally, both the method (Exhibit 6) and the reactor (Exhibit 10) for continuous hydrolysis of raffinose in Steffen molasses has been patented.

(5) Methods for detection of the substance in food

(a) Sucrose: Not applicable

(b) The enzyme:

The detection of residues, if any, of the enzyme in intermediate solutions and the final product is by enzymatic activity.

The general method for measurement of activity of alpha-galactosidase is set forth in Exhibits 11 and 12. These describe the procedure using alpha-galactosidase in enzyme pellets.

The same procedure can be used to analyze for enzymatic activity in intermediate stages of processing Steffen molasses to sugar, if preparatory steps are taken. See Exhibit 13 for these steps.

The enzyme process has been studied in pilot plant experiments which demonstrate that there is no likelihood that the enzyme activity will survive the sugar production process. See Exhibit 14. Sensitivity limits of the method (50 units) in thin juice were determined (Exhibit 15). The absence of residual enzyme in the intermediate (thin juice) stage of processing assures the absence of residual enzyme in the end product.

The same method can be used to analyze for enzymatic

activity in sucrose, if the following preparatory steps are taken:

1. Granulated sugar (10g) is dissolved in 10 - 20 ml of 0.05 M phosphate buffer at 6.8 pH and 4 - 10°C.

2. The sucrose solution is transferred to dialysis tubing and dialyzed 24 hours against 5 l of 0.05 M phosphate buffer at 6.8 pH and 4 - 10°C. The volume of the solution inside the dialysis bag increases as sucrose is replaced by dialyzing buffer.

3. To the solution from inside the dialysis bag, saturated ammonium sulfate solution is slowly added with stirring to 55% saturation.

4. The solution is allowed to stand at least 2 hours at 4 - 10°C.

5. The precipitated proteins if present would be sedimented by centrifugation at 4 - 10°C and 30,000 x g for 30 minutes.

6. The supernatant is carefully decanted and the pellet dissolved in a minimum volume of 0.05 M phosphate buffer at pH 6.8 and 4 - 10°C.

7. The solutions are transferred to dialysis tubing and dialyzed 2 hours against 5 l of 0.05 M phosphate buffer at pH 6.8 and 4 - 10°C.

8. The dialyzed solution is assayed for alpha-galactosidase activity by the standard method except that 2.0 ml of the reaction supernatant (rather than 0.5 ml normally) is used for the glucose oxidase determination.

Pilot plant procedures for sample collection and analytical steps are described in Exhibit 16. Microbiological analysis procedures are set forth in Exhibit 17.

(6) Safety

(a) Sucrose:

The safety and functionality of sucrose in food is unquestioned.

The sucrose produced by this method is identical to sucrose produced by the ordinary refining process. Indeed, sucrose produced by this method goes through every step of the ordinary refining process. The only difference is the addition of one step: conversion of raffinose to sucrose.

(b) The enzyme :

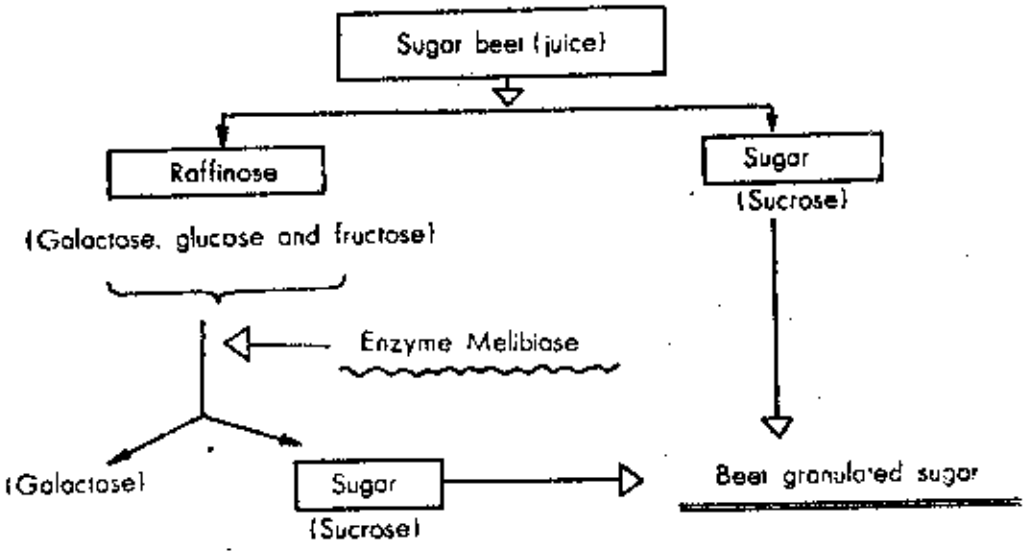
The filtration of the Steffen molasses after hydrolysis of raffinose with alpha-galactosidase, and the process of extracting sucrose from the molasses, eliminates all alpha-galactosidase from the end product, sucrose. Indeed, as is shown in Exhibits 13 and 14, no alpha-galactosidase remains in the intermediate steps of processing.

Moreover, alpha-galactosidase is so innocuous that direct intubation of 10g/kg of animal weight of the enzyme into the stomachs of rats resulted in only mild, transient effects. See Exhibit 22. The overall safety of the enzyme itself, as well as sugar and molasses produced employing the enzyme process, have been confirmed by the studies presented as exhibits in Part III of this petition. See Exhibits 18, 19, 20, 21, 22, 23, 24, 25, 26, and 27.

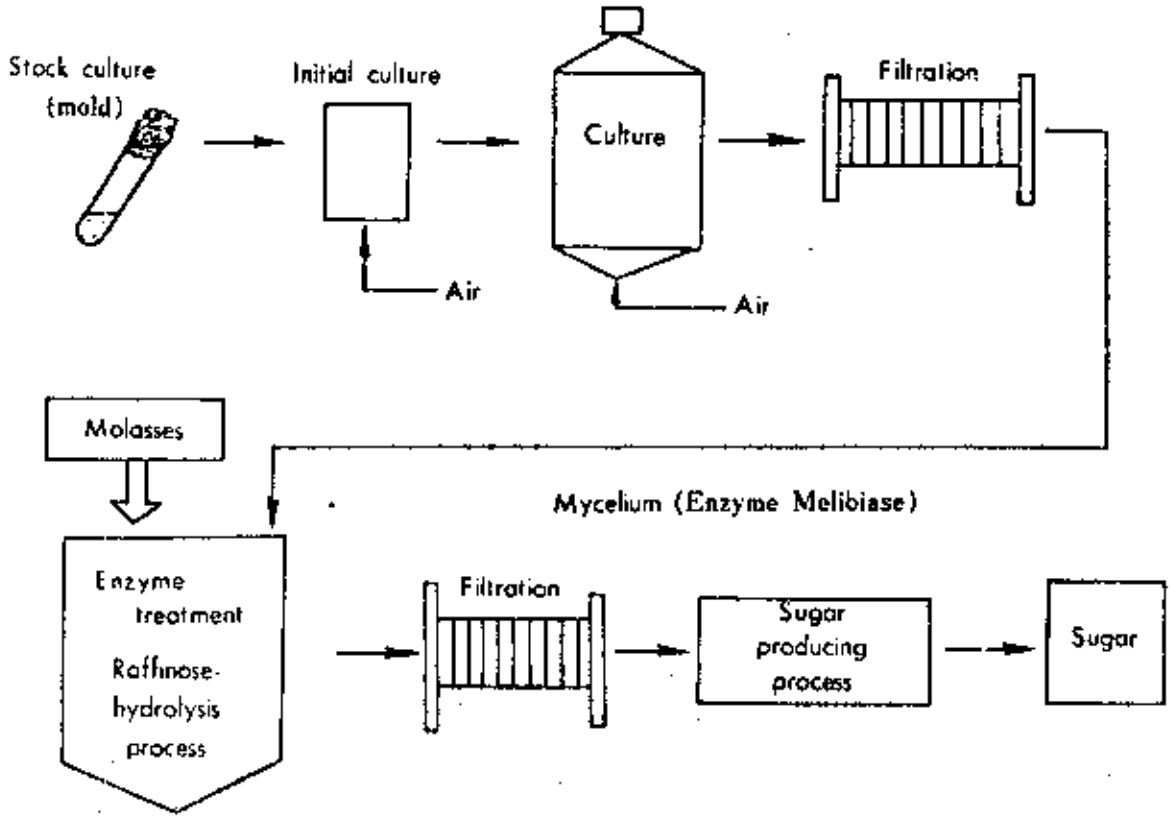
(7) Label of the Enzyme

The enzyme is supplied to the sugar factory in 20 kilogram bags. A draft of the label for these bags is attached as Exhibit 28. Final printed labels will be submitted as soon as available.

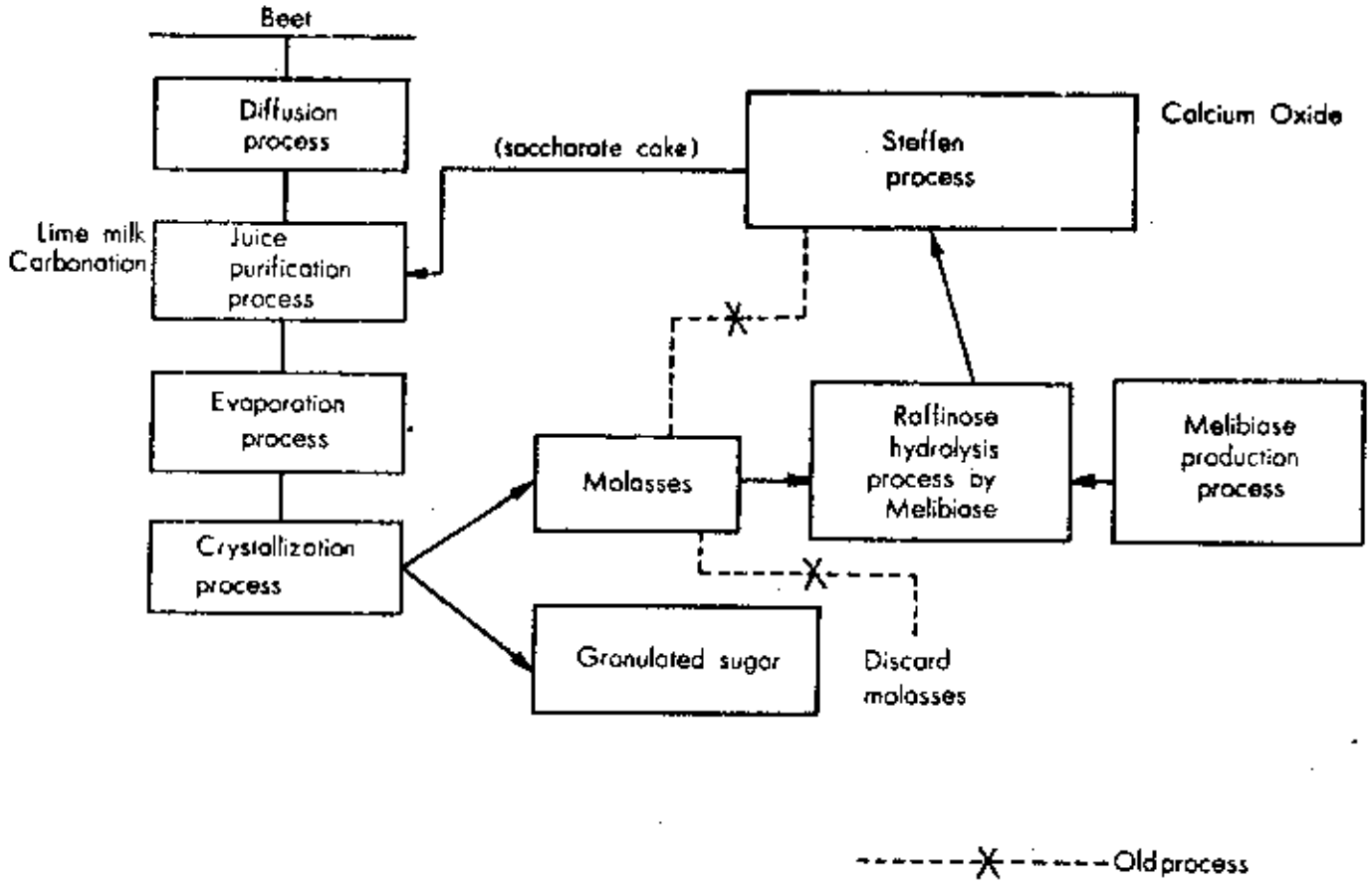
Increased-yield in sugar by using new enzyme



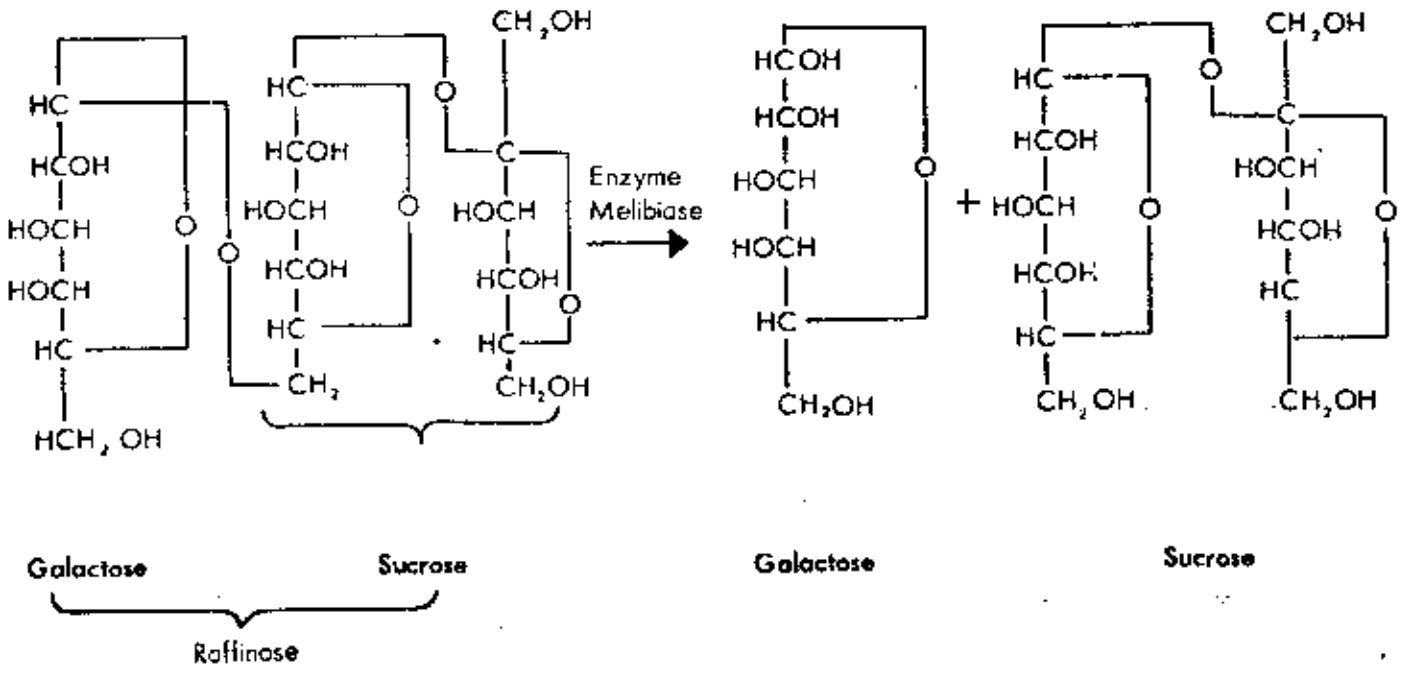
Production process of Melibiase



Improved method in beet sugar manufacturing by the application of Melibiase



Hydrolysis of Raffinose by Enzyme Melibiase



Industrial BIO-TEST *Laboratories, Inc.*
1810 FRONTAGE ROAD
NORTHBROOK, ILLINOIS 60062

REPORT TO

GREAT WESTERN SUGAR COMPANY

ACUTE ORAL TOXICITY STUDY WITH MELIBIA-D (MELIBIASE ENZYME) IN ALBINO RATS

P.O. NO. 41393

OCTOBER 2, 1972

IBT NO. A2148

Industrial BIO-TEST *Laboratories, Inc.*
1810 FRONTAGE ROAD
NORTHBROOK, ILLINOIS 60062

October 2, 1972

Mr. Richard H. Coffey
Manager, Quality Assurance
The Great Western Sugar Company
P. O. Box 5308, Terminal Annex
Denver, Colorado 80217

Dear Mr. Coffey:

Re: IBT No. A2148 - Acute Oral Toxicity Study with Melibia-D (Melibiase Enzyme) P. O. No. 41393

We are submitting herewith our laboratory report dated October 2, 1972, prepared in connection with the above study.

Very truly yours,

J. C. Calandra
President

JCC/kjl

REPORT TO

GREAT WESTERN SUGAR COMPANY

ACUTE ORAL TOXICITY STUDY WITH MELIBIA-D (MELIBIASE ENZYME) IN ALBINO RATS

P.O. NO. 41393

OCTOBER 2, 1972

IBT NO. A2148

I. Introduction

At the request of Great Western Sugar Company, an acute oral toxicity study in albino rats was conducted with a sample identified as Melibia-D (Melibiase Enzyme).

II. Summary

An acute oral toxicity study was conducted with Melibia-D (Melibiase Enzyme). The test material was administered at a dose level of 10.0 g/kg. Each dose was weighed individually, and the test material was flushed through the intubating needle with 10 to 12 cc of corn oil. Eight albino rats (four males and four females) were tested. The acute oral median lethal dose (LD_{50}) was found to be greater than 10.0 g/kg. General signs of intoxication exhibited by the rats following dosing included hypoactivity, ruffed fur, diarrhea, and diuresis. Necropsy revealed no gross pathologic alterations among any of the animals.

Respectfully submitted,

INDUSTRIAL BIO-TEST LABORATORIES, INC.

Report prepared by: Beverley Kretchmar
Beverley Kretchmar
Technician
Acute Toxicity

Report approved by: Carmen Mastri
Carmen Mastri, B.S.
Senior Group Leader
Acute Toxicity

M. L. Keplinger
M. L. Keplinger, Ph.D.
Manager, Toxicology

sjn

III. Investigational Procedures

Young albino rats of the Sprague-Dawley strain* were used as test animals. All animals were kept under observation for five days prior to experimental use, during which period they were checked for general physical health and suitability as test animals. The animals were housed in stock cages and were permitted a standard laboratory diet plus water ad libitum, except during the 16-hour period immediately prior to oral intubation when food was withheld.

Eight albino rats (four males and four females) were administered the test material at a a dose level of 10.0 g/kg. All doses were administered directly into the stomachs of the rats using a hypodermic syringe equipped with a ball-tipped intubating needle.

After oral administration of the test material, the rats were housed individually in suspended, wire-mesh cages and observed for the following 14 days. Initial and final body weights and reactions were recorded. A necropsy was conducted on all animals sacrificed at the end of the 14-day observation period.

At the end of the observation period, the acute oral median lethal dose (LD_{50}) of the test material was determined.

* ARS/Sprague-Dawley, Madison, Wisconsin.

IV. Results

A. Mortality and Body Weights

Individual mortality and body weight data are presented in Table I.

TABLE I

TEST MATERIAL: Melibia-D (Melibiase Enzyme)

Acute Oral Toxicity Study - Albino Rats

Mortality and Body Weight Data

Dose* (g/kg)	Animal Number and Sex	Individual Body Weights (grams) Test Day Number: 0	14	Number Dead / Number Tested	Percent Dead
10.0	1-M	206	323	0/8	0
	2-M	190	321		
	3-M	201	313		
	4-M	193	278		
	5-F	194	206		
	6-F	205	228		
	7-F	199	224		
	8-F	203	235		

Acute Oral LD_{50} > 10.0 g/kg

* Each dose was weighed individually and the test material was flushed through the intubating needle with 10 to 12 cc of corn oil.

B. Reactions

The untoward reactions exhibited by the rats following dosing are presented in Table II.

Necropsy revealed no gross pathologic alterations among any of the animals.

TABLE II

TEST MATERIAL: Melibia-D (Meliblase Enzyme)

Acute Oral Toxicity Study - Albino Rats

Summary of Reactions

Dose (g/kg)	Reaction	Time of Onset Following Dose Administration	Duration of Reaction	Time of Death Following Dose Administration
10.0	Hypoactivity	4 hours	3 days	-
	Ruffed fur	4 hours	3 days	
	Diarrhea	4 hours	6-22 hours	
	Diuresis	6-22 hours	2 days	

EXHIBIT 23

Acute Lethal Level of Sucrose
by Intragastric Single Administration to Rodents

(Ref. of E-7.)

NATIONAL INSTITUTE OF HEALTH
10-35, 2-Chome, Kamiosaki, Shinagawa-ku, Tokyo, Japan

KOKURITSUYOKEN
Cable Address: KOKURITU YOKEN
Telephone: (444) 2181

CERTIFICATION

This is to certify, on the basis of the biological test performed at the National Institute of Health of Japan, that the results of acute toxicological tests using rats and mice indicate no differences in the LD_{50} values between Melibiase treated beet granulated sugar presented and ordinarily processed beet granulated sugar.

Samples: (1) Beet granulated sugar manufactured by the Melibiase treatment process.

(2) Beet granulated sugar manufactured by ordinary process.

Manufacturer: Hokkaido Sugar Co., Ltd.
1, 2-chome, Kanda Jinbocho, Chiyoda-ku,
Tokyo, Japan

Test animal: Rat (Wister, Male) 50
Mouse (DDYS, Male) 212

Administration: Oral administration

Date of tests finished: May 10, 1972

RESULTS

Test animal	Beet granulated sugar (1) Melibiase treated	(2) Ordinarily treated
Rat (6 weeks old)	40.7g/kg	42.1g/kg
Mice (6 weeks old)	40.6g/kg	39.6g/kg

Ken Yanagisawa

Ken YANAGISAWA
Director
National Institute of Health
of Japan

ACUTE LETHAL LEVEL OF SUCROSE BY INTRAGASTRIC SINGLE ADMINISTRATION TO RODENTS

National Institute of Health of Japan

Test MATERIALS

Two different types of sucrose samples were obtained from HOKKAIDO SUGAR Co., Ltd.

One of them is a product of the conventional processing system and the other is a new product processed by the enzymatic treatment.

A hundred grams of sucrose was dissolved in 50 ml of hot water and treated by a Bransonic to make a complete solution. The final volume was 114 ml. at room temperature.

Concentration of sucrose in the test solutions was 87.7% (W/V) and their osmotic pressures were 2640 mOsm (control sucrose) and 2612 mOsm (enzymatic processed sucrose).

ANIMALS AND DIET

Wistar rats (5-wk-old) and ddY mice (5-wk-old) were purchased from Shimuchi Farm for Experimental purposes.

All the animals were given a standard pellet diet (of Oriental Yeast Co., Ltd.,) for a week before commencing the oral studies. The average body weight of rats used at the time of the oral study was 110 grs. and that of mice was 21.8 grs.

EXPERIMENTAL DESIGN

All the test animals were starved for 18 hrs. to empty the stomach prior to the administration of sucrose. The sucrose solution was given through an intragastric cannula attached to a syringe in 36.0, 36.5, 37.0, 37.5, 38.0, 38.5, 39.0, 39.5, 40.0, 40.5, 41.0, 41.5, 42.0, 43.0 and 44.0 g/k. body weight. Each dose level was given to at least from 4 animals to 10 animals.

Diet and drinking water for the test animals would be given *ad lib.* 2 hours after the administration of sucrose.

Acute lethal level indicated by LD 50 value 48 hours after the administration of sucrose was calculated according to the method of probability unit, the so called "probit method".

RESULTS

The results are summerized in the following table.

TABLE I. ACUTE LETHAL LEVEL OF SUCROSE BY INTRAGASTRIC SINGLE ADMINISTRATION TO RODENTS

Sample A: Conventional processed sucrose

Sample B: Enzymatic processed sucrose

RATS

Sample	Dose	Mortality	(%)	LD50 (g/kg, c.f. 95%)
A	38.5	1/6	16.7	40.6 (35.2--43.7)
	39.5	2/4	50.0	
	40.3	4/6	66.7	
B	38.0	1/5	20.0	39.6 (36.4--39.6)
	38.6	3/8	37.5	
	39.9	5/7	71.4	
MICE				
A	38.0	1/6	16.7	40.7 (35.4--41.8)
	40.0	3/7	42.9	
	41.3	6/9	66.7	
B	40.0	1/7	14.3	42.1 (39.8--43.7)
	42.0	4/7	57.1	
	43.1	5/7	71.4	

The "test-group" (B), showed somewhat loose feces, but compared with the "control group" (A), the difference in body weight was negligible.

REFERENCES

Boyd, E.M., et al. Acute oral toxicity of sucrose. Toxic. appl. Pharmac. 7,600, 1965

Constantopoulos, G. et al. Maximal tolerated amounts of sucrose given by daily intragastric administration to albino rats. Fd Cosmet. Toxicol. 6,717, 1968

EXHIBIT 24

Acute Toxicity of Beet Sugar Using Mice

ACUTE TOXICITY TEST OF BEET SUGAR USING MICE

HOKKAIDO SUGAR CO., LTD.

Acute toxicity of beet sugar produced by applying enzyme process was tested using mice.
1,000 and 2,000 mg of beet sugar per Kg of body weight per day were injected to the vein of mice for 14 days. Four mice were used for one group.
The result has been shown in Table I.
It is concluded that LD_{50} is much more than 2.0 g per Kg, per day.

TABLE I

	Quantity Injected mg/Kg Day	Body Weight Before Injection gr.	Mortality	Body Weight After 14 Days gr.
Beet Sugar (enzyme used)	2,000	18.7	0/4	25.3
	1,000	17.0	0/4	20.6
Beet Sugar (no enzyme used)	2,000	20.6	0/4	22.5
	1,000	21.6	0/4	24.1

EXHIBIT 25

Biological Screen of Reacted Molasses for Aflatoxins Using Peking Ducklings

CONFIDENTIAL

HRC 1-137
735-394

BIOLOGICAL SCREEN OF REACTED MOLASSES FOR AFLATOXINS USING PEKING DUCKLINGS

Mr. Hirotoshi Konishi
Managing Director
Hokkaido Sugar Co., Ltd.
Iwanami Fudosan Jinbocho Building
1, 2 - Chome, Jinbocho Kanda
Chiyoda-Ku
Tokyo, Japan

Huntingdon Research Center
Post Office Box 6857
Baltimore, Maryland 21204
U. S. A.

SUMMARY

One-day old Peking ducklings were orally intubated with various levels of Melibiase enzyme reacted molasses to screen for the presence of aflatoxins. No adverse effects were noted in behavior, bodyweight gain or in the livers examined microscopically.

Principal Investigator:

Paul J. Upman, B.A.
Laboratory Manager,
Toxicology

Approved by:

L. E. Van Petten, D. V. M.
Director
Pathology and Toxicology
June 25, 1973

EXPERIMENTAL PROCEDURE

Animal Management

Forty Peking ducklings were obtained from Skippack Farms in Pennsylvania immediately after hatching. Prior to feeding, the birds were transferred to Huntingdon Research Center; Baltimore, Maryland.

The ducklings were identified, weighed and randomly assigned to the following treatment groups:

Group	No. Ducklings	Treatment Via Gastric Intubation
1	10	*1ml/bird tap water
2	10	*1ml/bird reacted molasses
3	10	½ml/bird reacted molasses
4	10	¼ml/bird reacted molasses

* Maximum tolerated volume of 1-day old ducks

Treatment

Each duckling was dosed one time upon arrival via the gastric tube with the appropriate volume of reacted molasses or water (control).

Compound

Melibiase enzyme reacted molasses was received from the Hokkaido Sugar Company; Tokyo, Japan; as a dark brown viscous material.

The molasses was given to the ducklings as received.

Housing and Feeding

The ducklings were gang housed in double tiered electrically heated cages. Growena duck feed and water were available after dosing and until termination on an ad libitum basis.

Observations

1. All ducklings were observed frequently after dosing and daily for 7 days.

2. Bodyweights were recorded prior to dosing and after 7 days.

3. All ducklings dying or sacrified after 7 days were subjected to a gross necropsy.

4. Liver samples were preserved in 10% neutral buffered formalin; livers of the control and high dose molasses groups were subjected to histopathological examination.

RESULTS

1. No adverse behavior or signs of illness were noted in any of the ducks dosed except for the following deaths:

Group	Treatment/Bird	Deaths/No. Dosed	Mean Dose ml/kg bodyweight
1	1ml distilled water	0/10	20.7
2	1ml molasses	3/10	20.6
3	½ml molasses	2/10	20.7
4	¼ml molasses	0/10	21.8

The LD_{50} of the reacted molasses in one-day old Peking ducklings is judged to be greater than 20ml/kg bodyweight.

2. Bodyweight gain during the 7-day observation period ranged from an average of 85 to 93 grams per bird and was comparable throughout all groups.

3. No gross pathology was noted at sacrifice.

4. Liver sections from the high dose and control birds were examined microscopically. No treatment-related microscopic changes were seen in the livers. Incidental findings are noted in Appendix 2, pages 6 - 9.

corrected copy

APPENDIX 1
(continued)

Individual Duckling Data

Duck No.	Group/Level	Weight (grams) Day 0	Weight (grams) Day 7	Treatment ml/kg bodyweight
21	3 (½ Maximum)	58	Dead	8.6
22	½ml molasses/bird	41	105	12.2
23		51	135	9.8
24		43	125	11.7
25		49	125	10.2
26		45	Dead	11.1
27		51	164	9.8
28		46	143	10.8
29		52	143	9.6
30		53	126	9.4
MEAN		48.9	133.5	10.3
31	4 (¼ Maximum)	53	142	4.7
32	¼ml molasses/bird	48	140	5.2
33		44	130	5.7
34		47	125	5.3
35		39	125	6.4
36		47	132	5.3
37		45	140	5.5
38		48	165	5.2
39		46	118	5.4
40		44	135	5.7
MEAN		46.1	135.2	5.4

corrected copy

APPENDIX I
(continued)

Individual Duckling Data

Duck No.	Group/Level	Weight (grams) Day 0	Weight (grams) Day 7	Treatment ml/kg bodyweight
21	3 (½ Maximum)	58	Dead	8.6
22	½ml molasses/bird	41	105	12.2
23		51	135	9.8
24		43	125	11.7
25		49	125	10.2
26		45	Dead	11.1
27		51	164	9.8
28		46	143	10.8
29		52	143	9.6
30		53	126	9.4
MEAN		48.9	133.5	10.3
31	4 (¼ Maximum)	53	142	4.7
32	¼ml molasses/bird	48	140	5.2
33		44	130	5.7
34		47	125	5.3
35		39	125	6.4
36		47	132	5.3
37		45	140	5.5
38		48	165	5.2
39		46	118	5.4
40		44	135	5.7
MEAN		46.1	135.2	5.4

APPENDIX 2

Pathology Summary

HRC-1-137

PATHOLOGY SUMMARY

Two sections of liver from ten ducks dosed orally with 1 ml. of Reactivated Molasses were examined microscopically. A similar set of liver sections were examined from ten ducks dosed orally with 1 ml. of distilled water and served as controls.

No treatment related microscopic changes were seen in the livers from the ducks trated with Reactivated Molasses. Incidental microscopic alterations were seen in the livers from both the control and treated ducks. These changes were characterized by focal areas of hepatocyte necrosis surrounded by an infiltration of mononuclear macrophages. A minimal (Grade 1) bile duct proliferation was seen in several control and treated duck livers.

WILLIAM M. BUSEY, D.V.M., Ph.D
Pathologist

KEY FOR INCIDENCE TABLE

0 = Not Present

N = No Section

A = Autolysis

1 = Minimal

2 = Slight

3 = Moderate

4 = Moderately Severe/High

5 = Severe/High

HRC-1-137-B

DETAILED HISTOPATHOLOGY INCIDENCE TABLE

	Control										Reactivated Molasses									
Animal No	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20
LIVER																				
Bild Duct Proliferation	1	0	1	0	1	1	0	1	0	0	1	1	0	A	1	1		1	1	1
Focal Mononuclear Infiltration	1	1	2	2	2	2	1	2	1		1	2	2		1	1		2	2	2
Focal Necrosis		1	1	2	3	2	2	3	1		2	3	3		1			2	3	3
Acute Necrosis																3	3			

EXHIBIT 26

Six-Month Dietary Administration of Reacted Molasses to Albino Rats

CONFIDENTIAL

1-314
727-290

FINAL REPORT

SIX-MONTH DIETARY ADMINISTRATION OF REACTED MOLASSES TO ALBINO RATS

Mr. Hirotoshi Konishi
Managing Director
Hokkaido Sugar Co., Ltd.
Iwanami Fudosan Jinbocho Building
1, 2 - Chome, Jinbocho Kanda
Chiyoda-Ku
Tokyo, Japan

Huntingdon Research Center
Post Office Box 6857
Baltimore, Maryland 21204
U. S. A.

Six-Month Dietary Administration of Reacted Molasses to Albino Rats

TABLE OF CONTENTS

	Pages
SUMMARY	1
EXPERIMENTAL PROCEDURE	1 - 3
RESULTS	4 - 5
FIGURES	
1 1. Cage and Group Arrangement in Batteries	6
2. Growth of Male Rats	7
3. Growth of Female Rats	8
TABLES	
1. Group Mean Bodyweights	9 - 11
2. Group Mean Food Consumption	12 - 14
3. Group Mean Food Conversion Ratios	15
4. Group Mean Water Consumption	16
4A. Group Mean Water Consumption (ml/kg bodyweight/day)	17
5. Group Mean Hematology	18
6. Group Mean Biochemistry	19
7. Mesophile and pH Record of Molasses Samples	20
8. Group Mean Organ Weights in Grams	21
9. Group Mean Organ Weights as a Percent of Bodyweight x 100	22

Pages

APPENDICES

1.	Individual Bodyweights	23 - 47
2.	Individual Cage Food Consumption	48 - 50
3.	Individual Hematology	51 - 54
4.	Individual Urinalysis	55 - 57
5.	Individual Biochemistry	58 - 59
6.	Ophthalmoscopy	60 - 63
7.	Individual Organ Weights in Grams	64 - 71
8.	Individual Organ Weights as a Percent of Bodyweight x 100	72 - 79
9.	Macroscopic Pathology of Rats Sacrificed at 26 Weeks	80 - 82
10.	Pathology Summary	83 - 123

Six-Month Dietary Administration of Reacted Molasses to Albino Rats

SUMMARY

One hundred and sixty albino rats were divided into four groups and treated as follows:

Group	No. Rats Male	No. Rats Female	% Reacted Molasses (Meliblase) in diet
1	20	20	0
2	20	20	12.5
3	20	20	25.0
4	20	20	50.0

No treatment-related changes were noted in the rate of growth of the animals or in the clinical chemistry, hematology or urinalysis of the rats examined during the 26-week feeding experiment.

Histopathological examination revealed no abnormalities attributable to the feeding of the reacted molasses.

Submitted by:

Paul J. Upman

Paul J. Upman, B.A.
Senior Supervisor, Toxicology

Approved by:

Luis E. Van Petten

L. E. Van Petten, D.V.M.
Director
Pathology and Toxicology
June 20, 1973

EXPERIMENTAL PROCEDURE

Animal Management

One hundred and sixty albino weanling rats (COBS®) were obtained from the Charles River Breeding Laboratory. Following a two-week acclimatization period, the animals were weighed and randomized into the following treatment groups:

Group	Male	Female	%Reacted Molasses (Melibiase) in diet
1	20	20	0
2	20	20	12.5
3	20	20	25.0
4	20	20	50.0

Animals were housed five to a cage in stainless steel, wire-mesh cages randomly assigned to the battery rack. The appropriate diet and water were available *ad libitum*.

Diet Preparation

A special high-protein diet was formulated to insure a balanced diet at all levels and to insure that each group of rats received the same basic ingredients:

% Ingredients	Group 1	Group 2	Group 3	Group 4
Water	10.0	7.5	5.0	-
Sucrose	35.0	26.2	17.5	-
Salt mix	5.0	3.8	2.5	-
Molasses	-	12.5	25.0	50.0
High-protein feed	50.0	50.0	50.0	50.0
TOTAL DIET	100.0	100.0	100.0	100.0

Observations and Laboratory Estimates

a) Clinical signs - Physical appearance and behavior were observed daily, and any abnormalities noted were recorded.

b) Mortalities - All animals found dead were necropsied and tissues were preserved for histopathology.

c) Bodyweight - All animals were weighed on a weekly basis. Group mean values were calculated.

d) Food consumption - The quantity of food consumed by each cage of rats was recorded weekly. The group mean food consumption was calculated from these data.

e) Water consumption - Total consumption was measured for two consecutive days on the first 10 males and 10 females of the high dose group and the control group prior to treatment and after 13 and 26 weeks of treatment.

f) Ophthalmic examination - The eyes of all rats were dilated with 1% Mydriacyl and examined with an indirect ophthalmoscope prior to treatment, after 13 and 26 weeks of treatment.

g) Hematology - Blood samples from the first 10 males and 10 females of the high dose and control groups were taken from the tail vein prior to treatment and after 4 and 13 and 26 weeks of treatment for measurement of the following parameters:

1) Hematocrit (Hct) - as microhematocrit
2) Hemoglobin concentration (Hb) - by cyanmethemoglobin of Cannon, 1958; Am. J. Clin. Path., 30: 211.
3) Red cell count (RBC) - Coulter counter
4) White cell count (WBC) - Coulter counter
5) Differential white cell count - by direct visual count of smear after staining with Wright's stain. Counts were recorded under the following categories:

N	=	Neutrophils
L	=	Lymphocytes
E	=	Eosinophils
B	=	Basophils
M	=	Monocytes
S	=	Stabs

h) Urinalysis - Urine samples from the first 10 male and 10 females of the high dose and control groups were obtained after 4, 13 and 26 weeks of treatment for the following:

1) Volume - measured to nearest ml
2) pH - Labstix with final reading using Beckman Research Model pH meter (to nearest .1 pH)
3) Specific Gravity (SG) - by refractometry using American Optical Company TS meter
4) Protein - Labstix®, if positive confirmed by precipitation with sulfosalicylic acid and quantitative estimation against turbidimetric standards
5) Glucose - Labstix®, graded negative (-) .25%, present .5%
6) Ketones - Labstix®, if positive confirmed by Rothera's Test; graded negative, trace or positive (-, Tr, +)
7) Blood Pigments - Labstix®, graded negative, small moderate, large (-, S, M, L)
8) Microscopy - Clay Adams "Sedi-Cal" Procedure, deposits examined for:

epithelial cells	(E)
polymorphonuclear leucocytes	(P)
mononuclear leucocytes	(M)
erythrocytes	(R)
organisms	(O)
casts	(C)
amorphous sediment	(A)

Gradings of cell frequency in deposit were recorded as follows:

0	=	signifies none observed
1	=	signifies few in some fields
2	=	signifies few in all fields
3	=	signifies many in all fields
3+	=	signifies field obscured by crystals

i) Blood Chemistry - At termination, blood samples were collected from the descending aorta following ether anesthesia on 10 male and 10 female rats at all levels for the following:

1) Serum glutamate-pyruvate transaminase (SGPT) Coleman Spec. to DSA, Sigma 505
2) Serum glutamate-oxalacetic transaminase (SGOT) Coleman Spec. to DSA, Sigma 505
3) Urea nitrogen - DSA Fermco Urease - New England Reagent Lab.
4) Glucose - DSA 560, Calbiochem. Academic Press, 1965

5) Alkaline phosphatase - DSA, Bessey, Lowry, Brock. J. Biol. Chem, 164: 321; 1946
6) Serum proteins - DSA 560, American Monitor, Clin. Chem., 2: 441; 1965
7) Serum electrolytes - sodium, potassium and chloride

j) Monitoring of Reacted Molasses

1) The pH of the molasses was monitored when the container was first opened and periodically during its period of use.

2) Mesophile count was checked on the first molasses sample from each shipment according to the following procedure:

a) A 1 gram sample of molasses was collected and placed in 9 ml of sterile distilled water

b) Serial tenfold dilutions were made ranging from 10^{-1} through 10^{-10}

c) A "spread plate" technique was used to plate .1 ml of each serial dilution onto petri plates containing 20 ml of Beef Extract Agar. Plates were prepared in duplicate

d) The plates were incubated at 28°C and read after 24 hours, 72 hours and 10 days

k. Terminal Sacrifice

1) After 26 weeks of treatment, the rats were anesthetized with ether and bled from the abdominal aorta. All animals were subject to a full macroscopic examination, including organ weight analysis; but histopathological examination of the tissues was limited in the first place to all high dose level animals together with half the animals in the control group and also any definitely abnormal tissues found in the animals in other groups on macroscopic examination.

a) Following macroscopic examination, the following organs were removed and weighed:

brain	lungs	adrenals
pituitary	heart	gonads
thyroid	liver	uterus
spleen	kidney	

b) Samples of the following tissues from all rats were preserved routinely in 10% buffered formaldehyde in order to be available for subsequent microscopic examination:

eye	lungs	brain (medullary, cerebellar and cortical sections)
thymus (where present)	bladder	heart
lymph nodes (cervical and mesenteric)	pancreas	liver
ileum	spleen	duodenum
pituitary	kidney	thyroid
uterus	adrenals	gonads
femur	mid-colon	stomach (glandular and non-glandular)
	salivary gland	
	seminal vesicles	
	sciatic nerve	

c) Fixed tissue samples were embedded in paraffin wax and sections cut at 5μ and stained with hematoxylin and eosin. Additional sections of liver and kidney were also stained with Oil Red "O" and P.A.S.

RESULTS

a) b) Clinical Signs and Mortality

Other than a few episodes of wheezing, no signs of illness were noted. There were no adverse reactions to treatment except that the fecal droppings of the animals at the 25% and 50% treatment levels were noted to be darker in color than the low dose and control animals. There were no deaths noted during the 26-week treatment period.

c) Bodyweight (Table 1, pages 9 - 11; Appendix 1, pages 23 - 47)

All animals in the treatment groups maintained a rate of growth comparable to the control rats. No adverse effects were attributable to the treatment.

d) Food Consumption (Table 2, pages 12 - 14; Appendix 2, pages 48 - 50)

Mean food consumptions remained acceptable for all groups during the 26-week treatment period. Food conversion ratios (Table 3, page 15) calculated during the first 16 weeks of treatment indicate a comparable utilization of diets fed to all groups of animals.

e) Water Consumption (Table 4, page 16)

Monitored on animals from the high test group and the control group of rats show a general decrease in the volume consumed as compared to the pretreatment values. This is probably due to the high water content of the diets fed.

When examined on a ml/kg of bodyweight basis (Table 4A, page 17), the rats at the 50% test level consumed a greater volume as compared to the control rats. No explanation can be offered for this difference at this time.

f) Ophthalmic Examinations

Abnormalities noted are outlined in Appendix 6, pages 60 - 63, none of which were attributable to the administration of the test material.

g) Hematology

A slight decrease in neutrophils (males) and a corresponding increase in leucocytes is probably due to a stabilization to the laboratory environment and is not considered to be an adverse effect. All other parameters are comparable with those of the control rats.

h) Urinalysis (Appendix 4, pages 55 - 57)

Samples examined from the high test level and the control rats revealed no significant differences when compared.

i) Blood Biochemistry (Table 6, page 19; Appendix 5, pages 58 - 59)

Examined on representative rats of each group and sex. Data appears to be within normal ranges for rats utilized in this laboratory.

j) Mesophile Count and pH (Table 7, page 20)

Monitored at selected intervals on each batch of molasses received were all within the acceptable ranges of less than 3,963 mesophiles/gm of molasses cultured and less than 7.7 pH.

k) Terminal Studies

1. Gross abnormalities observed at necropsy are listed in Appendix 9, pages 80 - 82. There is no compound related trend noted in these data. Abnormal tissues were subjected to histological examination and are included in the histopathology report.

a. Individual absolute and relative organ weights with mean values appear in Appendices 7 and 8, pages 64 to 79. Mean absolute and relative liver and kidney weights from high dose males and females show a slight increase over the control weights. In addition, mean absolute and relative adrenal gland weights in high dose females show a slight increase when compared to the female control adrenal gland weights. The significance of these findings is unclear since microscopic morphology showed no significant differences between high dose and control livers, kidneys and adrenals.

b. The pathologic-anatomical evaluation was done by Dr. A. J. Newman; Head, Department of Pathology; Huntingdon Research Centre, England. His report appears in Appendix 10, pages 83 to 123. No histopathological changes or variations from normal were seen in the tissues examined that were considered to be of toxicological significance.

FIGURE 1

Cage and Group Arrangement in Batteries

Cage	Group

#18 1♀	#23 2♀
#22 2♀	#27 3♀
#26 3♀	#31 4♀
#30 4♀	#20 1♀
#19 1♀	#24 2♀

#1 1♂	#6 2♂
#5 2♂	#10 3♂
#9 3♂	#14 4♂
#13 4♂	#3 1♂
#2 1♂	#7 2♂

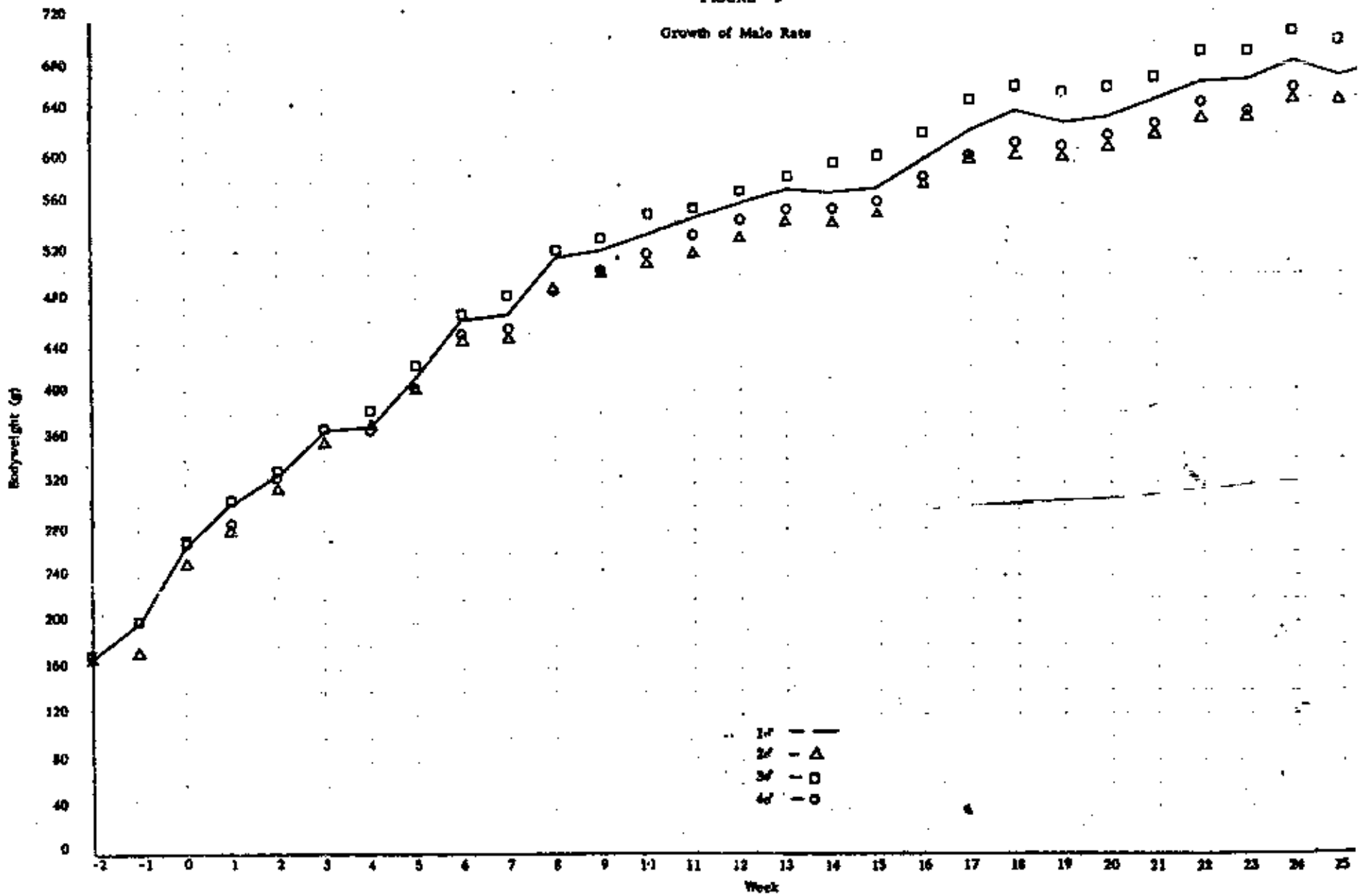
#11 3♂	#16 4♂
#15 4♂	#17 1♀
#4 1♂	#21 2♀
#8 2♂	#25 3♀
#12 3♂	#29 4♀

	#32 4♀
	#28 3♀

Six-Month Dietary Administration of Reacted Molasses to Albino Rats

FIGURE 2

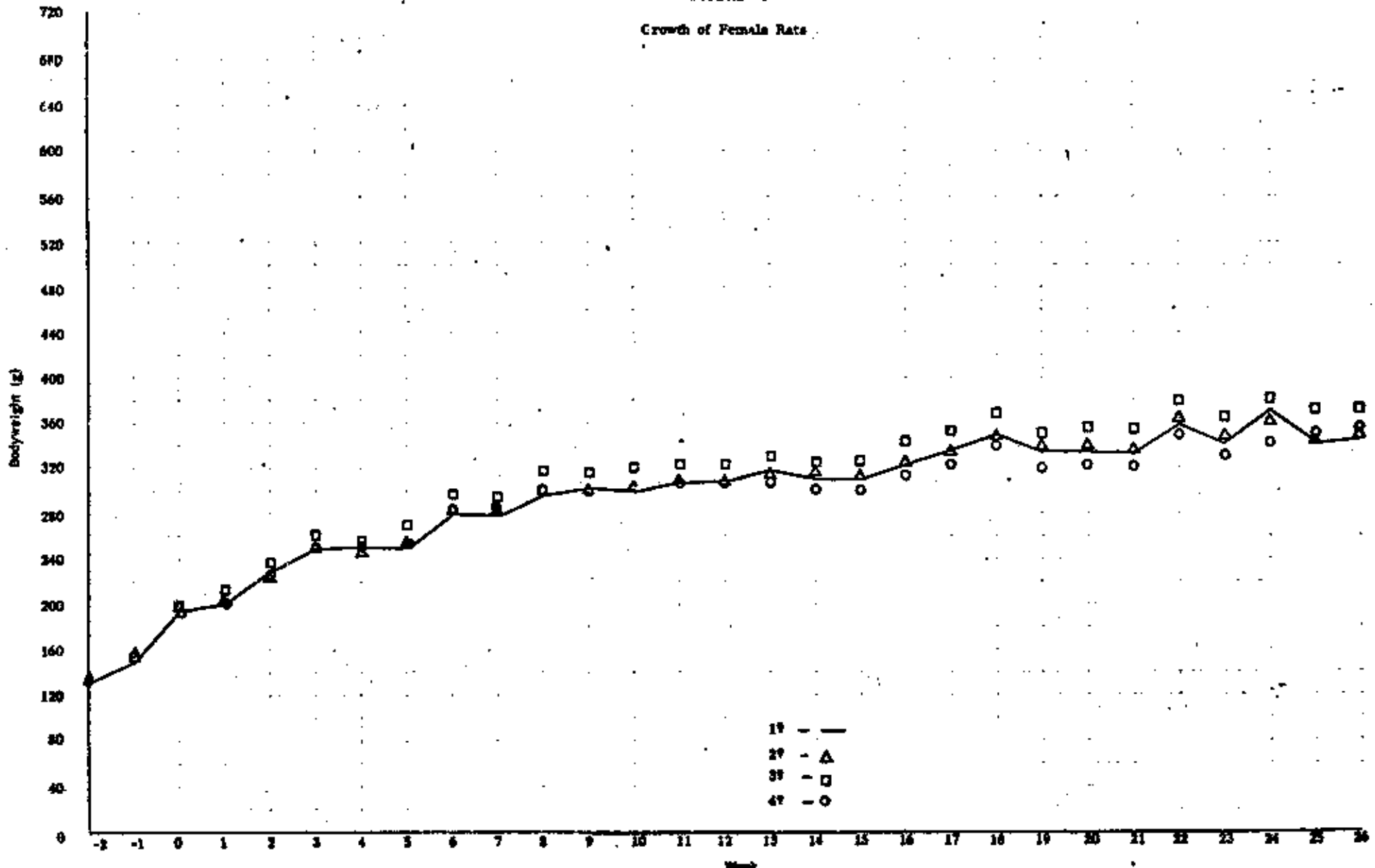
Growth of Male Rats



Six-Month Dietary Administration of Reacted Molasses to Albino Rats

FIGURE 3

Growth of Female Rats



Six-Month Dietary Administration of Reacted Molasses to Albino Rats

TABLE 1

Group Mean Bodyweight (g)

Group Sex	Week* -2	-1	0	1	2	3	4	5	6	7
1 ♂ Control	168	194	265	302	327	364	369	411	460	465
2 ♂ 12.25%	165	174	251	280	314	356	368	401	445	447
3 ♂ 25%	168	200	269	303	330	364	382	430	465	480
4 ♂ 50%	167	191	253	286	325	366	362	400	447	454
1 ♀ Control	134	151	197	201	228	247	251	252	280	277
2 ♀ 12.25%	136	155	195	202	222	247	242	252	281	279
3 ♀ 25%	137	155	197	211	235	260	255	270	296	295
4 ♀ 50%	136	150	191	206	224	252	253	254	284	285

* All groups fed control feed prior to week 0

TABLE 1
(continued)

Group Mean Bodyweight (g)

Group Sex	8	9	10	11	12	13	14	15	16	17
1 ♂ Control	511	520	536	550	562	576	569	577	600	625
2 ♂ 12.5%	489	502	512	520	533	548	545	557	578	598
3 ♂ 25%	519	533	551	556	572	585	592	604	623	651
4 ♂ 50%	487	520	517	533	544	555	556	566	585	605
1 ♀ Control	297	300	300	309	311	315	309	309	322	334
2 ♀ 12.5	300	300	305	310	310	316	316	313	325	333
3 ♀ 25%	317	315	319	326	325	332	327	328	344	353
4 ♀ 50%	301	298	301	308	307	309	301	299	315	323

TABLE 1
(continued)

Group Mean Bodyweight (g)

Group Sex	Week 18	19	20	21	22	23	24	25	26
1♂ Control	640	633	637	650	666	666	681	670	680
2♂ 12.5%	607	603	610	621	637	637	653	648	654
3♂ 25%	663	657	662	668	691	691	709	700	710
4♂ 50%	614	611	619	628	647	640	660	648	666
1♀ Control	347	336	337	335	359	341	372	341	343
2♀ 12.5%	349	340	340	337	367	349	360	344	350
3♀ 25%	368	349	355	352	378	367	381	370	371
4♀ 50%	338	319	323	321	351	331	343	330	333

TABLE 2

Group Mean Food Consumption (g)

Group Sex	Week * -1	0	1	2	3	4	5	6	7
1 ♂ Control	147	190	189	198	198	206	213	221	214
2 ♂ 12.25%	140	187	182	202	204	208	216	215	210
3 ♂ 25%	144	166	176	202	202	207	216	229	218
4 ♂ 50%	141	176	184	196	204	207	207	219	208
1 ♀ Control	120	149	180	185	149	159	157	159	163
2 ♀ 12.25%	138	160	178	188	157	151	153	158	137
3 ♀ 25%	114	159	165	183	156	152	151	160	155
4 ♀ 50%	109	167	139	161	147	151	129	156	144

* All groups fed control feed prior to week 0

TABLE 2
(continued)

Group Mean Food Consumption (g)

Group Sex	Week 8	9	10	11	12	13	14	15	16	17
1 ♂ Control	222	209	219	218	225	209	210	220	218	214
2 ♂ 12.5%	220	202	222	204	232	216	210	222	234	226
3 ♂ 25%	225	211	234	206	235	228	213	233	249	216
4 ♂ 50%	247	200	223	214	212	204	201	217	229	208
1 ♀ Control	158	145	161	155	159	161	156	157	143	149
2 ♀ 12.5%	147	137	148	151	157	146	151	161	147	146
3 ♀ 25%	158	144	161	154	147	150	150	158	160	141
4 ♀ 50%	98	132	166	136	140	140	152	152	131	136

TABLE 2
(continued)

Group Mean Food Consumption (g)

Group Sex	Week 18	19	20	21	22	23	24	25	26
1♂ Control	202	210	218	223	229	227	227	208	211
2♂ 12.5%	201	220	225	227	235	221	231	193	230
3♂ 25%	216	237	236	240	239	239	241	235	241
4♂ 50%	195	209	217	221	217	220	222	201	222
1♀ Control	138	147	151	169	162	155	158	138	140
2♀ 12.5%	139	154	156	161	165	148	147	130	157
3♀ 25%	138	151	165	163	164	158	157	152	154
4♀ 50%	131	142	151	148	144	141	131	126	143

Six-Month Dietary Administration of Reacted Molasses to Albino Rats

TABLE 3

Group Mean Food Conversion Ratios ($\frac{\text{Body wt}}{\text{Food cons}}$)

Week	GROUP ♂ Control	12.5%	25.0%	50.0%	GROUP ♀ Control	12.5%	25.0%	50.0%
1	1.6	1.5	1.7	1.6	1.1	1.1	1.8	1.5
2	1.7	1.6	1.6	1.6	1.2	1.2	1.3	1.4
3	1.8	1.7	1.8	1.8	1.7	1.6	1.7	1.7
4	1.8	1.8	1.8	1.7	1.6	1.6	1.7	1.7
Mean	1.7	1.7	1.7	1.7	1.4	1.4	1.5	1.6
5	1.9	1.9	1.9	1.9	1.6	1.6	1.8	2.0
6	2.1	2.1	2.0	2.0	1.7	1.8	1.9	1.8
7	2.2	2.1	2.2	2.2	1.7	2.0	1.9	2.0
8	2.3	2.2	2.3	2.0	1.9	2.0	2.0	3.1
Mean	2.1	2.1	2.1	2.0	1.7	1.9	1.9	2.2
9	2.5	2.5	2.5	2.5	2.1	2.2	2.2	2.3
10	2.4	2.3	2.4	2.3	1.9	2.1	2.0	1.8
11	2.5	2.5	2.7	2.5	2.0	2.1	2.1	2.8
12	2.5	2.3	2.4	2.6	2.0	2.0	2.2	2.2
Mean	2.5	2.4	2.5	2.5	2.0	2.1	2.1	2.2
13	2.8	2.5	2.6	2.7	2.0	2.2	2.2	2.2
14	2.7	2.6	2.8	2.8	2.0	2.1	2.2	2.0
15	2.6	2.5	2.6	2.6	2.0	1.9	2.1	2.0
16	2.8	2.5	2.5	2.5	2.3	2.2	2.2	2.4
Mean	2.7	2.5	2.6	2.7	2.1	2.1	2.2	2.2

Six-Month Dietary Administration of Reacted Molasses to Albino Rats

TABLE 4

Group Mean Water Consumption

Group/Sex Level	Pretreat Day 1	Pretreat Day 2	Pretreat Average	Week 13 Day 1	Week 13 Day 2	Week 13 Day 3	Week 13 Average	Week 26 Day 1	Week 26 Day 2	Week 26 Day 3	Week 26 Average
1 ♂ Control	43.0	34.4	38.7	82.5	63.0	78.5	74.5	64.6	72.1	79.3	72.0
4 ♂ 50%	31.0	33.0	32.0	74.5	57.5	75.5	68.5	83.3	91.4	91.9	88.9
1 ♀ Control	39.4	26.1	32.8	52.0	45.5	52.5	50.0	34.0	52.0	50.7	45.6
4 ♀ 50%	46.9	30.3	38.6	63.0	62.5	67.5	64.5	50.6	79.7	71.1	67.1

TABLE 4A

Group Mean Water Consumption
(ml/kg bodyweight/day)

Week	Group ♂			Group ♀		
	Control	50.0%	Difference	Control	50.0%	Difference
-1	199	167	-32	217	257	+40
13	130	124	-6	159	210	+51
26	106	133	+27	133	202	+79
Change from Pretreat to Week 26:						
	-98	-34		-84	-55	

Six-Month Dietary Administration of Reacted Molasses to Albino Rats

TABLE 5

Hematology - Group Mean Values

Group Sex	Interval	Hct	Hgb g%	RBC $x10^6$ cmm	WBC $x10^3$ cmm	Differential N	S	L	E	B	M
1♂ Control	Pretreat	48	16.0	8.10	12.4	26.5	0.3	73.0	0.1	0.0	0.1
	Week 4	50	16.6	8.10	16.7	21.6	0.2	78.1	0.1	0.0	0.0
	Week 13	49	16.4	7.46	14.8	17.6	0.6	80.2	1.5	0.0	0.1
	Week 26	49	14.5	8.08	12.9	16.8	0.5	82.1	0.6	0.0	0.0
4♂ 50%	Pretreat	47	15.6	7.45	12.6	21.5	0.1	78.2	0.2	0.0	0.0
	Week 4	52	17.3	7.81	16.3	16.9	0.5	82.6	0.0	0.0	0.0
	Week 13	51	17.1	7.17	14.2	22.4	0.4	75.5	1.4	0.0	0.3
	Week 26	49	14.5	7.93	14.1	18.7	0.3	80.0	1.0	0.0	0.0
1♀ Control	Pretreat	49	15.9	7.95	13.2	27.5	0.6	71.7	0.1	0.1	0.0
	Week 4	49	16.3	7.85	10.1	24.3	0.7	74.1	0.9	0.0	0.0
	Week 13	46	16.3	7.06	13.5	18.5	0.6	79.7	1.1	0.0	0.1
	Week 26	45	14.1	7.02	7.8	19.9	0.0	79.2	0.9	0.0	0.0
4♀ 50%	Pretreat	50	16.2	7.93	11.7	28.6	0.2	70.7	0.4	0.1	0.0
	Week 4	51	16.7	7.72	10.2	27.9	0.2	70.8	1.1	0.0	0.0
	Week 13	46	16.1	7.11	11.2	22.3	0.2	74.8	2.5	0.0	0.2
	Week 26	46	14.1	7.29	9.6	31.2	0.4	67.9	0.5	0.0	0.0

TABLE 6

Group Mean Biochemistry (Week 26)

Group Sex	SGOT SF U/ml	SGPT SF U/ml	SAP BLB U/ml	BUN mg%	Glucose mg%	Na^+	K^+	Total Protein
1 ♂ Control	23	19	2.5	17.7	134	146	5.0	6.3
2 ♂ 12.5%	21	18	2.3	17.0	153	145	5.1	6.2
3 ♂ 25%	17	14	2.2	15.5	152	149	4.6	6.4
4 ♂ 50%	15	14	2.5	14.5	160	149	4.5	6.8
1 ♀ Control	20	17	1.5	27.6	121	148	4.7	6.6
2 ♀ 12.5%	15	16	1.5	31.5	132	149	4.0	6.7
3 ♀ 25%	15	12	1.6	20.0	125	146	4.1	6.5
4 ♀ 50%	16	10	1.6	23.6	114	152	4.9	6.8

TABLE 7

Mesophile Count and pH Record of Molasses

Date	Week	pH	Mesophile Count (per gram)	Batch
8-11-72	-2	6.85	acceptable limits (500)	1 240kg
8-19-72	0	6.90		1
11- 2-72	6	6.80		1
11-10-72	7	6.91	acceptable limits	2 75kg
11-28-72	9	6.70	acceptable limits (290)	3 240kg
1-17-73	17	6.70	acceptable limits (2,300)	4 240kg
2-20-73	23	6.90	acceptable limits	5 85.6kg
3-12-73	26	6.88		5

Six-Month Dietary Study of Administration of Reacted Molasses to Albino Rats

TABLE 8

Group Mean Organ Weights in Grams

Group/ Sex	Body Wt.	Heart	Lung	Liver	Spleen	Kidneys	Adrenals	Gonads	Thyroids	Brain	Pituitary	Uterus
1 ♂ Control	625	1.68	2.05	16.30	0.69	3.89	0.056	3.37	0.029	2.24	0.017	-
2 ♂ 12.5%	614	1.85	1.90	14.72	0.71	4.07	0.058	3.42	0.032	2.31	0.019	-
3 ♂ 25%	666	1.98	2.03	16.90	0.80	4.16	0.060	3.54	0.033	2.30	0.019	-
4 ♂ 50%	616	1.67	1.93	17.15	0.74	4.12	0.063	3.11	0.08	2.23	0.017	-
1 ♀ Control	321	1.12	1.41	8.88	0.49	2.40	0.077	0.097	0.027	2.14	0.020	0.805
2 ♀ 12.5%	315	1.25	1.38	8.80	0.49	2.38	0.072	0.118	0.025	2.08	0.018	0.905
3 ♀ 25%	343	1.18	1.38	9.20	0.54	2.56	0.075	0.115	0.031	2.12	0.021	0.830
4 ♀ 50%	306	1.13	1.46	9.26	0.54	2.52	0.114	0.116	0.030	2.14	0.020	1.089

Six-Month Dietary Administration of Reacted Molasses to Albino Rats

TABLE 9.

Group Mean Organ Weights as a Percent of Bodyweight x 100

Group/ Sex	Heart	Lung	Liver	Spleen	Kidneys	Adrenals	Gonads	Thyroids	Brain	Pituitary	Uterus
1 ♂ Control	.27	.32	2.60	.11	.62	.009	.54	.005	.36	.003	-
2 ♂ 12.5%	.30	.31	2.39	.11	.66	.009	.56	.005	.37	.003	-
3 ♂ 25%	.29	.30	2.54	.12	.62	.009	.53	.005	.35	.003	-
4 ♂ 50%	.27	.31	2.78	.12	.67	.010	.50	.005	.36	.003	-
1 ♀ Control	.35	.44	2.77	.15	.75	.024	.03	.008	.67	.006	.25
2 ♀ 12.5%	.39	.44	2.79	.16	.76	.023	.04	.008	.66	.006	.29
3 ♀ 25%	.34	.40	2.68	.16	.75	.022	.03	.009	.62	.006	.24
4 ♀ 50%	.37	.48	3.03	.18	.82	.037	.04	.009	.69	.006	.35

APPENDIX 1

Individual Bodyweights

BODYWEIGHT (g) Experiment: 1-314

Group: 1st Control (50% Sucrose)

CAGE	Animal No.	Week No. -2 (9-1)	-1 (9-8)	0 (9-15)	1 (9-22)	2 (9-29)	3 (10-6)	4 (10-13)	5 (10-20)	6 (10-27)	7 (11-3)
		PED Control									
1	1	146	193	251	300	319	358	378	402	430	450
	5	144	143	202	266	263	334	350	369	423	431
	9	148	142	201	269	302	319	340	354	412	430
	13	148	211	251	306	318	359	361	382	443	424
	17	148	211	167	317	333	396	420	438	482	480
Total											
Mean											
2	21	177	225	291	312	304	329	346	367	411	469
	25	171	291	281	312	304	365	376	419	465	460
	29	161	230	291	337	351	374	387	418	424	482
	33	159	194	239	289	333	352	361	389	446	441
	37	168	161	259	319	322	336	354	400	451	458
Total											
Mean											
3	41	178	210	279	289	346	380	368	412	468	470
	45	180	113	289	349	393	404	391	459	500	520
	49	170	205	269	319	313	349	355	425	488	496
	53	160	122	219	269	262	319	313	368	407	418
	57	176	152	229	310	304	378	369	432	492	511

BODYWEIGHT (g) Experiment: 1-314

Group: 1st Control (50% Sucrose)

	Animal No.	Week No. -2	-1	0	1	2	3	4	5	6	7
4	61	181	250	309	309	342	384	380	444	472	470
	65	168	210	261	299	357	400	404	468	511	538
	69	191	252	317	339	314	392	389	436	488	480
	73	174	238	299	319	366	370	366	425	480	480
	77	174	284	309	339	370	394	377	409	458	458
Total											
Mean											
Total		9362	3875	5293	6046	6548	7282	7382	8214	9201	9306
Mean		168	194	265	302	327	364	369	411	460	465

BODYWEIGHT (g) Experiment: F-314

Group: 1 ♂ Control (50% Sucrose)

CAGE	ANIMAL NUMBER	11/6/12 Week No. 8	11/13/12 9	11/20/12 10	12/4 11	12/11 12	12/18 13	12/22 14	12/28 15	1/3/13 16	1/10/13 17
1	1	499	508	527	529	533	552	536	541	561	586
	5	480	482	516	513	528	551	535	539	564	590
	9	470	483	514	519	540	548	534	553	585	604
	13	469	480	510	518	530	537	529	537	560	575
	17	530	543	560	568	578	589	579	524	615	650
Total											
Mean											
2	21	451	462	473	500	513	521	519	526	540	566
	25	501	515	530	540	550	551	550	534	562	586
	29	544	555	560	585	595	612	601	608	637	656
	33	500	510	530	530	540	547	540	550	569	605
	37	500	516	531	545	550	566	552	557	578	619
Total											
Mean											
3	41	512	527	540	560	575	603	610	615	635	664
	45	559	502	513	530	530	552	541	541	546	602
	49	555	570	577	600	600	617	606	619	626	656
	53	460	482	500	512	528	546	541	546	556	593
	57	557	572	584	610	622	650	651	623	635	717

BODYWEIGHT (g) Experiment: F-314

Group: 1 ♂ Control (50% Sucrose)

CAGE	ANIMAL NUMBER	11/6/12 Week No. 8	11/13/12 9	11/20/12 10	12/4 11	12/11 12	12/18 13	12/22 14	12/28 15	1/3/13 16	1/10/13 17
4	61	511	519	528	536	548	559	567	583	611	616
	65	599	593	611	633	660	676	660	700	743	741
	69	520	539	550	564	572	579	581	587	607	633
	73	521	532	554	561	590	601	601	607	623	652
	77	500	506	523	540	550	555	551	560	587	593
Total		10219	10375	10719	10993	11232	11502	11334	11551	11865	12504
Mean		511	520	536	550	562	576	569	577	600	625

BODYWEIGHT (g) Experiment: 1-314

Group: 2F 12.5 %

	Animal No.	Week No.	8-8 -1	8-15 0	8-22 1	8-29 2	9-6 3	9-13 4	9-20 5	9-27 6	10-3 7
5	2		140	186	241	290	311	357	389	412	456	458
	6		150	218	261	312	337	385	393	416	462	460
	10		148	198	241	300	320	378	381	412	453	454
	14		142	186	241	262	270	311	316	332	388	394
	18		145	201	261	304	321	359	381	396	437	436
Total												
Mean												
6	22		170	216	274	318	334	390	400	444	492	480
	26		173	224	291	330	311	379	378	429	480	484
	30		154	204	261	312	337	341	337	371	425	422
	34		168	205	209	269	288	313	335	366	421	423
	38		176	172	249	289	317	344	341	363	401	408
Total												
Mean												
7	42		171	123	227	268	319	335	341	362	405	400
	46		175	146	289	232	294	312	335	383	428	438
	50		171	171	269	189	244	308	351	412	462	462
	54		113	113	286	320	318	390	384	414	452	441
	58		119	119	217	265	302	331	343	365	411	427
	62											

BODYWEIGHT (g) Experiment: 1-314

Group: 2F 12.5 %

	Animal No.	Week No.	-1	0	1	2	3	4	5	6	7
8	62		194	253	310	339	370	411	417	464	506	510
	66		187	220	297	315	349	390	391	421	452	450
	70		175	170	257	289	336	363	378	407	438	445
	74		116	145	219	269	323	352	370	414	465	470
	78		126	95	169	219	274	379	389	427	465	472
Total												
Mean												
Total			3294	3421	5019	5601	6285	7122	7350	7910	8889	8934
Mean			165	174	251	270	314	356	368	401	445	447

BODYWEIGHT (g)

Experiment: I-314

Group: 2 ♂ 12.5 %

CAGE	ANIMAL NUMBER	WEEK No. 8	11/19/72 9	11/26/72 10	12/1 11	12/8 12	12/15 13	12/22 14	12/29 15	1/5/73 16	1/12/73 17
5	2	501	511	510	520	532	544	537	557	561	577
	6	504	517	519	540	540	565	567	578	586	619
	10	500	506	515	530	543	557	548	559	570	587
	14	444	457	470	470	500	524	521	537	550	578
	18	479	493	500	530	545	560	561	577	579	612
Total											
Mean	22	536	537	539	541	553	563	560	561	570	590
6	26	512	529	538	551	543	543	539	552	581	588
	30	462	473	460	478	500	519	530	537	537	577
	34	459	425	478	474	480	500	493	487	513	542
	38	441	446	440	452	455	469	472	468	496	506
Total											
Mean	42	441	467	474	500	510	519	486	500	520	546
7	46	493	500	515	520	530	548	529	535	552	581
	50	511	534	560	566	568	580	574	589	630	648
	54	482	488	481	502	505	523	520	527	550	569
	58	481	478	501	500	530	555	571	572	603	626

BODYWEIGHT (g)

Experiment: I-314

Group: 2 ♂ 12.5 %

CAGE	ANIMAL NUMBER	WEEK No. 8	11/19/72 9	11/26/72 10	12/1 11	12/8 12	12/15 13	12/22 14	12/29 15	1/5/73 16	1/12/73 17
8	62	543	557	578	581	600	602	606	622	654	650
	66	489	515	538	530	553	577	584	628	629	650
	70	480	492	511	518	535	546	550	577	612	629
	74	522	545	568	576	590	611	590	607	668	672
	78	506	521	534	535	552	553	569	567	573	604
Total		9776	10042	10249	10402	10663	10755	10927	11140	11534	11951
Mean		489	502	512	520	533	538	546	557	578	598

BODYWEIGHT (g) Experiment: 1-314

Group: 2♂ 12.5%

Cage	Rat No.	1/12 Week No. 18	1/26 19	2/2 20	2/9 21	2/16 22	2/23 23	3/2 24	3/9 25	3/16 26	3/23/23 27
5	2	582	571	595	608	608	610	619	607	620	
	6	635	636	635	642	668	670	680	670	692	
	10	604	601	610	615	621	618	640	650	660	
	14	573	570	592	601	620	618	631	630	642	
	18	611	605	600	600	620	615	629	618	618	
Total											
Mean											
6	22	602	587	600	609	616	620	640	621	617	
	26	601	606	600	612	621	639	655	636	647	
	30	562	574	575	590	609	609	625	618	617	
	34	550	537	558	580	590	591	601	601	600	
	38	525	511	525	540	550	553	560	568	560	
Total											
Mean											
7	42	562	542	572	575	591	590	609	590	600	
	46	593	577	582	592	600	600	621	601	608	
	50	674	669	690	702	726	726	749	755	768	
	54	589	584	595	629	629	630	650	631	634	
	58	627	626	640	652	665	655	661	640	662	
Total											
Mean											

BODYWEIGHT (g) Experiment: 1-314

Group: 2♂ 12.5%

Cage	Rat No.	1/12 Week No. 18	1/26 19	2/2 20	2/9 21	2/16 22	2/23 23	3/2 24	3/9 25	3/16 26	3/23/23 27
8	62	662	650	672	662	680	670	699	687	684	
	66	647	650	655	655	697	700	725	734	740	
	70	650	644	644	651	682	684	702	701	725	
	74	680	684	695	700	728	720	742	738	736	
	78	604	604	604	600	620	615	628	637	650	
Total		12133	12054	12209	12416	12941	12728	13060	12953	13080	
Mean		607	603	610	621	637	637	653	648	654	
Total											
Mean											
Total											
Mean											

BODYWEIGHT (g)

Experiment: 1-314

Group: 3F 25 %

	Animal No.	Week No. -2	-1	0	1	2	3	4	5	6	7
9.	3	150	213	264	298	296	336	331	357	394	406
	7	146	215	281	302	318	390	396	444	480	482
	11	150	198	241	302	299	357	365	409	463	469
	15	138	187	234	295	288	334	331	366	407	413
	19	151	232	281	298	335	388	397	455	500	520
Total											
Mean											
10	23	161	218	274	315	331	362	371	387	436	433
	29	171	225	291	331	328	393	421	429	474	475
	31	162	230	299	307	332	400	428	445	490	482
	35	159	211	199	239	344	390	421	436	501	498
	39	141	221	270	339	351	324	376	345	414	422
Total											
Mean											
11	43	181	220	270	339	381	412	444	471	506	528
	47	171	118	256	315	341	269	322	362	413	422
	51	175	182	216	282	330	328	371	388	430	451
	55	162	228	229	288	341	396	411	445	500	583
	59	175	119	229	319	325	384	405	427	469	488

BODYWEIGHT (g)

Experiment: 1-314

Group: 3F 25 %

	Animal No.	Week No. -2	-1	0	1	2	3	4	5	6	7
12.	63	187	240	299	325	347	344	361	437	478	525
	67	174	235	292	327	347	341	351	389	457	441
	71	170	238	297	337	362	368	384	452	448	540
	75	157	259	339	307	367	385	401	471	500	531
	79	157	256	307	340	360	372	401	466	514	522
Total											
Mean											
Total		9358	3994	5377	6069	6595	7273	7650	8373	9294	9591
Mean		168	200	269	303	330	364	382	420	465	480

BODYWEIGHT (g) Experiment: 1-314

Group: 3 ♂ 25 %

CAGE	ANIMAL NUMBER	11/6/72 Week No. 8	11/13/72 9	11/24/72 10	12/1 11	12/8 12	12/15 13	12/22 14	12/29 15	1/5/73 16	1/12/73 17
9	3	437	444	458	480	483	505	502	520	531	550
	7	521	530	538	545	560	570	626	573	582	620
	11	515	527	540	550	568	582	590	592	600	625
	15	448	460	475	490	508	527	555	550	567	592
	19	556	577	595	600	620	636	652	681	700	719
Total											
Mean	23	481	481	502	510	525	542	556	568	586	614
10	27	525	542	530	545	570	587	585	611	622	643
	31	523	530	542	550	580	601	601	616	633	666
	35	540	560	575	568	605	616	616	632	655	675
	39	456	490	500	510	522	552	550	565	584	612
Total											
Mean	43	561	580	605	598	600	621	629	642	666	677
11	47	468	476	510	514	532	547	540	576	594	609
	51	493	516	551	552	577	580	585	611	623	642
	55	564	578	606	615	626	634	641	655	635	711
	59	528	533	555	558	573	598	593	615	643	650

BODYWEIGHT (g) Experiment: 1-314

Group: 3 ♂ 25 %

CAGE	ANIMAL NUMBER	11/6/72 Week No. 8	11/13/72 9	11/24/72 10	12/1 11	12/8 12	12/15 13	12/22 14	12/29 15	1/5/73 16	1/12/73 17
12	63	573	570	618	605	615	612	622	643	592	[illegible]
	67	471	482	508	511	540	538	538	545	576	[illegible]
	71	578	585	600	608	620	614	619	634	655	702
	75	562	573	600	597	600	601	601	601	614	653
	79	583	595	618	612	625	629	631	642	677	737
Total											
Mean											
Total		10383	10654	11025	11121	11444	11682	11532	12073	12465	13015
Mean		519	533	551	556	572	585	592	604	623	651

BODYWEIGHT (g) Experiment: 1-314

Group: 3♂ 25%

Cage	Rat No.	1/12 Week No. 18	1/26 19	2/2 20	2/9 21	2/16 22	2/23 23	3/2 24	3/9 25	3/16 26	3/23/23 27
9	3	550	556	550	565	678	680	600	580	592	
	7	630	610	615	640	680	660	665	650	660	
	11	650	693	650	668	681	690	706	712	726	
	15	600	600	608	610	625	639	652	648	656	
	19	731	731	721	750	760	760	780	770	784	
Total											
Mean											
16	23	619	619	620	630	635	640	651	643	650	
	27	660	666	670	685	704	700	729	708	714	
	31	675	676	680	690	710	700	730	732	730	
	35	693	691	691	705	730	720	740	730	730	
	39	620	630	620	644	647	651	670	664	670	
Total											
Mean											
11	43	693	680	696	699	720	730	750	740	744	
	47	631	625	636	636	670	669	689	650	694	
	51	663	652	657	657	678	675	690	698	713	
	55	725	717	718	720	752	752	771	758	770	
	59	662	656	674	670	655	690	710	701	718	
Total											

BODYWEIGHT (g) Experiment: 1-314

Group: 3♂ 25%

Cage	Rat No.	1/12 Week No. 18	1/26 19	2/2 20	2/9 21	2/16 22	2/23 23	3/2 24	3/9 25	3/16 26	3/23/23 27
12	63	720	702	707	700	730	730	778	768	792	
	67	616	612	620	605	621	610	640	590	601	
	71	718	707	722	710	730	730	770	768	771	
	75	672	682	660	660	676	665	709	701	713	
	79	737	722	727	731	729	730	760	764	784	
Total		13250	13147	13242	13365	13521	13321	14180	14600	14222	
Mean		663	657	662	668	671	691	709	700	710	
Total											
Mean											

BODYWEIGHT (g)

Group: 4 ♂ 50%

Experiment: 2-374

	Animal No.	Week No. -2	-1	0	1	2	3	4	5	6	7
	4	146	153	211	257	261	324	342	342	400	410
	8	150	217	281	340	352	398	401	423	473	480
13	12	147	200	261	247	255	367	371	374	439	437
	16	145	177	251	305	332	363	361	367	412	419
	20	145	176	241	300	311	370	370	382	436	442
Total											
Mean											
	24	167	202	291	307	346	369	367	400	463	465
	28	165	207	271	241	307	374	372	414	458	481
	32	172	222	207	297	335	389	374	413	470	483
14	36	162	203	257	307	342	363	358	401	450	450
	40	160	207	277	309	255	368	363	397	448	464
Total											
Mean											
	44	181	123	197	277	328	390	402	447	490	496
	48	181	118	261	307	352	386	403	433	466	478
15	52	178	210	209	294	350	412	420	464	503	531
	56	173	176	246	240	327	358	365	387	420	425
	60	174	110	167	207	238	278	264	284	339	339

BODYWEIGHT (g)

Group: 4 ♂ 50%

Experiment: 1-374

	Animal No.	Week No. -2	-1	0	1	2	3	4	5	6	7
	64	170	124	209	257	313	322	311	384	421	417
	68	175	254	314	287	335	375	344	413	461	452
16	72	173	145	277	209	314	381	366	444	475	479
	76	181	248	307	327	352	376	350	431	425	475
	80	176	240	297	310	342	353	344	407	442	462
Total		3349	3811	5061	5720	6493	7316	7246	9007	9231	9085
Mean		167	191	253	286	325	366	362	400	447	454

C

BODYWEIGHT (g) Experiment: 1-314

Group: 4 ♂ 50 %

CAGE	ANIMAL NUMBER	11/6/72 Week No. 8	11/13/72 9	11/24/72 10	12/1 11	12/8 12	12/15 13	12/22 14	12/29 15	1/5/73 16	1/12/73 17
	4	403	408	410	420	430	444	436	441	457	476
	8	515	532	550	565	578	574	555	556	584	604
13	12	471	476	480	510	512	521	511	504	522	544
	16	454	471	480	495	508	522	510	512	534	550
	20	490	494	500	530	531	533	520	527	553	571
Total											
Mean	24	510	513	540	545	560	575	577	588	610	634
	28	527	535	550	570	595	615	611	621	660	681
	32	513	521	535	550	560	575	580	584	600	623
	36	500	511	518	530	540	550	548	550	560	584
14	40	511	511	529	548	564	575	576	586	600	619
Total											
Mean	44	526	559	582	600	612	631	650	659	686	711
	48	502	530	550	561	568	577	564	584	605	612
	52	543	561	584	586	600	606	607	626	638	651
15	56	454	467	490	509	519	531	537	542	570	584
	60	363	385	410	420	440	457	467	467	480	500

BODYWEIGHT (g) Experiment: 1-314

Group: 4 ♂ 50 %

CAGE	ANIMAL NUMBER	11/6/72 Week No. 8	11/13/72 9	11/24/72 10	12/1 11	12/8 12	12/15 13	12/22 14	12/29 15	1/5/73 16	1/12/73 17
	64	438	446	479	501	512	503	508	521	542	552
	68	485	500	529	554	560	565	574	600	600	623
16	72	513	528	533	542	590	601	614	629	652	683
	76	511	528	550	553	565	574	586	607	620	640
	80	500	511	535	558	538	569	577	614	626	650
Total											
Mean											
Total		9731	9991	10333	10657	10352	11103	11110	11318	11697	12092
Mean		487	500	517	533	544	555	556	566	585	605

BODYWEIGHT (g) Experiment: 1-314

Group: 4 ♂ 50%

Cage	Rat No.	1/12 Week No. 18	1/26 19	2/2 20	2/9 21	2/16 22	2/23 23	3/2 24	3/9 25	3/16 26	3/23/23 27
13	4	493	500	512	530	501	529	541	517	535	
	8	614	613	620	630	649	660	680	657	680	
	12	560	550	560	565	598	585	610	592	605	
	16	555	547	555	565	580	572	599	580	587	
	20	583	573	585	585	600	595	625	597	607	
Total											
Mean											
14	24	648	656	650	675	689	689	711	670	700	
	28	696	711	705	719	743	732	769	750	770	
	32	618	616	620	623	642	630	642	624	630	
	36	600	600	590	608	610	610	630	620	620	
	40	641	638	642	659	680	665	691	654	689	
Total											
Mean											
15	44	715	700	710	720	741	632	635	660	767	
	48	606	602	605	615	637	640	661	665	670	
	52	654	640	645	665	693	695	709	718	730	
	56	587	590	603	600	623	623	640	640	650	
	60	504	502	520	515	532	525	548	550	559	
Total											
Mean											

BODYWEIGHT (g) Experiment: 1-314

Group: 4 ♂ 50%

Cage	Rat No.	1/12 Week No. 18	1/26 19	2/2 20	2/9 21	2/16 22	2/23 23	3/2 24	3/9 25	3/16 26	3/23/23 27
16	64	577	570	582	590	610	610	630	609	630	
	68	637	625	650	648	678	680	719	690	700	
	72	684	680	700	705	740	740	760	757	765	
	76	651	643	660	665	690	695	710	698	708	
	80	659	656	666	672	706	702	692	712	718	
Total		12266	12212	12389	12559	12812	12809	13202	12946	13316	
Mean		614	611	619	628	641	640	660	648	666	
Total											
Mean											

BODYWEIGHT (g) Experiment: 1-314

Group: 1 ♀ Control

	Animal No.	Week No. -2	-1	0	1	2	3	4	5	6	7
17	51	142	162	197	226	273	295	296	306	326	324
	56	136	156	189	145	206	205	236	239	266	270
	69	140	161	207	213	237	244	252	236	263	272
	73	140	170	219	145	177	233	242	252	270	273
	77	138	150	194	188	227	233	244	229	264	260
Total											
Mean											
18	101	127	155	184	204	236	244	250	243	267	270
	105	131	171	219	219	229	270	265	262	300	288
	109	133	120	189	211	235	246	250	266	290	286
	113	130	156	181	192	203	210	222	238	252	250
	117	130	135	191	184	233	250	253	238	271	267
Total											
Mean											
19	121	144	135	201	219	235	262	261	272	290	291
	125	140	172	199	216	229	248	245	237	261	259
	149	147	182	209	225	222	256	261	267	287	281
	133	140	108	159	191	206	230	233	233	262	250
	137	150	199	239	182	241	292	304	311	338	336

BODYWEIGHT (g) Experiment: 1-314

Group: 1 ♀ Control

	Animal No.	Week No. -2	-1	0	1	2	3	4	5	6	7
20	141	120	120	219	241	253	288	279	286	321	310
	145	128	155	189	200	229	234	236	250	287	272
	149	124	161	189	209	238	246	244	249	286	271
	153	120	158	186	184	205	226	227	219	246	245
	157	123	142	159	171	214	226	224	215	250	255
Total		2683	3011	3933	4021	4565	4938	5024	5048	5587	5530
Mean		134	151	197	201	228	247	251	252	280	277

BODYWEIGHT (g) Experiment: 1-314

Group: 1 ♀ Control

CAGE	ANIMAL NUMBER	11/6/72 Week No. 8	11/13/72 9	11/24/72 10	12/1 11	12/8 12	12/15 13	12/22 14	12/29 15	1/5/73 16	1/12/73 17
17	81	350	342	342	350	351	353	353	339	371	391
	85	288	276	270	288	285	277	280	284	295	300
	89	287	290	294	304	310	313	307	312	323	348
	93	298	300	290	300	300	301	300	300	312	350
	97	286	294	287	295	300	305	300	303	336	337
Total											
Mean	101	292	298	300	300	307	303	300	300	285	310
18	105	302	311	311	326	323	334	321	315	341	350
	109	297	313	309	314	320	330	312	313	333	344
	113	270	266	278	276	276	276	267	268	280	289
	117	280	281	290	300	310	312	304	305	313	323
Total											
Mean	121	313	305	315	322	330	333	319	319	326	328
19	125	277	281	283	290	297	303	281	293	305	310
	149	304	314	318	335	340	349	342	350	359	373
	133	281	283	279	295	300	296	304	306	313	321
	137	361	356	360	375	372	367	366	365	381	391

BODYWEIGHT (g) Experiment: 1-314

Group: 1 ♀ Control

CAGE	ANIMAL NUMBER	11/6/72 Week No. 8	11/13/72 9	11/24/72 10	12/1 11	12/8 12	12/15 13	12/22 14	12/29 15	1/5/73 16	1/12/73 17
20	141	329	333	325	330	325	330	326	320	321	333
	145	294	302	308	305	308	316	319	303	317	331
	149	295	304	300	305	305	320	311	311	327	342
	153	266	264	268	270	275	289	274	270	277	293
	157	270	280	290	290	292	302	300	300	316	319
Total											
Mean											
Total		5942	5993	6003	6170	6222	6309	6181	6176	6431	6693
Mean		297	300	300	309	311	315	309	309	322	334

BODYWEIGHT (g) Experiment: 1-314

Group: 14 Control

Cage	Rat No.	Week No. 18	1/20 19	1/27 20	2/4 21	2/11 22	2/18 23	2/25 24	3/4 25	3/11 26	3/18/23 27
17	81	420	390	392	392	400	395	400	390	386	
	85	320	310	312	309	330	305	318	301	298	
	89	353	333	343	340	360	360	365	337	343	
	93	356	336	332	332	352	330	340	338	320	
	97	353	336	344	352	392	360	365	374	357	
Total											
Mean											
18	101	319	315	310	304	334	310	330	317	330	
	105	363	345	350	355	390	360	380	355	370	
	109	350	350	352	358	378	360	381	352	350	
	113	304	304	300	303	319	292	318	290	298	
	117	326	325	325	330	344	335	350	330	328	
Total											
Mean											
19	121	341	333	330	331	357	331	349	330	334	
	125	344	322	318	315	325	310	325	317	320	
	129	392	380	370	379	412	409	437	428	436	
	133	315	310	310	310	340	310	328	320	394	
	137	413	400	409	385	411	390	411	384	404	
Total											
Mean											

BODYWEIGHT (g) Experiment: 1-314

Group: 14 Control

Cage	Rat No.	Week No. 18	1/20 19	1/27 20	2/4 21	2/11 22	2/18 23	2/25 24	3/4 25	3/11 26	3/18/23 27
20	141	352	335	340	330	356	340	361	342	340	
	145	336	336	339	338	361	340	357	328	335	
	149	340	340	345	340	370	349	359	345	340	
	153	302	298	292	292	311	301	320	315	311	
	157	319	320	328	320	341	330	339	330	332	
Total		6938	6718	6746	6705	7183	6817	7433	6813	6856	
Mean		347	336	337	335	359	341	372	341	343	
Total											
Mean											
Total											
Mean											

C

DYWEIGHT (g) Experiment: 1-314

oup: 21 12.5%

	Animal No.	Week No. -2	-1	0	1	2	3	4	5	6	7
	82	132	100	169	178	209	216	211	219	243	251
	86	144	176	197	189	217	249	221	262	281	280
	90	136	167	277	225	216	245	241	255	225	280
21	94	140	175	220	217	244	260	225	268	294	296
	98	134	170	209	208	235	248	229	259	296	281
otal											
ean											
	102	131	118	179	159	213	238	221	232	261	250
	106	127	125	179	158	211	219	233	222	252	249
12	110	122	157	179	203	223	231	226	228	255	246
	114	136	167	201	221	206	258	246	260	274	278
	118	130	186	161	190	202	229	227	238	263	260
otal											
ean											
	122	141	189	171	216	213	260	254	241	270	275
	126	144	185	178	183	231	268	269	266	302	305
13	130	152	130	178	223	229	255	255	257	296	296
	134	146	122	177	214	211	259	261	267	290	297
	138	142	169	179	206	222	236	252	259	279	286

BODYWEIGHT (g) Experiment: 1-314

Group: 29 12.5%

	Animal No.	Week No. -2	-1	0	1	2	3	4	5	6	7
	142	133	178	207	217	223	268	270	272	307	306
	146	126	163	200	219	241	250	257	259	294	295
	150	130	179	206	227	241	268	277	271	325	305
24	154	138	96	169	179	230	260	258	277	311	308
	158	124	195	179	196	187	216	214	216	242	240
Total											
Mean											
Total		2710	3100	3903	4032	4434	4933	4849	5038	5610	5587
Mean		136	155	195	202	222	247	242	252	281	279

BODYWEIGHT (g) Experiment: I-314

Group: 2 ♀ 12.5 %

CAGE	ANIMAL NUMBER	11/6/72 Week No. 8	11/13/72 9	11/20/72 10	11/27 11	12/4 12	12/11 13	12/18 14	12/25 15	1/5/73 16	1/12/73 17
	82	269	269	258	275	278	274	268	268	282	289
	86	303	306	300	315	315	311	314	309	333	326
	90	307	293	300	316	316	325	333	345	352	365
21	94	316	314	320	326	326	327	324	324	341	342
	98	298	298	301	314	340	321	314	315	338	336
Total											
Mean	102	277	281	300	290	288	300	300	303	303	316
	106	267	270	271	270	262	271	271	273	276	296
	110	265	269	280	278	278	282	282	286	300	305
	114	300	303	309	308	306	329	374	354	350	345
22	118	280	275	295	300	294	300	301	303	307	319
Total											
Mean	122	295	291	311	314	310	313	300	301	325	323
	126	328	320	325	328	330	341	335	337	346	364
	130	318	308	318	332	332	347	351	335	319	368
23	134	314	317	320	321	324	329	325	317	325	341
	138	320	308	312	311	310	328	328	323	328	339

BODYWEIGHT (g) Experiment: I-314

Group: 2 ♀ 12.5 %

CAGE	ANIMAL NUMBER	11/6/72 Week No. 8	11/13/72 9	11/20/72 10	11/27 11	12/4 12	12/11 13	12/18 14	12/25 15	1/5/73 16	1/12/73 17
	142	320	321	322	332	325	333	325	333	353	354
	146	301	312	311	315	313	305	304	291	309	310
	150	326	340	350	350	355	376	375	373	389	394
24	154	330	329	333	340	335	340	336	331	349	352
	158	261	261	263	270	268	275	262	255	275	285
Total											
Mean											
Total		6002	5995	6098	6205	6205	6327	6322	6276	6498	6662
Mean		300	300	305	310	310	316	316	313	325	333

BODYWEIGHT (g) Experiment: 1-314

Group: 2 ♀ 12.5%

Cage	Rat No.	Week No. 18	1/26 19	2/2 20	2/9 21	2/16 22	2/23 23	3/2 24	3/9 25	3/16 26	3/23 27
21	82	303	282	300	290	320	300	305	291	291	
	86	340	330	350	346	369	355	362	347	348	
	90	375	344	344	342	370	350	355	352	360	
	94	362	362	320	360	391	375	382	366	367	
	98	344	340	357	342	366	360	370	350	350	
Total											
Mean											
22	102	321	314	311	311	348	320	335	324	330	
	106	307	297	300	301	322	310	330	311	308	
	110	314	305	300	309	338	331	325	301	312	
	114	367	360	360	352	378	370	380	348	360	
	118	332	325	311	318	359	320	352	325	337	
Total											
Mean											
23	122	349	342	342	340	360	340	361	309	343	
	126	380	369	365	360	398	380	383	375	374	
	130	375	344	352	350	409	380	392	390	397	
	134	356	354	362	365	385	370	390	357	384	
	138	361	350	332	335	362	350	361	340	346	
Total											
Mean											

BODYWEIGHT (g) Experiment: 1-314

Group: 2 ♀ 12.5%

Cage	Rat No.	Week No. 18	1/26 19	2/2 20	2/9 21	2/16 22	2/23 23	3/2 24	3/9 25	3/16 26	3/23 27
24	142	380	370	365	355	385	370	381	375	384	
	146	329	330	332	330	346	325	331	334	328	
	150	411	390	392	385	418	410	411	405	420	
	154	372	370	360	369	385	370	387	375	371	
	158	300	270	290	290	315	290	309	290	287	
Total		6978	6795	6795	6744	7364	6976	7202	6885	6997	
Mean		349	340	340	337	367	349	360	344	350	
Total											
Mean											
Total											
Mean											

BODYWEIGHT (g)

Experiment: 1-314

Group: 1 ♂ Control (50% Sucrose)

CAGE	ANIMAL NUMBER	1/19 Week No 18	1/26 19	2/2 20	2/9 21	2/16 22	2/23 23	3/2 24	3/9 25	3/16 26	3/23/73 27
1	1	600	588	602	611	619	626	633	618	630	
	5	617	615	620	650	648	650	660	660	664	
	9	609	617	615	634	639	640	661	640	642	
	13	600	600	610	630	632	628	640	632	640	
	17	662	665	670	680	701	706	718	705	707	
Total											
Mean											
2	21	575	574	580	590	615	600	618	598	614	
	25	621	600	611	630	650	645	660	650	663	
	29	670	672	670	690	711	705	720	704	727	
	33	611	614	620	632	638	650	666	648	667	
	37	630	633	632	639	669	665	680	660	680	
Total											
Mean											
3	41	671	691	670	690	696	700	715	714	720	
	45	606	600	600	605	601	621	618	575	586	
	49	664	672	670	694	703	691	721	720	724	
	53	610	604	620	630	648	641	660	670	668	
	57	727	715	725	747	780	762	788	782	791	

BODYWEIGHT (g)

Experiment: 1-314

Group: 1 ♂ Control (50% Sucrose)

Cage	Rat No.	1/19 Week No 18	1/26 19	2/2 20	2/9 21	2/16 22	2/23 23	3/2 24	3/9 25	3/16 26	3/23/73 27
4	61	640	522	540	570	602	610	621	606	600	
	65	775	765	773	773	805	800	820	816	834	
	69	640	635	637	640	660	660	680	668	670	
	73	663	650	670	661	691	690	710	698	707	
	77	602	600	606	601	621	625	630	628	640	
Total		12793	12660	12741	12998	13328	13315	13619	13398	13592	
Mean		640	633	637	650	666	666	681	670	680	
Total											
Mean											
Total											
Mean											

BODYWEIGHT (g) Experiment: 1-314

Group: 3♂ 25%

	Animal No.	Week No. -2	-1	0	1	2	3	4	5	6	7
25	83	144	173	209	226	245	250	221	288	303	300
	87	144	145	199	211	223	243	211	266	288	284
	91	116	98	162	200	222	228	212	232	255	260
	95	137	152	187	238	252	248	214	265	294	293
	99	142	178	217	237	266	268	200	296	318	311
Total											
Mean											
26	103	134	162	209	197	247	278	277	292	303	318
	107	130	167	209	199	223	230	236	243	286	260
	111	130	148	189	212	210	252	247	249	284	290
	115	135	170	181	223	236	251	256	268	291	291
	119	132	190	218	227	243	268	262	277	305	290
Total											
Mean											
27	123	152	161	198	173	220	251	261	258	286	283
	127	141	156	199	224	231	290	287	292	337	332
	131	150	175	177	224	247	293	254	303	322	331
	135	146	184	207	226	223	264	267	269	300	300
	139	142	188	219	222	241	280	250	269	301	292

BODYWEIGHT (g) Experiment: 1-314

Group: 3♂ 25%

	Animal No.	Week No. -2	-1	0	1	2	3	4	5	6	7
28	143	136	161	177	207	238	250	253	258	275	276
	147	124	178	201	218	234	274	272	274	300	301
	151	121	116	169	170	264	289	302	306	336	353
	155	136	98	167	189	216	243	234	253	292	280
	159	129	156	189	204	221	243	241	242	268	268
Total											
Mean											
Total		2749	3106	3930	4229	4705	5103	5107	5470	5722	5893
Mean		137	155	197	211	235	260	255	270	296	295

BODYWEIGHT (g) Experiment: F 314

Group: 3 ♀ 25 %

CAGE	ANIMAL NUMBER	Week No. 8	9	10	11	12	13	14	15	16	17
25	83	326	327	320	337	335	341	335	336	357	363
	87	308	312	308	319	350	322	320	321	352	350
	91	277	276	284	290	292	295	300	300	310	313
	95	314	318	319	326	325	313	312	310	317	330
	99	350	342	340	371	375	409	392	421	430	419
Total											
Mean	103	333	333	326	325	330	338	324	334	350	346
26	107	282	279	290	290	283	284	279	282	300	314
	111	311	312	312	320	322	330	324	315	336	350
	115	319	311	325	325	326	344	340	342	350	361
	119	322	323	329	330	325	334	322	323	326	332
Total											
Mean	123	312	300	289	300	295	297	274	287	300	317
27	127	354	344	355	370	365	370	371	366	390	394
	131	350	333	342	360	360	360	357	358	370	375
	135	317	311	316	325	320	321	320	312	329	340
	139	309	304	309	315	305	319	306	295	307	318

BODYWEIGHT (g) Experiment: F 314

Group: 3 ♀ 25 %

CAGE	ANIMAL NUMBER	Week No. 8	9	10	11	12	13	14	15	16	17
28	143	293	278	289	302	305	304	301	301	333	339
	147	318	328	335	339	340	351	360	352	372	385
	151	352	355	366	355	365	376	368	373	391	400
	155	296	303	314	308	320	333	339	327	355	364
	159	295	300	289	309	320	302	305	316	316	327
Total											
Mean											
Total		6333	6297	6374	6519	6508	6623	6554	6562	6977	7067
Mean		317	315	319	326	325	332	327	328	344	353

BODYWEIGHT (g) Experiment: 1-314

Group: 3♀ 25%

Cage	Rat No.	1/12 Week No. 18	1/26 19	2/2 20	2/9 21	2/16 22	2/23 23	3/2 24	3/9 25	3/16 26	3/23/75 27
	83	392	364	388	390	413	425	422	407	427	
	87	350	322	333	340	361	362	370	373	390	
	91	326	310	325	315	348	340	341	344	350	
25	95	342	327	331	330	355	340	351	344	342	
	99	447	444	450	450	488	481	493	500	498	
Total											
Mean											
	103	366	344	350	350	371	360	370	358	360	
	107	331	300	300	310	328	310	331	320	320	
	111	353	350	355	350	370	355	376	368	360	
26	115	375	360	360	360	381	370	380	372	364	
	119	352	340	345	342	372	365	370	366	370	
Total											
Mean											
	123	341	316	322	320	345	315	335	318	320	
	127	415	405	408	400	435	419	429	418	418	
	131	400	381	388	383	412	392	401	378	398	
	135	344	320	331	335	357	341	361	320	327	
27	139	324	319	320	321	346	320	332	320	312	
Total											
Mean											

BODYWEIGHT (g) Experiment: 1-314

Group: 3♀ 25%

Cage	Rat No.	1/12 Week No. 18	1/26 19	2/2 20	2/9 21	2/16 22	2/23 23	3/2 24	3/9 25	3/16 26	3/23/75 27
	143	342	320	331	320	345	340	350	334	332	
	147	421	386	390	383	405	401	419	403	404	
	151	422	400	400	391	418	418	452	444	425	
28	155	378	358	361	361	378	370	390	374	344	
	157	342	320	311	312	332	318	350	340	334	
Total		7368	6986	7079	7048	7560	7342	7623	7401 PK	7425	
Mean		368	349	355	352	378	367	381	370	371	
Total											
Mean											
Total											
Mean											

BODYWEIGHT (g) Experiment: 1-314

Group: 41 50%

	Animal No.	Week No. -2	-1	0	1	2	3	4	5	6	7
29	84	144	121	179	201	217	243	236	235	263	263
	88	138	141	219	227	261	272	275	277	301	306
	92	140	131	179	208	223	248	244	244	270	274
	96	142	126	179	197	221	227	227	217	250	250
	100	134	119	169	189	219	231	233	239	264	269
Total											
Mean											
30	104	140	171	219	229	247	263	264	255	281	280
	108	131	133	189	207	233	251	243	251	310	287
	112	121	149	201	171	203	242	251	241	282	281
	116	126	156	191	212	219	250	253	251	289	278
	120	125	130	171	200	217	231	233	229	264	270
Total											
Mean											
31	124	143	182	211	224	223	271	271	275	303	305
	128	150	159	179	221	254	282	283	281	321	330
	132	150	179	219	221	246	272	275	277	307	304
	136	146	125	169	200	206	243	244	235	272	271
	140	141	152	199	190	199	274	269	268	303	320

BODYWEIGHT (g) Experiment: 1-314

Group: 47 50%

	Animal No.	Week No. -2	-1	0	1	2	3	4	5	6	7
32	144	130	163	179	199	222	242	252	255	276	275
	148	127	163	181	209	229	248	244	263	[illegible]	284
	152	126	161	206	219	235	250	258	259	[illegible]	270
	156	126	170	169	175	203	231	245	256	[illegible]	283
	160	131	168	209	209	230	255	256	269	280	283
Total		2711	2994	3825	4112	4476	5031	5056	5077	5672	5723
Mean		136	150	191	206	224	252	253	254	284	285

BODYWEIGHT (g) Experiment: F-314

Group: 4 ♀ 50%

CAGE	ANIMAL NUMBER	11/6/92 Week No. 8	11/13/92 9	11/20/92 10	12/1 11	12/8 12	12/15 13	12/22 14	12/29 15	1/5/93 16	1/12/93 17
29	84	274	274	278	280	290	297	292	292	294	303
	88	312	318	312	328	328	315	314	310	328	327
	92	290	281	290	289	288	280	278	280	306	300
	96	270	266	270	288	295	306	314	302	322	333
	100	293	290	296	300	302	300	306	291	317	324
Total											
Mean											
30	104	304	304	307	305	304	306	304	279	309	324
	108	303	313	315	318	325	327	318	311	328	340
	112	296	300	310	300	300	303	290	290	302	321
	116	281	287	298	300	295	291	279	286	290	308
	120	282	282	290	290	290	301	296	289	300	300
Total											
Mean											
31	124	318	309	311	330	325	322	311	308	331	330
	128	339	336	342	340	341	340	338	344	355	356
	132	336	330	340	345	340	353	351	342	353	373
	136	273	282	288	285	280	287	277	267	290	294
	140	314	312	309	335	320	336	300	310	307	326

BODYWEIGHT (g) Experiment: F-314

Group: 4 ♀ 50%

CAGE	ANIMAL NUMBER	11/6/92 Week No. 8	11/13/92 9	11/20/92 10	12/1 11	12/8 12	12/15 13	12/22 14	12/29 15	1/5/93 16	1/12/93 17
32	144	291	296	288	296	290	289	291	286	292	302
	148	300	288	289	300	300	300	304	292	311	321
	152	311	299	290	301	303	308	302	304	317	334
	156	318	323	295	317	317	311	303	300	322	325
	160	309	301	300	308	308	302	300	301	321	323
Total											
Mean											
Total		6014	5952	6018	6155	6141	6172	6085	5984	6291	6464
Mean		301	298	301	308	307	309	301	299	315	323

BODYWEIGHT (g) Experiment: 1-314

Group: 49 50%

Cage	Rat No.	1/12 Week No. 18	1/26 19	2/2 20	2/9 21	2/16 22	2/23 23	3/2 24	3/9 25	3/16 26	3/23/23 27
29	54	327	308	312	301	340	310	319	312	307	
	58	352	327	340	351	379	350	360	347	350	
	92	312	300	308	305	331	300	308	301	309	
	96	340	316	328	329	349	340	345	333	340	
	100	340	320	325	330	352	335	358	348	340	
Total											
Mean											
30	104	346	312	320	320	346	325	331	314	320	
	108	360	320	320	302	348	328	341	330	345	
	112	341	320	320	320	355	320	339	328	329	
	116	307	300	309	308	340	311	322	306	307	
	120	319	312	318	318	345	330	341	302	328	
Total											
Mean											
31	124	341	327	327	312	368	360	360	340	354	
	128	373	350	350	350	380	352	371	357	360	
	132	372	372	368	360	392	372	390	380	390	
	136	319	272	281	295	328	300	321	298	300	
	140	335	312	325	330	352	340	340	327	330	
Total											
Mean											

BODYWEIGHT (g) Experiment: 1-314

Group:

Cage	Rat No.	1/12 Week No. 18	1/26 19	2/2 20	2/9 21	2/16 22	2/23 23	3/2 24	3/9 25	3/16 26	3/23/23 27
32	144	316	300	306	300	322	320	320	307	307	
	148	340	315	320	312	348	321	342	339	339	
	152	339	320	323	318	341	330	331	340	334	
	156	340	316	326	320	340	335	345	335	334	
	160	343	338	342	329	359	340	370	341	348	
Total		6267	6337	6468	6410	7015	6619	6860	6598	6651	
Mean		338	319	323	321	351	331	343	330	333	
Total											
Mean											
Total											
Mean											

APPENDIX 2

Individual Cage Food Consumption (gm/wk)

Group Sex	Cage No.	WEEK -1	0	1	2	3	4	5	6	7
1 ♂ Control	1	700	1005	974	1006	760	1015	926	1088	1000
	2	755	978	987	1045	1070	1028	1039	1142	1042
	3	800	901	766	890	1020	1030	1155	1097	1087
	4	680	911	1043	1021	1118	1035	1136	1095	1158
2 ♂ 12.5%	5	740	1250	1008	1019	940	1018	1000	1041	1004
	6	710	937	951	1062	1030	1026	1158	1129	983
	7	665	809	628	933	1000	1068	1066	1014	989
	8	675	742	1052	1021	1116	1038	1084	1110	1041
3 ♂ 25%	9	745	875	974	1024	920	1029	1037	1060	1028
	10	730	970	806	1008	1060	1020	963	1074	1016
	11	650	749	652	1006	932	954	1089	1116	1047
	12	760	729	1079	1000	1120	1127	1228	1270	1270
4 ♂ 50%	13	680	1050	959	911	1075	978	900	1046	1023
	14	695	904	939	1026	920	1050	991	1108	1067
	15	680	654	707	978	1070	1065	1026	1041	1024
	16	765	912	1072	1041	1000	1043	1225	1176	1050
1 ♀ Control	17	655	883	960	972	760	760	948	1090	1015
	18	625	801	888	827	720	676	689	846	814
	19	555	635	940	985	800	851	777	773	695
	20	570	664	804	922	700	889	732	671	741
2 ♀ 12.5%	21	675	841	800	953	750	766	700	742	713
	22	750	834	973	958	770	734	715	746	640
	23	750	834	973	958	770	734	715	746	726
	24	590	675	811	875	820	779	900	855	657
3 ♀ 25%	25	560	779	870	789	690	648	700	750	700
	26	650	949	924	958	770	830	730	769	700
	27	615	751	1035	986	850	808	863	908	1003
	28	445	701	465	930	800	739	727	779	695
4 ♀	29	590	827	586	763	700	762	600	789	696
	30	585	885	718	779	730	740	500	796	693
	31	570	873	978	893	800	720	750	766	778
	32	435	760	497	782	710	789	721	770	721

APPENDIX 2
(continued)

Individual Cage Food Consumption (gm/wk)

Group Sex	Cage No.	WEEK 8	9	10	11	12	13	14	15	16	17
1 ♂ Control	1	1067	1115	1076	1018	1100	1028	1062	1070	1078	1033
	2	1218	1027	1107	1212	1100	1034	952	1120	1128	1100
	3	989	1005	1128	1070	1150	1057	1093	1080	1080	1107
	4	1175	1038	1062	1052	1140	1063	1100	1130	1065	1088
2 ♂ 12.5%	5	1118	980	1079	1040	1170	1050	1097	1150	1192	1045
	6	1064	964	1043	965	1140	989	1073	1030	1100	1020
	7	1089	1018	1123	1018	1150	1043	1022	1080	1080	1110
	8	1127	1084	1200	1055	1170	1239	1000	1180	1304	1337
3 ♂ 25%	9	1150	992	1117	1035	1120	1042	1142	1090	1091	1044
	10	1116	1010	1026	1000	1170	1150	1125	1160	1200	1028
	11	1034	1170	1215	1046	1200	1114	726	1200	1317	1050
	12	1200	1055	1316	1040	1210	1250	1270	1200	1372	1200
4 ♂ 50%	13	1362	900	932	976	980	930	828	790	700	660
	14	1120	1032	1228	1217	1110	1080	992	1160	1283	1114
	15	1220	1067	1110	1100	1130	1020	1066	1070	1126	993
	16	1232	1000	1180	994	1010	1040	1137	1180	1150	1091
1 ♀ Control	17	872	858	1122	1000	964	1075	968	980	850	984
	18	905	654	704	678	760	718	689	760	670	668
	19	657	713	739	752	780	759	807	720	690	705
	20	734	665	647	664	680	667	665	670	650	617
2 ♀ 12.5%	21	736	663	705	750	780	635	717	790	700	660
	22	700	710	700	730	780	743	778	850	717	713
	23	745	700	789	780	790	800	788	780	770	788
	24	768	670	765	763	785	750	730	800	746	749
3 ♀ 25%	25	691	692	766	709	680	756	825	750	742	710
	26	817	650	755	726	750	723	700	750	740	624
	27	937	740	935	872	720	793	735	730	862	729
	28	722	802	770	768	790	730	735	820	850	750
4 ♀ 50%	29	494	685	848	718	730	700	669	750	709	680
	30	462	627	815	559	850	680	690	700	619	650
	31	480	692	900	730	750	738	1059	900	700	754
	32	530	639	750	712	670	690	612	680	600	632

APPENDIX 2
(continued)

Individual Cage Food Consumption (gm/wk)

Group Sex	Cage No.	18	19	20	21	22	23	24	25	26
1 ♂	1	950	1079	1100	1120	1225	1100	1120	975	940
Control	2	1034	1090	1175	1180	1165	1155	1100	1035	1160
	3	1013	1064	1089	1110	1130	1180	1120	1080	1080
	4	1041	972	995	1045	1060	1095	1195	1075	1040
2 ♂	5	989	1030	1062	1060	1140	1125	1050	710	1130
12.5%	6	983	1093	1155	1120	1200	1010	1130	1050	1080
	7	1077	1112	1163	1180	1120	1140	1180	1070	1270
	8	970	1170	1121	1180	1230	1235	1250	1025	1120
3 ♂	9	1030	1120	1128	1150	1140	1170	1180	1165	1160
25%	10	1084	1174	1157	1180	1200	1130	1180	1200	1200
	11	1099	1210	1200	1220	1210	1240	1225	1240	1240
	12	1097	1230	1234	1240	1230	1235	1240	1090	1220
4 ♂	13	892	983	1026	1065	1000	1010	1050	925	1010
50%	14	1021	1100	1130	1120	1080	1095	1235	970	1140
	15	923	1000	1017	1080	1100	1105	1055	1075	1100
	16	1057	1090	1167	1160	1160	1180	1110	1045	1180
1 ♀	17	782	869	779	1010	865	840	815	710	755
Control	18	633	692	814	760	740	800	800	670	660
	19	718	691	732	765	930	789	830	755	755
	20	626	683	700	850	700	680	720	620	625
2 ♀	21	636	717	757	775	810	700	670	635	745
12.5%	22	695	798	794	800	795	740	740	630	795
	23	727	789	779	835	930	770	760	670	815
	24	718	769	783	800	770	745	760	660	785
3 ♀	25	662	736	834	760	820	910	790	810	800
25%	26	626	734	735	730	690	715	720	785	700
	27	710	767	827	800	840	785	760	630	730
	28	761	792	900	960	930	750	860	815	850
4 ♀	29	668	750	764	710	745	720	700	615	740
50%	30	654	669	686	720	710	685	740	505	740
	31	689	748	825	820	770	710	680	690	715
	32	714	691	741	700	645	710	590	705	660

APPENDIX 3

Hematology - Individual Values (Pretreat)

Rat No.	Group	Week	Hct.	Hb g%	RBC x10^6 cmm	WBC x10^3 cmm	Differential % N	S	L	E	B	M
1	1 ♂	-1	47	15.6	7.43	14.9	10	0	90	0	0	0
5			60	19.0	9.10	6.1	34	0	66	0	0	0
9			43	14.4	9.96	8.8	27	0	73	0	0	0
13			48	16.2	7.91	17.7	61	0	39	0	0	0
17			57	18.5	9.81	13.0	17	1	81	0	0	1
21			55	17.4	8.31	11.5	17	0	83	0	0	0
25			49	16.6	8.06	16.2	13	1	85	1	0	0
29			41	14.0	5.71	17.3	28	0	72	0	0	0
33			44	14.7	8.08	7.3	22	0	78	0	0	0
37			39	13.6	6.65	10.8	36	1	63	0	0	0
4	4 ♂	-1	52	18.0	9.06	15.4	11	0	89	0	0	0
8			40	13.9	6.06	11.5	19	0	80	1	0	0
12			44	15.8	8.04	9.3	14	1	84	1	0	0
16			47	15.6	7.05	10.5	16	0	84	0	0	0
20			57	18.0	9.93	8.3	9	0	91	0	0	0
24			47	15.0	7.19	13.6	32	0	68	0	0	0
28			43	14.2	6.45	15.4	40	0	60	0	0	0
32			41	13.9	6.31	19.1	11	0	89	0	0	0
36			43	14.4	6.81	11.3	36	0	64	0	0	0
40			51	17.4	7.67	11.7	27	0	73	0	0	0
81	1 ♀	-1	45	14.7	7.72	12.4	23	0	77	0	0	0
85			49	16.7	8.47	10.0	19	0	81	0	0	0
89			44	14.2	8.16	11.1	26	0	74	0	0	0
93			49	15.3	8.44	11.8	9	0	91	0	0	0
97			54	17.4	8.91	20.1	50	0	50	0	0	0
101			45	15.0	7.70	9.5	13	1	86	0	0	0
105			42	14.2	7.15	15.9	37	2	60	0	0	1
109			44	15.0	7.80	9.2	27	0	73	0	0	0
113			60	19.0	8.12	16.3	27	1	71	1	0	0
117			53	17.0	7.07	15.4	44	2	54	0	0	0
84	4 ♀	-1	53	17.0	6.98	13.5	32	0	68	0	0	0
88			50	16.5	8.74	8.4	29	2	68	0	0	1
92			43	14.2	7.31	8.2	19	0	81	0	0	0
96			65	19.2	9.02	9.1	20	0	80	0	0	0
100			60	19.0	10.17	15.6	42	0	56	2	0	0
104			45	14.7	8.01	10.1	17	0	83	0	0	0
108			48	16.2	7.11	7.7	26	0	73	1	0	0
112			43	14.4	8.31	21.7	49	0	51	0	0	0
116			46	15.0	7.63	11.4	17	0	82	1	0	0
120			47	15.8	6.10	11.0	35	0	65	0	0	0

APPENDIX 3
(continued)

Hematology - Individual Values (Week 4)

Rat No.	Group	Week	Hct	Hb g%	RBC x10^6 cmm	WBC x10^3 cmm	Differential % N	S	L	E	B	M
1	1 ♂	4	48	15.8	8.37	22.8	18	2	80	0	0	0
5			50	16.7	8.84	15.4	14	0	86	0	0	0
9			49	16.2	8.79	12.9	26	0	74	0	0	0
13			52	17.0	7.18	15.2	40	0	60	0	0	0
17			49	15.8	7.96	24.3	25	0	75	0	0	0
21			51	17.0	7.91	15.0	16	0	84	0	0	0
25			52	17.4	8.64	15.9	16	0	84	0	0	0
29			48	15.8	7.36	16.7	17	0	82	1	0	0
33			48	16.2	8.55	18.8	18	0	82	0	0	0
37			53	17.6	7.44	9.9	26	0	74	0	0	0
4	4 ♂	4	53	17.6	8.18	15.0	23	0	77	0	0	0
8			50	16.5	6.87	17.7	11	1	88	0	0	0
12			51	17.0	8.93	12.6	13	0	87	0	0	0
16			50	16.7	8.12	14.9	21	1	78	0	0	0
20			55	18.0	8.82	10.7	14	2	84	0	0	0
24			52	17.0	8.02	15.7	17	0	83	0	0	0
28			52	17.4	7.15	18.5	20	0	80	0	0	0
32			56	18.5	7.74	20.5	18	0	82	0	0	0
36			53	17.6	7.31	15.6	15	1	84	0	0	0
40			49	16.2	6.98	21.6	17	0	83	0	0	0
81	1 ♀	4	52	17.4	8.68	12.3	21	0	79	0	0	0
85			49	15.8	7.40	7.9	13	1	85	1	0	0
89			52	17.0	8.41	11.9	19	0	79	2	0	0
93			50	16.7	8.64	7.8	34	0	66	0	0	0
97			52	17.4	8.63	9.4	23	2	72	3	0	0
101			46	14.7	6.72	11.6	18	2	79	1	0	0
105			47	15.8	7.66	8.6	35	1	64	0	0	0
109			48	16.7	7.83	8.9	23	0	77	0	0	0
113			47	15.0	7.68	9.1	14	1	83	2	0	0
117			50	16.7	6.92	12.1	43	0	57	0	0	0
84	4 ♀	4	51	16.7	7.21	8.3	27	0	71	2	0	0
88			53	17.4	8.66	12.1	32	0	68	0	0	0
92			50	16.5	7.43	11.2	17	0	82	1	0	0
96			55	18.0	8.29	7.7	30	0	70	0	0	0
100			51	17.0	7.20	9.5	17	0	83	0	0	0
104			55	18.0	8.76	11.0	36	1	63	0	0	0
108			50	16.5	7.09	7.7	39	0	61	0	0	0
112			43	14.7	8.63	15.5	33	1	65	1	0	0
116			48	15.6	7.03	11.1	19	0	79	2	0	0
120			52	17.0	6.90	8.0	29	0	66	5	0	0

APPENDIX 3
(continued)

Hematology - Individual Values (Week 13)

Rat No.	Group Sex	Week	Hct	Hb g%	RBC x10⁶ cmm	WBC x10³ cmm	Differential % N	S	L	E	B	M
1	1 ♂	13	48	15.8	8.10	13.7	17	0	81	2	0	0
5			50	17.0	8.89	14.3	15	1	84	0	0	0
9			48	15.6	8.64	13.4	28	1	70	1	0	0
13			49	16.2	6.28	16.9	17	1	81	1	0	0
17			47	16.2	8.29	14.1	22	0	77	1	0	0
21			52	17.4	7.78	18.8	14	1	83	2	0	0
25			48	16.5	6.27	19.1	9	1	87	3	0	0
29			49	16.5	6.14	12.8	12	0	87	1	0	0
33			49	16.5	8.11	14.5	27	1	72	0	0	0
37			50	16.7	6.05	10.3	15	0	80	4	0	1
4	4 ♂	13	50	17.0	7.12	11.9	38	2	56	3	0	1
8			49	16.5	5.17	17.7	18	0	81	1	0	0
12			50	16.7	7.30	13.7	18	0	79	3	0	0
16			52	17.4	8.27	14.7	25	2	70	2	0	1
20			50	17.4	7.51	10.2	21	0	79	0	0	0
24			49	16.5	6.83	16.4	21	0	78	1	0	0
28			59	18.5	8.95	14.5	33	0	63	3	0	1
32			50	17.0	7.53	17.1	15	0	84	1	0	0
36			51	17.0	6.79	12.0	20	0	80	0	0	0
40			51	17.0	6.27	13.5	15	0	85	0	0	0
81	1 ♀	13	48	16.7	7.40	17.6	27	0	73	0	0	0
85			46	16.5	6.83	11.7	17	0	83	0	0	0
89			46	16.2	7.00	12.7	10	1	87	2	0	0
93			42	15.0	6.38	28.1	8	0	88	4	0	0
97			48	16.2	8.00	11.7	19	1	79	1	0	0
101			46	16.2	6.25	12.3	10	1	86	3	0	0
105			46	16.7	7.53	10.4	27	0	73	0	0	0
109			46	16.2	6.83	10.7	15	1	82	1	0	1
113			47	16.2	7.02	9.5	10	0	90	0	0	0
117			48	16.7	7.34	11.1	42	2	56	0	0	0
84	4 ♀	13	47	16.0	7.09	12.9	20	0	77	3	0	0
88			47	16.0	7.80	8.6	27	0	72	1	0	0
92			45	16.2	6.98	12.3	20	1	77	2	0	0
96			46	16.2	7.62	12.4	31	0	66	3	0	0
100			48	15.9	6.83	12.8	26	0	72	2	0	0
104			45	16.2	7.50	7.7	19	0	77	3	0	1
108			44	16.2	6.78	11.5	19	0	78	3	0	0
112			44	16.2	8.00	13.4	23	0	76	1	0	0
116			44	15.5	6.51	8.4	10	0	85	4	0	1
120			45	16.2	5.96	11.5	28	1	68	3	0	0

APPENDIX 3
(continued)

Hematology - Individual Values (Week 26)

Rat No.	Group	Week	Hct.	Hb g%	RBC x10⁶ cmm	WBC x10³ cmm	Differential % N	S	L	E	B	M
1	1 ♂	26	47	13.9	7.88	23.4	11	0	89	0	0	0
5			53	14.2	8.30	14.7	24	1	73	2	0	0
9			46	13.9	6.96	12.1	13	0	87	0	0	0
13			52	15.0	9.10	18.8	27	2	71	0	0	0
17			49	14.7	8.15	9.9	13	1	86	0	0	0
21			52	15.8	8.91	16.6	12	1	87	0	0	0
25			50	14.7	7.87	9.7	15	0	83	3	0	0
29			47	14.4	7.48	9.2	16	0	83	1	0	0
33			47	13.9	8.16	8.1	26	0	74	0	0	0
37			48	14.4	8.02	6.2	11	0	89	0	0	0
4	4 ♂	26	48	14.4	7.71	15.6	42	0	58	0	0	0
8			48	14.4	7.35	14.8	21	0	77	2	0	0
12			50	14.7	8.20	8.0	10	0	88	2	0	0
16			51	14.2	7.90	16.6	16	0	82	2	0	0
20			46	14.4	7.43	5.7	18	0	82	0	0	0
24			48	14.2	7.72	21.6	24	1	74	1	0	0
28			49	15.3	8.58	13.0	17	0	82	1	0	0
32			45	14.7	7.95	24.8	6	0	92	0	0	0
36			47	14.2	8.34	8.8	19	0	80	1	0	0
40			49	14.7	8.16	11.8	12	2	85	1	0	0
81	1 ♀	26	40	13.3	6.57	12.5	26	0	73	1	0	0
85			44	14.2	7.16	7.6	22	0	78	0	0	0
89			47	14.2	7.14	7.7	12	0	85	3	0	0
93			44	14.2	6.45	7.9	12	0	88	0	0	0
97			44	14.4	6.94	9.1	18	0	81	1	0	0
101			43	13.9	6.74	5.5	17	0	83	0	0	0
105			43	13.6	6.74	7.0	33	0	66	1	0	0
109			47	13.9	7.42	7.5	21	0	79	0	0	0
113			44	14.2	7.14	5.9	20	0	77	3	0	0
117			51	15.0	7.93	7.6	18	0	82	0	0	0
84	4 ♀	26	47	13.9	6.99	8.4	32	0	68	0	0	0
88			49	15.8	7.46	8.9	44	2	53	1	0	0
92			43	13.6	6.72	9.8	23	0	75	2	0	0
96			50	14.4	8.31	13.2	20	0	80	0	0	0
100			44	14.2	7.52	13.1	48	0	52	0	0	0
104			46	13.9	6.96	6.0	24	0	75	1	0	0
108			49	14.7	7.40	9.1	26	0	74	0	0	0
112			39	12.8	6.91	12.8	38	1	61	0	0	0
116			43	13.6	7.42	9.8	22	0	77	1	0	0
120			45	13.6	7.23	4.5	35	1	64	0	0	0

APPENDIX 4

Urinalysis Data (Week 4)

	Rat No.	SG	pH	Color & Turb.	Protein	Sugar	Ke-tones	Occult Blood	Vol.	Microscopy E	P	M	R	O	C	A
1♂	1	1.034	6.6	1-	10mg%	.50%	-	-	15ml	1	0	0	0	0	0	2
	5	1.031	7.5	1-	40mg%	.25%	-	-	14ml	1	0	0	0	0	0	3+
	9	1.025	7.1	1-	20mg%	.25%	-	-	19ml	1	0	0	0	0	0	3
	13	1.046	7.0	1-	10mg%	.50%	-	-	7ml	1	0	1	0	0	0	1
	17	1.044	7.1	1-	30mg%	.50%	-	-	13ml	1	0	0	0	0	0	3+
	21	1.029	7.1	1-	10mg%	-	-	-	15ml	1	0	0	0	0	0	3+
	25	1.030	7.2	1+	20mg%	-	-	-	14ml	1	0	0	0	0	0	2
	29	1.042	6.9	1-	40mg%	.50%	-	-	14ml	1	0	0	0	0	0	2
	33	1.036	7.4	1-	50mg%	.50%	-	-	12ml	1	0	0	0	0	0	3+
	37	1.035	6.9	1-	40mg%	.50%	-	-	13ml	1	0	0	0	0	0	2
4♂	4	1.036	7.4	1+	40mg%	.50%	-	-	12ml	1	0	0	0	0	0	3
	8	1.040	7.4	2-	20mg%	.50%	-	-	10ml	2	0	0	0	0	0	3
	12	1.040	7.0	2-	40mg%	.75%	-	-	8ml	1	0	0	0	0	0	2
	16	1.058	7.2	1-	30mg%	.50%	-	-	12ml	1	0	0	0	0	0	3+
	20	1.038	7.0	2-	10mg%	.75%	-	-	12ml	1	0	0	0	0	0	3
	24	1.030	8.0	1-	5mg%	.50%	-	-	16ml	1	0	0	0	0	0	2
	28	1.040	7.2	1-	5mg%	.75%	-	-	12ml	2	0	0	0	0	0	3
	32	1.035	7.4	1-	10mg%	.75%	-	-	13ml	1	0	0	0	0	0	3+
	36	1.030	7.3	1-	20mg%	.25%	-	-	12ml	1	0	0	0	0	0	3+
	40	1.033	7.3	1-	30mg%	.50%	-	-	13ml	1	0	0	0	0	0	3
1♀	81	1.035	6.7	2+	20mg%	-	-	-	6ml	1	0	0	0	0	0	2
	85	1.030	6.6	2-	5mg%	.25%	-	-	9ml	1	0	1	0	0	0	1
	89	1.048	8.3	2+	10mg%	.25%	-	-	6ml	1	0	0	0	0	0	3+
	93	1.036	8.6	2-	10mg%	-	-	-	8ml	1	0	0	0	0	0	3+
	97	1.062	7.4	3-	5mg%	.25%	-	-	3ml	1	0	0	0	0	0	3+
	101	1.070	6.9	3-	5mg%	-	-	-	3ml	1	0	1	0	0	0	2
	105	1.058	7.7	2+	10mg%	.25%	-	-	4ml	1	0	1	0	0	0	3+
	109	1.044	8.5	2-	5mg%	-	-	S	10ml	1	0	0	0	0	0	3+
	113	1.022	7.6	1+	5mg%	-	-	-	21ml	1	0	0	0	0	0	1
	117	1.064	8.4	2-	5mg%	.25%	-	-	5ml	1	0	0	0	0	0	3+
4♀	84	1.052	7.7	3-	5mg%	.25%	-	-	8ml	1	0	0	0	0	0	3
	88	1.054	7.8	3-	5mg%	.25%	-	-	6ml	1	0	0	0	0	0	3+
	92	1.056	7.4	3-	10mg%	.25%	-	-	5ml	1	0	1	0	0	0	3
	96	1.075	8.4	3-	5mg%	.25%	-	-	3ml	1	0	1	0	0	0	3
	100	1.060	7.8	2-	5mg%	.25%	-	-	6ml	1	0	0	0	0	0	1
	104	1.040	7.4	2-	5mg%	-	-	-	7ml	1	0	0	0	0	0	3+
	108	1.068	8.4	2-	5mg%	.25%	-	-	7ml	1	0	0	0	0	0	3
	112	1.052	8.9	1-	50mg%	-	-	M	29ml	2	0	2	0	0	0	1
	116	1.048	8.5	2-	10mg%	-	-	-	9ml	1	0	0	0	0	0	3
	120	1.064	6.9	2-	10mg%	-	-	-	7ml	1	0	0	0	0	0	2

APPENDIX 4
(continued)

Urinalysis Data (Week 13)

Rat No.	SG	pH	Color & Turb.	Protein	Sugar	Ke-tones	Occult Blood	Vol.	Microscopy S	P	M	R	O	C	A
1♂ 1	1.019	7.1	1-	*	-	-	-	20ml	1	0	0	0	2	0	1
5	1.017	7.0	1-	10mg%	-	-	-	20ml	1	0	0	0	2	0	2
9	1.044	6.8	1-	50mg%	.25%	-	-	9ml	0	0	0	0	2	0	1
13	1.040	6.8	1-	30mg%	.25%	-	-	8ml	0	0	0	0	2	0	1
17	1.034	7.1	1-	10mg%	.25%	-	-	12ml	0	0	0	0	2	0	2
21	1.020	6.9	1-	10mg%	-	-	-	22ml	0	0	0	0	1	0	2
25	1.060	6.8	1-	(100)75mg%	-	-	-	10ml	0	0	0	0	1	1	2
29	1.030	6.9	1-	40mg%	-	-	-	13ml	0	0	0	0	2	0	2
33	1.018	6.4	1-	10mg%	-	-	-	25ml	0	0	0	0	1	0	2
37	1.030	6.9	1-	25mg%	.25%	-	-	13ml	0	0	0	0	2	0	2
4♂ 4	1.040	6.0	2-	20mg%	.25%	-	-	10ml	0	0	0	0	2	0	3
8	1.056	6.3	2-	50mg%	.25%	-	-	8ml	0	0	0	0	3	0	2
12	1.031	6.9	1-	50mg%	-	-	-	14ml	0	0	0	0	3	0	1
16	1.030	6.3	1-	40mg%	.25%	-	-	14ml	0	0	0	0	2	0	1
20	1.019	6.3	1-	50mg%	.25%	-	-	28ml	1	0	1	0	2	0	1
24	1.025	7.6	1-	5mg%	-	-	L	27ml	1	0	0	0	2	0	2
28	1.024	6.4	1-	10mg%	-	-	-	25ml	0	0	0	0	2	0	2
32	1.035	6.7	1-	50mg%	-	-	-	12ml	0	0	0	0	3	0	2
36	1.025	6.5	1+	5mg%	.25%	-	-	19ml	0	0	0	0	3	0	2
40	1.040	7.3	2+	50mg%	.25%	-	-	10ml	0	0	0	0	2	0	2
1♀ 81	1.052	6.5	2-	10mg%	.25%	-	-	7ml	0	0	0	0	2	1	1
85	1.024	6.6	1-	*	-	-	-	11ml	1	0	0	0	3+	0	1
89	1.058	6.4	2-	5mg%	.25%	-	-	3ml	0	0	0	0	2	0	1
93	1.048	7.0	2-	*	.25%	-	-	5ml	0	0	0	0	2	0	1
97	1.021	6.7	1-	*	-	-	-	18ml	0	0	0	0	3	0	2
101	1.025	6.7	1-	5mg%	-	-	-	12ml	0	0	0	0	3	0	1
105	1.018	8.0	1+	10mg%	-	-	-	19ml	0	0	0	0	3	0	2
109	1.020	6.9	1-	*	-	-	-	21ml	1	0	0	0	3	0	1
113	1.046	6.5	2+	5mg%	.25%	-	-	5ml	0	0	0	0	2	0	2
117	1.062	6.3	2+	5mg%	.25%	-	-	25ml	0	0	0	0	2	0	1
4♀ 84	1.060	5.6	2-	5mg%	.25%	-	-	5ml	1	0	0	0	2	0	2
88	1.054	6.4	2-	5mg%	.25%	-	-	7ml	0	0	0	0	2	1	3
92	1.050	7.6	2-	5mg%	.50%	-	-	7ml	1	0	0	0	3	0	3
96	1.052	6.5	2-	*	.25%	-	-	7ml	0	0	0	0	2	0	2
100	1.048	6.8	2-	5mg%	.25%	-	-	7ml	0	0	0	0	3	0	1
104	1.040	6.0	2-	5mg%	.25%	-	-	8ml	1	0	0	0	2	0	1
108	1.030	6.1	2-	*	.25%	-	-	15ml	0	0	0	0	2	0	2
112	1.021	9.0	2+	(40)150mg%	-	-	-	26ml	0	0	3	0	1	0	3
116	1.046	6.2	2-	5mg%	.25%	-	-	5ml	0	0	0	0	2	0	2
120	1.038	7.3	2+	*	.25%	-	-	9ml	0	0	0	0	2	0	1

* Less than 5mg%
( ) Repeated value

\# 25 had 2 granular casts
\# 81 had 1 granular cast
\# 88 had many amorphous urates and 1 granular cast

APPENDIX 4
(continued)

Urinalysis Data (Week 26)

Rat No.	SG	pH	Color & Turb.	Protein	Sugar	Ketones	Occult Blood	Vol.	Microscopy E	P	M	R	O	C	A
1♂ 1	1.018	6.5	1-	-	-	-	M	25ml	0	0	0	1	2	0	3
5	1.016	7.0	1+	-	-	-	-	32ml	1	0	0	0	2	0	2
9	1.025	6.5	1-	-	.25%	-	-	20ml	0	0	0	0	1	0	1
13	1.054	6.5	2+	30mg%	.25%	-	-	10ml	2	1	1	0	2	0	3
17	1.035	7.0	2-	30mg%	.25%	-	-	15ml	0	0	0	0	3	0	3
21	1.017	6.5	1-	-	-	-	-	18ml	0	0	0	0	1	0	1
25	1.034	7.0	2-	-	.25%	-	-	11ml	1	0	0	0	2	0	3
29	1.038	6.5	2-	Tr	-	-	-	13ml	0	0	0	0	1	0	1
33	1.023	6.0	1-	-	-	-	-	28ml	1	0	0	0	2	0	1
37	1.046	6.5	1+	-	.25%	-	-	10ml	2	1	1	0	1	0	1
4♂ 4	1.046	6.0	2-	-	.25%	-	-	10ml	0	0	0	0	1	0	1
8	1.031	6.0	2-	-	.25%	-	-	17ml	1	1	1	0	0	0	1
12	1.032	6.0	2-	-	.25%	-	-	13ml	1	0	1	0	2	0	1
16	1.036	5.5	2-	-	-	-	-	10ml	0	0	0	0	0	0	1
20	1.035	6.0	2+	-	.25%	-	-	15ml	2	1	1	0	2	0	1
24	1.040	7.0	2+	(30) 30mg%	.25%	-	-	13ml	2	2	1	0	3	0	3
28	1.021	6.5	1-	-	-	-	-	24ml	1	0	0	0	2	0	2
32	1.034	6.0	1-	30mg%	-	-	-	14ml	1	0	0	0	2	0	1
36	1.020	6.0	2-	-	.25%	-	-	8ml	0	0	0	0	0	0	1
40	1.023	7.5	2-	-	-	-	-	21ml	0	0	0	0	2	0	3
1♀ 81	1.031	6.0	1-	-	.25%	-	-	13ml	1	0	0	0	1	0	2
85	1.050	6.0	1+	-	.25%	-	-	5ml	2	1	1	0	2	0	2
89	1.054	6.0	2-	-	.25%	-	-	5ml	2	0	2	0	2	0	1
93	1.037	6.5	1+	-	-	-	-	6ml	1	0	0	0	3	0	3
97	1.020	6.0	1+	-	-	-	-	25ml	1	0	0	0	1	0	1
101	1.027	5.5	1+	-	-	-	-	17ml	1	0	0	0	0	0	0
105	1.026	7.0	1-	-	-	-	-	11ml	1	0	0	0	2	0	2
109	1.019	6.5	1-	-	-	-	-	20ml	1	0	0	0	1	0	1
113	1.025	6.0	1-	-	-	-	-	10ml	1	0	1	0	3	0	3
117	1.048	6.0	2-	-	.25%	-	-	2ml	2	0	1	0	2	0	1
4♀ 84	1.040	6.0	2-	Tr	-	-	-	5ml	2	0	0	0	1	0	1
88	1.040	6.5	2-	-	.25%	-	-	7ml	2	0	1	0	2	0	1
92	1.026	7.0	2+	-	-	-	-	7ml	0	0	0	0	1	0	1
96	1.056	6.0	2+	-	.25%	-	-	7ml	1	0	2	0	2	0	1
100	1.044	6.0	2-	-	.25%	-	M	4ml	1	0	1	2	2	0	1
104	1.019	7.0	1+	-	-	-	-	19ml	1	0	0	0	1	0	1
108	1.027	7.5	2++	-	-	-	-	15ml	0	0	0	0	2	0	2
112	1.016	9.0	1-	30mg%	-	-	L	27ml	1	1	3	2	1	0	2
116	1.020	6.5	2+	-	-	-	-	10ml	1	1	0	0	1	0	1
120	1.015	6.5	1-	-	-	-	-	8ml	1	0	0	0	1	0	2

APPENDIX S

Individual Biochemistry (Week 26)

Animal No.	Group Sex	SGOT RF U/ml	SGPT RF U/ml	BLB SAP U/ml	BUN mg%	Glucose mg%	Na^+	K^+	Total Protein
1	1♂	36	15	2.6	12.0	137	156*	5.6	6.6
5	Control	30	24	2.2	16.6	122	152	5.4	6.0
13		20	18	2.7	28.5	134	136	3.6	6.1
17		22	15	2.9	21.8	130	144	5.2	6.2
37		30	26	2.4	14.7	103	151	5.1	6.1
41		18	17	1.9	14.1	169	140	4.7	6.2
53		22	21	3.2	12.2	158	154	4.3	6.1
61		28	23	2.4	9.0*	151	153	5.4	6.3
69		18	12	2.4	23.9	113	141	5.2	6.2
73		16	14	2.1	23.9*	123	136	5.1	6.7
2	2♂	26	24	2.4	12.6	130	139	417	6.4
10	12.5%	16	15	2.5	22.5	142	137	5.2	6.1
14		18	12	2.3	17.9	134	155	5.7	6.2
26		30	19	4.5	17.6	119	150	5.8	6.1
42		22	27	2.1	5.9	94	154	7.3	6.9
46		26	15	1.8	17.0	197	148	4.4	6.1
54		12	19	2.8	25.8	171	139	3.9	6.2
70		16	12	1.6	3.8*	136	140	4.6	5.7
74		20	21	2.0	24.6	203*	140	4.2	5.8
78		24	19	2.1	22.0	202*	147	4.9	6.1
7	3♂	16	7	2.4	7.7	140	152	4.8	6.2
15	25%	12	15	2.2	5.7	136	143	4.4	5.8
19		10	9	2.7	12.7	154	154	4.9	5.7
31		17	29	1.8	12.7	142	147	4.3	6.6
35		20	12	1.8	26.5	143	148	5.0	6.5
39		12	12	2.2	18.2	153	154	4.9	6.2
59		26	23	2.7	13.5	170	150	5.3	6.6
63		12	5*	1.9	25.5	180*	149	4.7	6.7
67		24	17	2.1	16.3	124	143	3.7	7.0
79		22	15	1.8	16.5	177	147	3.9	6.4
4	4♂	34	20	2.8	27.5	145	156*	5.4	6.4
16	50%	14	9	2.4	16.9	179	146	4.7	6.1
32		10	7	2.4	16.2	152	154	5.6	7.2
40		8	5	2.4	15.6	162	152	5.3	6.5
44		14	19	2.2*	4.4	153	152	4.3	10.4*
52		12	5*	1.8*	4.6	138	136	3.7	5.8
60		14	18	4.6	8.8	141	151	4.2	5.8
68		20	12	1.1	6.0	167	152	4.5	6.5
72		10	28	2.6	16.9	176	144	3.7	6.6
80		16	12	2.4	27.9	184*	145	3.9	6.7

* Values checked

APPENDIX 5
(continued)

Individual Biochemistry (Week 26)

Animal No.	Group Sex	SGOT RF U/ml	SGPT RF U/ml	BLB SAP U/ml	BUN mg%	Glucose mg%	Na^+	K^+	Total Protein
81	1♀	18	27	1.5	39.2	122	147	4.7	6.6
85	Control	14	9	1.4	18.4	109	149	4.1	6.7
89		20	30	1.4	14.6	167	144	3.9	6.9
93		30	30	1.3	29.6	140	147	4.4	5.6
97		15	7	2.1	28.5	129	156*	4.7	6.9
105		22	25	0.9*	20.8	133	154	4.9	6.8
109		16	5*	1.6	35.4	74*	148	4.7	7.0
113		29	17	1.4	24.1	90	147	6.2	6.7
121		20	9	1.2	17.5	135	149	3.3	6.1
149		18	15	1.9	47.4*	113	142	4.4	6.8
82	2♀	20	19	2.0	49.2*	124	144	4.5	6.7
86	12.5%	20	16	1.6	30.4	140	152	3.7	6.7
90		18	15	1.4	49.4*	110	147	3.9	7.1
98		16	14	1.2	29.5	137	153	4.6	6.7
102		12	12	1.7	10.8	114	149	3.7	6.2
114		8	21	1.1	7.8	120	149	3.9	6.1
118		16	10	1.8	31.3	147	146	3.7	6.4
126		22	20	0.9*	34.8	153	147	3.6	6.7
146		10	15	1.4	42.9	151	153	4.1	7.3
150		12	19	1.5	28.5	126	145	4.1	7.3
83	3♀	20	15	2.1	13.6	112	144	4.6	6.4
99	25%	16	17	1.5	15.0	149	137	3.8	6.3
107		8	7	1.6	10.2	126	153	3.7	6.7
123		16	18	2.3	18.3	122	151	4.0	6.6
127		10	10	1.6	13.9	111	147	4.2	6.3
139		14	9	1.7	23.7	99	138	4.1	6.5
143		12	10	1.7	46.4*	124	144	3.6	6.6
151		20	12	1.1	10.9	135	148	4.3	6.4
155		20	15	0.7*	13.4	123	154	4.5	6.8
159		10	5*	1.4	34.1	147	140	4.0	6.8
100	4♀	14	9	1.3	22.7	108	145	4.6	6.6
108	50%	20	12	1.6	9.4	103	148	4.7	7.1
112		26	16	1.5	10.0	78*	154	5.3	7.6
116		10	5*	1.7	26.3	135	158*	5.6	7.4
120		20	10	2.2	23.9	95	156*	4.8	6.8
136		14	9	1.7*	35.1	110	157*	5.0	6.3
140		16	18	1.2	31.7	124	158*	5.8	6.4
148		8	7	1.7	42.2*	149	149	4.0	6.4
152		12	5*	1.9	14.9	94	145	4.9	7.1
156		16	12	1.5	19.3	142	149	4.4	6.7

APPENDIX 6

Ophthalmoscopy

LIONEL F. RUBIN, V.M.D.
415 MONTGOMERY AVENUE
MERION STATION, PENNSYLVANIA 19066

MOHAWK 4-8018

October 3, 1972

Huntingdon Research Corporation
Box 6651
Baltimore, Maryland 21204

Attn: Mr. Uttman

Week 0 ophthalmoscopic examination of 160 project 1-314 rats (+4 extra rats) was performed September 22, 1972. An anterior synechia was present in rat 67 (left eye). No other abnormality was seen.

Lionel F. Rubin, V.M.D.

LFR/gj

LIONEL F. RUBIN, V.M.D.
418 MONTGOMERY AVENUE
MERION STATION, PENNSYLVANIA 19066

MOHAWK 4-5016

January 4, 1973

Huntingdon Research Corporation
Box 6851
Baltimore, Maryland 21204

Attn: Dr. G. Owen

Ophthalmoscopic examination of project 1-314 rats was performed December 19, 1972. The following abnormalities were seen:

1) Conjunctivitis in the right eye of rats 127 (cage 27) and 45 (cage 3). This is probably secondary to dental abnormalities.

2) Retinopathy, probably infectious in origin, in the left eye of rat 54 (cage 7).

In my opinion none of the ocular abnormalities are attributable to the administration of the test compound.

Lionel F. Rubin, V.M.D.

LFR/gj

LIONEL F. RUBIN, V.M.D.
415 MONTGOMERY AVENUE
MERION STATION, PENNSYLVANIA 19066

MOHAWK 4-5016

March 7, 1973

Huntingdon Research Corporation
Box 6851
Baltimore, Maryland 21204

Attn: Mr. Upman

Ophthalmoscopic examination of project 1-314 rats was performed March 6, 1973. The following abnormalities were seen:

1) Conjunctivitis, probably secondary to dental abnormalities, in rats
   123 (cage 27) - right eye
   45 (cage 3) - right eye
   4 (cage 13) - right eye

2) Posterior subcapsular cataract in rats:
   27 (cage 10) - right eye
   45 (cage 3) - both eyes
   9 (cage 1) - both eyes
   There is no evidence of dose relationship of these abnormalities

3) Retinal degeneration, probably infectious in origin, in the left eye of rat 54 (cage 7).

In my opinion, none of the ocular abnormalities are attributable to the administration of the test compound.

Lionel F. Rubin, V.M.D.

LFR/gj

Six-Month Dietary Administration of Reacted Molasses to Albino Rats

APPENDIX 7

Individual Organ Weights in Grams

Group 1♂ - Control

#	bwt	hrt	lng	liv	spl	kid	adr	gon	thy	brn	pit
1	583.	1.8	1.6	15.6	0.7	3.5	.050	3.6	.044	2.4	.018
5	622.	1.7	2.7	16.3	0.7	3.4	.054	3.8	.024	2.4	.016
9	622.	1.5	2.0	17.3	0.6	3.6	.057	3.2	.034	2.3	.017
13	610.	1.8	1.8	13.9	0.8	3.7	.058	3.8	.027	2.3	.014
17	666.	1.6	1.9	15.8	0.8	3.9	.072	3.6	.036	2.1	.017
21	543.	1.3	2.0	14.2	0.4	3.4	.050	3.1	.030	2.4	.023
37	600.	1.8	2.1	12.9	0.5	3.8	.055	3.2	.020	2.1	.024
41	670.	1.8	1.7	16.7	0.7	3.8	.060	3.1	.027	2.3	.020
45	520.	1.7	1.8	11.5	0.6	3.3	.060	3.6	.022	2.3	.020
49	670.	1.7	2.4	17.4	0.9	4.3	.063	3.6	.034	2.1	.014
53	630.	1.5	1.7	12.9	0.7	3.8	.048	1.5	.033	2.2	.021
57	725.	1.7	2.8	24.6	0.7	4.7	.062	3.2	.026	2.4	.018
61	555.	1.8	1.5	11.6	0.7	3.6	.063	3.2	.035	2.3	.014
65	800.	1.8	2.7	28.4	0.7	5.3	*	3.6	.049	2.4	.010
69	645.	1.7	1.9	17.0	0.8	4.1	.075	3.4	.036	2.0	.019
73	680.	1.9	1.6	16.6	0.8	4.2	.059	3.9	.036	2.3	.016
77	606.	1.9	1.7	20.0	0.7	3.9	.043	3.2	.016	2.3	.015
25	593.	1.7	1.8	15.5	0.6	3.8	.071	4.0	*	2.3	.014
29	650.	1.3	2.2	14.1	0.7	4.1	.067	3.5	.024	1.9	.022
33	525.	1.6	2.1	13.7	0.7	3.6	.057	3.3	.033	2.1	.010

* not weighed - technical error

APPENDIX 7

Individual Organ Weights in Grams

Group 1º - Control

#	bwt	hrt	lng	liv	spl	kid	adr	gon	thy	brn	pit	utr
81	380.	1.3	1.5	9.5	0.6	2.4	.078	.121	.012	2.3	.014	0.5
85	290.	1.2	1.3	8.7	0.5	2.3	.065	.090	.030	2.2	.018	0.8
89	335.	1.3	1.1	8.5	0.5	2.3	.081	.080	.026	2.1	.018	0.8
93	312.	1.0	1.6	9.2	0.5	2.4	.073	.134	.025	2.1	.022	0.9
97	340.	1.1	1.7	8.2	0.5	2.1	.085	.088	.022	2.2	.019	0.7
101	306.	1.0	1.1	7.2	0.5	2.1	.077	.110	.044	2.0	.015	0.7
105	340.	1.3	1.6	8.9	0.4	2.5	.090	.073	.033	2.2	.024	0.9
109	323.	1.0	1.3	8.7	0.4	2.7	.091	.075	.020	2.2	.026	0.9
113	270.	0.9	0.8	6.9	0.4	2.0	.072	.096	.032	2.2	.040	1.0
117	300.	0.9	1.6	8.3	0.4	2.5	.070	.103	.030	2.1	.012	0.8
121	310.	1.0	1.2	8.4	0.5	2.5	.064	.110	.029	2.1	.016	0.7
125	290.	1.0	1.6	8.5	0.5	2.0	.067	.094	.035	2.1	.029	0.9
129	400.	1.4	1.4	11.3	0.6	2.7	.085	.111	.028	2.1	.036	0.7
133	300.	1.0	1.3	9.0	0.5	2.6	.097	.078	.028	2.3	.011	0.9
137	375.	1.4	1.8	8.8	0.6	2.5	.082	.137	.036	2.2	.015	0.7
141	320.	1.0	1.5	10.8	0.5	2.8	.063	.064	.024	2.0	.033	0.7
145	315.	1.1	1.8	8.9	0.4	2.3	.077	.137	.027	2.3	.015	0.9
149	320.	1.4	1.3	9.2	0.6	2.6	.070	.080	.027	2.0	.012	1.2
153	300.	1.0	1.5	9.2	0.5	2.3	.082	.066	.033	2.0	.024	0.7
157	313.	1.1	1.5	9.5	0.5	2.4	.079	.112	.017	2.1	.011	0.7

APPENDIX 7

Individual Organ Weights in Grams

Group 2♂ - 12.5%

#	bwt	hrt	lng	liv	spl	kid	adr	gon	thy	brn	pit
2	590.	1.8	2.0	18.5	0.7	4.0	.055	.400	.021	2.4	.021
6	660.	1.7	2.4	17.2	0.6	4.3	.056	.200	.048	2.5	.021
10	620.	2.1	1.6	15.7	0.9	4.1	.057	.000	.024	2.3	.013
14	600.	2.5	1.7	15.9	0.7	4.0	.072	.600	.036	2.4	.024
18	588.	2.1	2.0	14.8	0.8	4.1	.063	.300	.048	2.3	.014
22	581.	1.6	2.5	14.0	0.7	4.2	.057	.600	.048	2.4	.021
26	606.	1.8	1.8	15.3	0.6	3.8	.054	.300	.021	2.4	.016
30	622.	1.9	1.7	16.6	0.7	4.3	.072	.500	.045	2.1	.031
34	590.	1.5	2.4	19.4	0.7	4.6	.057	.400	.040	2.2	.022
38	535.	1.7	1.6	13.7	0.8	3.7	.056	.300	.031	2.4	.015
42	565.	1.5	1.8	15.5	0.5	4.2	.042	.400	.027	2.2	.021
46	575.	1.9	1.6	14.2	0.7	3.8	.078	.100	.027	2.2	.020
50	700.	2.1	2.1	20.9	0.6	4.6	.034	.700	.030	2.2	.026
54	590.	1.9	1.7	17.7	0.9	4.1	.059	.600	.033	2.3	.013
58	620.	1.9	1.6	13.4	0.6	3.7	.044	.400	.023	2.2	.022
62	632.	1.9	1.7	15.7	0.8	4.2	.073	.800	.031	2.4	.021
66	680.	1.8	1.9	17.4	0.8	3.9	.059	.500	.021	2.6	.016
70	665.	1.7	1.8	1.3	0.7	3.7	.052	.800	.035	2.3	.020
74	671.	2.0	2.4	17.3	0.7	4.1	.076	.000	.020	2.1	.021
78	594.	1.7	1.7	0.	0.7	3.9	.059	.500	.048	2.3	.010

APPENDIX 7

Individual Organ Weights in Grams

Group 2♀ - 12.5%

#	bwt	hrt	lng	liv	spl	kid	adr	gon	thy	brn	pit	utr
82	280.	1.1	1.2	7.4	0.4	2.2	.069	.126	.025	2.1	.018	0.9
86	335.	1.2	1.1	8.3	0.4	2.5	.072	.122	.017	2.1	.022	1.0
90	332.	1.2	1.3	8.7	0.5	2.5	.073	.146	.033	2.1	.010	1.0
94	350.	1.1	1.3	8.5	0.5	2.2	.062	.129	.027	2.2	.015	1.2
98	335.	1.2	1.5	8.3	0.4	2.4	.081	.103	.027	2.1	.020	0.8
102	303.	1.3	1.3	7.7	0.4	2.2	.060	.120	.036	2.1	.017	1.0
106	290.	0.9	1.5	7.9	0.4	1.9	.066	.140	.017	1.9	.019	0.8
110	288.	2.1	1.8	11.1	0.5	2.5	.085	.087	.025	1.8	.017	1.0
114	333.	1.0	1.5	8.7	0.5	2.3	.072	.128	.023	2.1	.021	0.7
118	305.	1.2	1.2	8.2	0.5	2.2	.061	.130	.024	2.2	.018	1.0
122	302.	1.1	1.0	6.4	0.5	1.9	.059	.121	.020	2.0	.010	0.7
126	343.	1.6	1.4	8.2	0.5	3.1	.088	.100	.020	2.1	.016	0.6
130	350.	1.2	1.4	8.8	0.5	2.2	.076	.119	.026	2.1	.011	0.7
134	340.	1.2	1.6	10.5	0.6	2.7	.079	.134	.033	2.1	.022	0.9
138	322.	1.1	1.3	7.7	0.5	2.4	.106	.152	.031	2.1	.021	1.1
142	258.	1.3	1.6	10.1	0.6	2.5	.080	.102	.018	2.0	.017	1.2
146	310.	1.3	1.2	8.9	0.5	2.1	.047	.078	.021	2.1	.014	0.8
150	309.	1.3	1.7	11.7	0.5	3.1	.069	.104	.029	2.1	.022	0.8
154	350.	1.4	1.6	11.5	0.7	2.8	.088	.132	.033	2.3	.020	0.8
158	268.	1.3	1.1	7.4	0.4	1.9	.064	.098	.021	2.1	.030	1.1

APPENDIX 7

Individual Organ Weights in Grams

Group 3♂ - 25%

#	bwt	hrt	lng	liv	spl	kid	adr	gon	thy	brn	pit
3	564.	1.6	2.3	17.4	0.7	3.8	.047	3.9	.039	2.5	.024
7	626.	2.0	2.1	19.2	0.9	4.9	.075	3.3	.032	2.4	.020
11	685.	2.0	1.6	0.	0.7	0.	.	3.5	.032	2.2	.017
15	638.	2.1	1.7	15.8	0.8	4.4	.055	3.9	.032	2.4	.017
19	744.	2.1	2.1	20.4	0.8	5.1	.063	3.4	.039	2.3	.022
23	610.	1.8	1.5	18.2	0.8	4.3	.071	3.3	.030	2.3	.018
27	670.	2.7	1.9	18.2	0.9	5.0	.066	2.2	.032	2.4	.022
31	688.	2.1	1.8	19.1	0.8	4.6	.064	3.4	.029	2.5	.017
35	690.	2.0	1.5	19.9	0.8	4.4	.066	3.4	.048	2.4	.012
39	648.	2.0	1.6	15.7	0.7	3.8	.069	4.0	.038	2.3	.018
43	700.	1.8	4.3	18.6	0.7	4.1	.062	3.7	.023	2.2	.018
47	650.	2.0	2.5	17.8	0.7	3.9	.044	3.9	.033	2.2	.018
51	652.	1.8	1.8	19.4	0.8	3.9	.049	3.7	.043	2.3	.020
55	725.	1.7	3.0	21.6	0.8	4.2	.060	3.4	.030	2.3	.026
59	670.	2.1	1.8	18.6	0.8	4.6	.075	3.9	.032	2.2	.025
63	730.	1.7	1.9	17.8	0.8	4.4	.088	3.6	.030	2.0	.019
67	550.	1.7	1.4	11.9	1.0	3.3	.065	3.4	.026	2.2	.018
75	663.	2.2	2.2	15.8	0.9	4.9	.070	3.5	.029	2.0	.020
71	710.	2.1	1.8	16.1	0.9	4.9	.063	3.9	.042	2.4	.018
79	710.	2.1	1.9	16.6	0.8	4.8	.058	3.6	.034	2.5	.019

APPENDIX 7

Individual Organ Weights in Grams

Group 32 - 25%

#	bwt	hrt	lng	liv	spl	kid	adr	gon	thy	brn	pit	utr
83	388.	1.1	1.6	10.0	0.5	2.3	.073	.180	.039	2.1	.020	0.6
87	350.	1.1	1.1	8.5	0.5	2.2	.076	.087	.020	2.2	.015	1.1
91	320.	1.1	1.3	9.5	0.5	3.1	.057	.059	.034	2.2	.034	0.8
95	326.	1.1	1.6	8.6	0.5	2.5	.067	.113	.018	2.1	.016	0.6
99	450.	1.5	1.4	10.7	0.6	2.7	.033	.180	.035	2.2	.019	0.9
103	330.	1.2	1.3	8.6	0.6	2.2	.077	.137	.026	2.0	.017	1.1
107	297.	1.0	1.4	8.5	0.5	2.0	.068	.104	.036	2.0	.022	0.8
111	331.	1.2	1.2	7.8	0.5	2.4	.075	.131	.032	2.1	.033	0.9
115	336.	1.2	1.6	10.9	0.6	3.0	.078	.153	.037	2.1	.027	1.0
119	334.	1.0	1.3	8.3	0.6	2.3	.070	.086	.026	2.1	.031	0.7
123	300.	1.1	1.3	8.3	0.6	2.3	.077	.111	.037	2.1	.012	1.0
127	395.	1.1	1.7	10.0	0.6	2.9	.080	.126	.036	2.2	.016	0.7
131	375.	1.3	1.4	9.3	0.6	2.9	.097	.142	.025	2.1	.012	0.8
135	312.	1.1	1.3	8.9	0.5	2.4	.093	.110	.028	2.1	.014	0.6
139	305.	1.2	1.3	8.5	0.5	2.3	.070	.123	.037	2.1	.019	0.7
143	303.	1.2	1.3	9.1	0.5	2.7	.067	.082	.035	2.2	.035	0.7
147	375.	1.4	1.6	10.0	0.5	3.1	.089	*	.029	2.1	.017	1.0
151	394.	1.5	1.2	10.4	0.7	3.2	.084	.148	.043	2.2	.039	0.9
155	346.	1.1	1.5	9.0	0.5	2.5	.089	.122	.025	2.1	.019	1.0
159	302.	1.1	1.2	9.2	0.5	2.2	.091	.118	.035	2.1	.022	0.7

* not weighed - technical error

APPENDIX 7

Individual Organ Weights in Grams

Group 4♂ - 50%

#	bwt	brt	lng	liv	spl	kid	adr	gon	thy	brn	pit
4	486.	1.4	2.0	12.1	0.9	3.4	.049	3.4	.036	2.3	.012
8	630.	1.6	2.3	14.3	0.8	4.5	.054	3.4	.022	2.2	.021
12	566.	1.6	2.0	19.1	0.6	4.3	.070	1.8	.028	2.1	.020
16	560.	1.6	1.7	14.3	0.6	3.4	.043	2.9	.036	2.3	.017
20	570.	1.4	1.9	19.4	0.7	4.3	.069	3.4	.022	2.2	.012
24	630.	1.7	1.8	15.8	0.8	3.9	.073	3.2	.043	2.1	.010
28	685.	1.6	1.3	17.3	0.8	4.3	.084	1.9	.021	1.9	.017
32	572.	1.8	1.8	17.1	0.8	4.1	.065	3.4	.033	2.3	.014
36	570.	1.7	1.6	16.7	0.7	4.2	.050	2.9	.024	2.2	.021
40	615.	1.6	2.1	17.8	0.7	4.1	.064	2.4	.032	2.2	.017
44	725.	1.7	1.9	20.6	0.8	4.9	.067	3.7	.041	2.2	.017
48	640.	1.8	2.0	18.8	0.8	4.5	.061	3.9	.054	2.3	.011
52	684.	1.9	1.9	19.8	0.9	4.2	.075	3.4	.043	2.4	.016
56	620.	1.9	1.8	16.4	0.8	4.3	.039	3.4	.034	2.2	.014
60	545.	1.5	1.5	14.6	0.7	3.0	.050	2.0	.054	2.5	.020
64	563.	1.5	2.0	18.3	0.6	3.9	.062	3.2	*	2.5	.015
68	650.	1.7	2.1	17.5	0.8	4.5	.073	3.4	.026	1.9	.019
72	700.	1.9	2.1	16.8	0.9	3.9	.086	3.8	.027	2.4	.015
76	650.	1.9	2.7	17.4	0.5	4.5	.059	3.5	.029	2.1	.025
80	666.	1.7	2.2	19.0	0.6	4.2	.070	3.3	.031	2.3	.028

* not weighed - technical error

APPENDIX 7

Individual Organ Weights in Grams

Group 4♀ - 50%

#	bwt	hrt	lng	liv	spl	kid	adr	gon	thy	brn	pit	utr
84	290.	1.1	1.5	9.4	0.5	2.4	.080	.167	.024	2.2	.020	1.1
88	320.	1.2	1.5	9.6	0.6	2.5	.077	.141	.030	2.2	.020	1.0
92	280.	1.2	1.2	8.3	0.5	2.9	.075	.101	.033	2.2	.021	0.9
96	304.	1.2	1.4	10.3	0.5	2.7	.077	.121	.035	2.2	.021	1.3
100	310.	1.1	1.3	7.9	0.6	2.7	.080	.121	.025	2.3	.023	1.1
104	300.	1.1	1.4	8.7	0.5	2.1	.091	.132	.031	2.2	.016	0.8
108	310.	1.4	1.3	9.5	0.5	2.8	.108	.137	.029	2.1	.025	0.9
112	290.	1.1	1.4	9.2	0.	0.	*	*	.021	2.1	.029	0.
116	280.	1.1	1.4	8.7	0.5	2.4	.820	.120	.043	2.1	.017	1.3
120	295.	1.0	2.2	9.0	0.9	2.5	.093	.097	.038	2.0	.026	0.8
124	320.	1.0	1.4	9.2	0.7	2.7	.079	.013	.029	2.2	.018	0.9
128	342.	1.0	1.5	9.7	0.7	2.6	.081	.097	*	2.2	.016	1.3
132	350.	1.5	1.4	10.0	0.6	3.3	.084	.084	.024	2.1	.018	1.0
136	293.	1.1	1.3	8.5	0.6	2.4	.076	.137	.027	2.2	.012	1.5
140	312.	1.2	1.2	9.3	0.5	3.0	.081	.090	.038	2.1	.011	1.3
144	290.	0.9	1.3	9.1	0.6	2.7	.073	.141	.043	2.1	.010	1.1
148	300.	1.0	1.7	9.2	0.6	2.6	.090	.140	.035	2.1	.017	1.2
152	308.	1.2	1.7	10.5	0.4	2.9	.090	.135	.026	2.2	.039	0.8
156	304.	1.1	1.7	9.4	0.4	2.6	.063	.155	.034	1.9	.029	1.6
160	325.	1.1	1.4	9.8	0.7	2.6	.074	.093	.035	2.1	.016	0.8

* not weighed - not weighed

Six-Month Dietary Administration of Reacted Molasses to Albino Rats

APPENDIX 8

Individual Organ Weights as a Percent of Bodyweight x 100

Group 1♂ - Control

#	bwt	hrt	lng	liv	spl	kid	adr	gon	thy	brn	pit
1	583.	0.31	0.27	2.68	0.12	.6003	.0086	.6175	.008	.412	.0031
5	622.	0.27	0.43	2.62	0.11	.5466	.0087	.6109	.004	.386	.0026
9	622.	0.24	0.32	2.78	0.10	.5788	.0092	.5145	.005	.370	.0027
13	610.	0.30	0.30	2.28	0.13	.6066	.0095	.6230	.004	.377	.0023
17	666.	0.24	0.29	2.37	0.12	.5856	.0108	.5405	.005	.315	.0026
21	543.	0.24	0.55	2.63	0.07	.6262	.0092	.5709	.006	.442	.0042
37	600.	0.30	0.35	2.15	0.08	.6333	.0092	.5333	.002	.350	.0040
41	670.	0.27	0.25	2.49	0.10	.5672	.0090	.4627	.004	.343	.0030
45	520.	0.33	0.35	2.21	0.12	.6346	.0115	.6923	.004	.442	.0038
49	670.	0.25	0.36	2.60	0.13	.6418	.0094	.5373	.005	.313	.0021
53	630.	0.24	0.27	2.05	0.11	.6032	.0076	.2381	.005	.349	.0033
57	725.	0.23	0.39	3.39	0.10	.6483	.0086	.4414	.004	.331	.0025
61	555.	0.32	0.27	2.09	0.13	.6486	.0114	.5766	.006	.414	.0025
65	800.	0.22	0.34	3.55	0.09	.6625	*	.4500	.006	.300	.0013
69	645.	0.26	0.29	2.64	0.12	.6357	.0116	.5271	.006	.310	.0029
73	680.	0.28	0.24	2.44	0.12	.6176	.0087	.5735	.005	.338	.0024
77	606.	0.31	0.28	3.30	0.12	.6436	.0071	.5281	.003	.380	.0025
25	593.	0.29	0.30	2.61	0.10	.6408	.0120	.6745	*	.388	.0024
29	650.	0.20	0.34	2.17	0.11	.6308	.0103	.5385	.004	.292	.0034
33	525.	0.30	0.40	2.61	0.13	.6857	.0109	.6286	.006	.400	.0019

* not weighed - technical error

APPENDIX 8

Individual Organ Weights as a Percent of Bodyweight x 100

Group 1♀ - Control

#	bwt	hrt	lng	liv	spl	kid	adr	gon	thy	brn	pit	utr
81	380.	0.34	0.39	2.50	0.16	.6316	.0205	.0318	.003	.605	.0037	.1316
85	290.	0.41	0.45	3.00	0.17	.7931	.0224	.0310	.010	.759	.0062	.2759
89	335.	0.39	0.33	2.54	0.15	.6866	.0242	.0239	.008	.627	.0054	.2388
93	312.	0.32	0.51	2.95	0.16	.7692	.0234	.0429	.008	.673	.0071	.2885
97	340.	0.32	0.50	2.41	0.15	.6176	.0250	.0259	.006	.647	.0056	.2059
101	306.	0.33	0.36	2.35	0.16	.6863	.0252	.0359	.014	.654	.0049	.2288
105	340.	0.38	0.41	2.62	0.12	.7353	.0265	.0215	.010	.647	.0071	.2647
109	323.	0.31	0.40	2.69	0.12	.8359	.0282	.0232	.006	.681	.0080	.2786
113	270.	0.33	0.30	2.56	0.15	.7407	.0267	.0356	.012	.815	.0148	.3704
117	300.	0.30	0.53	2.77	0.13	.8333	.0233	.0343	.010	.700	.0040	.2667
121	310.	0.32	0.39	2.71	0.16	.8065	.0206	.0355	.009	.677	.0052	.2258
125	290.	0.34	0.55	2.93	0.17	.6897	.0231	.0324	.012	.724	.0100	.3103
129	400.	0.35	0.35	2.82	0.15	.6750	.0212	.0277	.007	.525	.0090	.1750
133	300.	0.33	0.43	3.00	0.17	.8667	.0323	.0260	.009	.767	.0037	.3000
137	375.	0.37	0.48	2.35	0.16	.6667	.0219	.0365	.010	.587	.0040	.1867
141	320.	0.31	0.47	3.37	0.16	.8750	.0197	.0200	.008	.625	.0103	.2188
145	315.	0.35	0.57	2.83	0.13	.7302	.0244	.0435	.009	.730	.0048	.2857
149	320.	0.44	0.41	2.87	0.19	.8125	.0219	.0250	.008	.625	.0038	.3750
153	300.	0.33	0.50	3.07	0.17	.7667	.0273	.0220	.011	.667	.0080	.2333
157	313.	0.35	0.48	3.04	0.16	.7668	.0252	.0358	.005	.671	.0035	.2236

APPENDIX 8

Individual Organ Weights as a Percent of Bodyweight x 100

Group 2♂ - 12.5%

#	bwt	hrt	lng	liv	spl	kid	adr	gon	thy	brn	pit
2	590.	0.31	0.34	3.14	0.12	.6780	.0093	.5763	.004	.407	.0036
6	660.	0.26	0.36	2.61	0.09	.6515	.0085	.3333	.007	.379	.0032
10	620.	0.34	0.26	2.53	0.15	.6613	.0092	.6452	.004	.371	.0021
14	600.	0.42	0.28	2.65	0.12	.6667	.0120	.6000	.006	.400	.0040
18	588.	0.36	0.34	2.52	0.14	.6973	.0107	.5612	.008	.391	.0024
22	581.	0.28	0.43	2.41	0.12	.7229	.0098	.6196	.008	.413	.0036
26	606.	0.30	0.30	2.52	0.10	.6271	.0089	.5446	.003	.396	.0026
30	622.	0.31	0.27	2.67	0.11	.6913	.0116	.5627	.007	.338	.0050
34	590.	0.25	0.41	3.29	0.12	.7797	.0097	.5763	.007	.373	.0037
38	535.	0.32	0.30	2.56	0.15	.6916	.0105	.6168	.006	.449	.0028
42	565.	0.27	0.32	2.74	0.09	.7434	.0074	.6018	.005	.389	.0037
46	575.	0.33	0.28	2.47	0.12	.6609	.0136	.5391	.005	.383	.0035
50	700.	0.30	0.30	2.99	0.09	.6571	.0049	.5286	.004	.314	.0037
54	590.	0.32	0.29	3.00	0.15	.6949	.0100	.6102	.005	.390	.0022
58	620.	0.31	0.26	2.16	0.10	.5968	.0071	.5484	.004	.355	.0035
62	632.	0.30	0.27	2.48	0.13	.6646	.0116	.6013	.005	.380	.0033
66	680.	0.26	0.28	2.56	0.12	.5735	.0087	.5147	.003	.382	.0024
70	665.	0.26	0.27	0.20	0.11	.5564	.0078	.5714	.005	.346	.0030
74	671.	0.30	0.36	2.58	0.10	.6110	.0113	.4471	.003	.313	.0031
78	594.	0.29	0.29	*	0.12	.6566	.0099	.5892	.008	.387	.0017

* not weighed - technical error

APPENDIX 8

Individual Organ Weights as a Percent of Bodyweight x 100

Group 2♀ - 12.5%

#	bwt	hrt	lng	liv	spl	kid	adr	gon	thy	brn	pit	utr
82	280.	0.39	0.43	2.64	0.14	.7857	.0246	.0450	.009	.750	.0064	.3214
86	335.	0.36	0.33	2.48	0.12	.7463	.0215	.0364	.005	.627	.0066	.2985
90	332.	0.36	0.39	2.62	0.15	.7530	.0220	.0440	.010	.633	.0030	.3012
94	350.	0.31	0.37	2.43	0.14	.6286	.0177	.0369	.008	.629	.0043	.3429
98	335.	0.36	0.45	2.48	0.12	.7164	.0242	.0307	.008	.627	.0060	.2388
102	303.	0.43	0.43	2.54	0.13	.7261	.0198	.0396	.012	.693	.0056	.3300
106	290.	0.31	0.52	2.72	0.14	.6552	.0228	.0483	.006	.655	.0046	.2759
110	288.	0.73	0.62	3.85	0.17	.8681	.0295	.0302	.009	.625	.0059	.3472
114	333.	0.30	0.45	2.61	0.15	.6907	.0216	.0384	.007	.631	.0063	.2102
118	305.	0.39	0.39	2.69	0.16	.7213	.0200	.0426	.008	.721	.0059	.3279
122	302.	0.36	0.33	2.12	0.17	.6291	.0195	.0401	.007	.662	.0033	.2318
126	343.	0.47	0.41	2.39	0.15	.9038	.0257	.0292	.006	.612	.0047	.1749
130	350.	0.34	0.40	2.51	0.14	.6286	.0217	.0340	.007	.600	.0031	.2000
134	340.	0.35	0.47	3.09	0.18	.7941	.0232	.0394	.010	.618	.0065	.2647
138	322.	0.34	0.40	2.39	0.16	.7453	.0329	.0472	.010	.652	.0065	.3416
142	258.	0.50	0.62	3.91	0.23	.9690	.0310	.0395	.007	.775	.0066	.4651
146	310.	0.42	0.39	2.87	0.16	.6774	.0152	.0252	.007	.677	.0045	.2581
150	309.	0.42	0.55	3.79	0.16	.0032	.0223	.0337	.009	.680	.0071	.2589
154	350.	0.40	0.46	3.29	0.20	.8000	.0251	.0377	.009	.657	.0057	.2286
158	268.	0.49	0.41	2.76	0.15	.7090	.0239	.0366	.008	.784	.0112	.4104

APPENDIX 8

Individual Organ Weights as a Percent of Bodyweight x 100

Group 3♂ - 25%

#	bwt	hrt	lng	liv	spl	kid	adr	gon	thy	brn	pit
3	564.	0.28	0.41	3.09	0.12	.6738	.0083	.6915	.007	.443	.0043
7	626.	0.32	0.34	3.07	0.14	.7827	.0120	.5272	.005	.383	.0032
11	685.	0.29	0.23	*	0.10	*	*	.5109	.005	.321	.0025
15	638.	0.33	0.27	2.48	0.13	.6897	.0086	.6113	.005	.376	.0027
19	744.	0.28	0.28	2.74	0.11	.6855	.0085	.4570	.005	.309	.0030
23	610.	0.30	0.25	2.98	0.13	.7049	.0116	.5410	.005	.377	.0030
27	670.	0.40	0.28	2.72	0.13	.7463	.0099	.3284	.005	.358	.0039
31	688.	0.31	0.26	2.78	0.12	.6686	.0093	.4942	.004	.363	.0025
35	690.	0.29	0.22	2.88	0.12	.6377	.0096	.4928	.007	.348	.0017
39	648.	0.31	0.25	2.42	0.11	.5864	.0106	.6173	.006	.355	.0028
43	700.	0.26	0.61	2.66	0.10	.5857	.0089	.5286	.003	.314	.0026
47	650.	0.31	0.38	2.74	0.11	.6000	.0068	.6000	.005	.338	.0028
51	652.	0.28	0.28	2.98	0.12	.5982	.0075	.5675	.007	.353	.0031
55	725.	0.23	0.41	2.98	0.11	.5793	.0083	.4690	.004	.317	.0036
59	670.	0.31	0.27	2.78	0.12	.6866	.0112	.5821	.005	.328	.0037
63	730.	0.23	0.26	2.44	0.11	.6027	.0121	.4932	.004	.274	.0026
67	550.	0.31	0.25	2.16	0.18	.6000	.0118	.6182	.005	.400	.0033
75	663.	0.33	0.33	2.38	0.14	.7391	.0106	.5279	.004	.302	.0030
71	710.	0.30	0.25	2.27	0.13	.6901	.0089	.5493	.006	.338	.0025
79	710.	0.30	0.27	2.34	0.11	.6761	.0082	.5070	.005	.352	.0027

* not weighed - technical error

APPENDIX 8

Individual Organ as a Percent of Bodyweight x 100

Group 3♀ - 25%

#	bwt	hrt	lng	spl	kid	adr	gon	thy	brn	pit	utr
83	388.	0.28	0.41	**0.13**	.5928	.0188	.0464	.010	.541	.0052	.1546
87	350.	0.31	0.31	**0.14**	.6286	.0217	.0249	.006	.629	.0043	.3143
91	320.	0.34	0.41	**0.16**	.9687	.0178	.0184	.011	.687	.0106	.2500
95	326.	0.34	0.49	**0.15**	.7669	.0206	.0347	.006	.644	.0049	.1840
99	450.	0.33	0.31	**0.13**	.6000	.0073	.0400	.008	.489	.0042	.2000
103	330.	0.36	0.39	**0.18**	.6667	.0233	.0415	.008	.606	.0052	.3333
107	297.	0.34	0.47	**0.17**	.6734	.0229	.0330	.012	.673	.0074	.2694
111	331.	0.36	0.36	**0.15**	.7251	.0227	.0396	.010	.634	.0100	.2719
115	336.	0.36	0.48	**0.18**	.8929	.0232	.0455	.011	.625	.0080	.2976
119	334.	0.30	0.39	**0.18**	.6886	.0210	.0257	.008	.629	.0093	.2096
123	300.	0.37	0.43	**0.20**	.7667	.0257	.0370	.012	.700	.0040	.3333
127	395.	0.28	0.43	**0.15**	.7342	.0203	.0319	.009	.557	.0041	.1772
131	375.	0.35	0.37	**0.16**	.7733	.0259	.0379	.007	.560	.0032	.2133
135	312.	0.35	0.42	**0.16**	.7692	.0298	.0353	.009	.673	.0045	.1923
139	305.	0.39	0.43	**0.16**	.7541	.0230	.0403	.012	.689	.0062	.2295
143	303.	0.40	0.43	**0.17**	.8911	.0221	.0271	.012	.726	.0116	.2310
147	375.	0.37	0.43	**0.13**	.8267	.0237	*	.008	.560	.0045	.2667
151	394.	0.38	0.30	**0.18**	.8122	.0213	.0376	.011	.558	.0099	.2284
155	346.	0.32	0.43	**0.14**	.7225	.0257	.0353	.007	.607	.0055	.2890
159	302.	0.36	0.40	**0.17**	.7285	.0301	.0391	.012	.695	.0073	.2318

* not weighed - techn error

APPENDIX 8

Individual Organ Weights as a Percent of Bodyweight x 100

Group 4♂ - 50%

♂	bwt	hrt	lng	liv	spl	kid	adr	gon	thy	brn	pit
4	486.	0.29	0.41	2.49	0.19	.6996	.0101	.6996	.007	.473	.0025
8	630.	0.25	0.37	2.27	0.13	.7143	.0086	.5397	.003	.349	.0033
12	566.	0.28	0.35	3.37	0.11	.7597	.0124	.3180	.005	.371	.0035
16	560.	0.29	0.30	2.55	0.11	.6071	.0077	.5179	.006	.411	.0030
20	570.	0.25	0.33	3.40	0.12	.7544	.0121	.5965	.004	.386	.0021
24	630.	0.27	0.29	2.51	0.13	.6190	.0116	.5079	.007	.333	.0016
28	685.	0.23	0.19	2.53	0.12	.6277	.0123	.2774	.003	.277	.0025
32	572.	0.31	0.31	2.99	0.14	.7168	.0114	.5944	.006	.402	.0024
36	570.	0.30	0.28	2.93	0.12	.7368	.0088	.5088	.004	.386	.0037
40	615.	0.26	0.34	2.89	0.11	.6667	.0104	.3902	.005	.358	.0028
44	725.	0.23	0.26	2.84	0.11	.6759	.0092	.5103	.006	.303	.0023
48	640.	0.28	0.31	2.94	0.12	.7031	.0095	.6094	.008	.359	.0017
52	684.	0.28	0.28	2.89	0.13	.6140	.0110	.4971	.006	.351	.0023
56	620.	0.31	0.29	2.65	0.13	.6935	.0063	.5484	.005	.355	.0023
60	545.	0.28	0.28	2.68	0.13	.5505	.0092	.3670	.010	.459	.0037
64	563.	0.27	0.36	3.25	0.11	.6927	.0110	.5684	*	.444	.0027
68	650.	0.26	0.32	2.69	0.12	.6923	.0112	.5231	.004	.292	.0029
72	700.	0.27	0.30	2.40	0.13	.5571	.0123	.5429	.004	.343	.0021
76	650.	0.29	0.42	2.68	0.08	.6923	.0091	.5385	.004	.323	.0038
80	666.	0.26	0.33	2.85	0.09	.6306	.0105	.4955	.005	.345	.0042

* not weighed - technical error

APPENDIX 8

Individual Organ Weights as a Percent of Bodyweight x 100

Group 4♀ - 50%

#	bwt	hrt	lng	liv	spl	kid	adr	gon	thy	brn	pit	utr
84	290.	0.38	0.52	3.24	0.17	.8276	.0276	.0576	.008	.759	.0069	.3793
88	320.	0.37	0.47	3.00	0.19	.7812	.0241	.0441	.009	.687	.0062	.3125
92	280.	0.43	0.43	2.96	0.18	.0357	.0268	.0361	.012	.786	.0075	.3214
96	304.	0.39	0.46	3.39	0.16	.8882	.0253	.0398	.012	.724	.0069	.4276
100	310.	0.35	0.42	2.55	0.19	.8710	.0258	.0390	.008	.742	.0074	.3548
104	300.	0.37	0.47	2.90	0.17	.7000	.0303	.0440	.010	.733	.0053	.2667
108	310.	0.45	0.42	3.06	0.16	.9032	.0348	.0442	.009	.677	.0081	.2903
112	290.	0.38	0.48	3.17	0	*	*	*	.007	.724	.0100	*
116	280.	0.39	0.50	3.11	0.18	.8571	.2929	.0429	.015	.750	.0061	.4643
120	295.	0.34	0.75	3.05	0.31	.8475	.0315	.0329	.013	.678	.0088	.2712
124	320.	0.31	0.44	2.87	0.22	.8437	.0247	.0041	.009	.687	.0056	.2812
128	342.	0.29	0.44	2.84	0.20	.7602	.0237	.0284	*	.643	.0047	.3801
132	350.	0.43	0.40	2.86	0.17	.9429	.0240	.0240	.007	.600	.0051	.2857
136	293.	0.38	0.44	2.90	0.20	.8191	.0259	.0468	.009	.751	.0041	.5119
140	312.	0.38	0.38	2.98	0.16	.9615	.0260	.0288	.012	.673	.0035	.4167
144	290.	0.31	0.45	3.14	0.21	.9310	.0252	.0486	.015	.724	.0034	.3793
148	300.	0.33	0.57	3.07	0.20	.8667	.0300	.0467	.012	.700	.0057	.4000
152	308.	0.39	0.55	3.41	0.13	.9416	.0292	.0438	.008	.714	.0127	.2597
156	304.	0.36	0.56	3.09	0.13	.8553	.0207	.0510	.011	.625	.0095	.5263
160	325.	0.34	0.43	3.02	0.22	.8000	.0228	.0286	.011	.646	.0049	.2462

* not weighed - technical error

APPENDIX 9

Macroscopic Pathology of Rats Sacrificed at 26 Weeks

Group No.	Rat No.	Observations
1 ♂ Control	1, 9, 17, 25, 29, 33, 41, 45 49, 61, 65, 69, 73, 77	No abnormalities seen.
	5	Liver has a rounded perimeter. Waxed plug (appears to be sperm) found in urinary bladder.
	13	Light beige nodule in abdominal fat, 1.0 x 0.5cm.
	21	Red areas on all lobes of lungs.
	37	Dark spots on all lobes of lungs.
	53	Yellow nodule in the fatty tissue around the epididymis. Testes are small, 1.5g, and each have white foci. Liver has a rounded perimeter.
	57	Liver has a rounded perimeter.
2♂ 12.5%	2, 10, 14, 18, 22, 26, 30, 38, 42, 46, 50, 54, 58, 62, 66, 70, 74	No abnormalities seen.
	6	Waxed plug (appears to be sperm) found in urinary bladder.
	34	Purple spots on all lobes of lungs.
	78	Firm red mass attached to the superior portion of the liver. Weight of liver and attached mass 15.2g.
3♂ 25%	3, 7, 15, 19, 27, 35, 39, 51, 55, 59, 63, 75, 71, 79	No abnormalities seen.
	11	Firm fatty tissue connecting the liver and right kidney.
	23	Dark foci on liver
	31	Yellow nodule in the fatty tissue around the epididymis.
	43	Dark areas on all lobes of lungs.
	47	Light areas on all lobes of lungs.
	67	Light brown areas on the left kidney.

APPENDIX 9
(continued)

Macroscopic Pathology of Rats Sacrificed at 26 Weeks

Group No.	Rat No.	Observations
4 ♂ 50%	4, 16, 20, 24, 28, 32, 36, 40, 44, 48, 52, 56, 60, 64, 68, 72, 80	No abnormalities seen.
	8	Dark areas on all lobes of lungs.
	12	Small left testis 0.5g.
	76	Beige mass attached to esophagus and spinal column
1 ♀ Control	81, 89, 93, 97, 101, 105, 109, 125, 133, 137, 141, 149, 153, 157	No abnormalities seen.
	85, 117	Dark areas on all lobes of lungs
	113	Purple areas on lungs. Swollen area at cervix of uterus.
	121	Clear fluid-filled cyts on ovaries.
	129, 145	Dark foci on cervical lymph nodes.
2 ♀ 12.5%	82, 90, 106, 114, 126, 134, 146, 150, 154	No abnormalities seen
	94, 158	Clear fluid-filled cysts on ovaries.
	86, 98, 102, 118, 142	Uterus distended with fluid.
	110	Purple areas on all lobes of lungs. Left kidney has a large pelvic region. Right adrenal is more triangular shaped then spherical.
	122	Clear fluid-filled cyst on left ovary.
	130	Red foci on small intestine.
	138	Dark areas on all lobes of lungs. Uterus distended with fluid.
3 ♀ 25%	83, 87, 91, 95, 99, 103, 111, 119, 123, 131, 135, 155	No abnormalities seen.

APPENDIX 9
(continued)

Macroscopic Pathology of Rats Sacrificed at 26 Weeks

Group No.	Rat No.	Observations
3 ♀ 25%	107, 127	Left adrenal has a dark area.
	115, 139, 147	Clear fluid-filled cysts on ovaries.
	143	Clear fluid-filled cysts on spleen.
	159	Clear fluid-filled cyst on right ovary.
	151	Uterus distended with fluid.
4 ♀ 50%	84, 88, 92, 100, 108, 116, 124, 128, 132, 148, 160	No abnormalities seen.
	96	Purple areas on all lobes of lungs. Uterus distended with fluid.
	104	Clear fluid-filled cyst on left ovary.
	112	Firm tissue connecting left kidney, spleen and left adrenal. Hard, stone-like structures in the pelvic region of the right kidney; stone-like structures in the urinary bladder. Yellow foci regions on the cortex of the left kidney. Pelvic region filled with clear fluid.
	120	Clear fluid-filled cyst on right ovary. Swollen area on left horn of uterus.
	136, 144	Swollen left horn of uterus.
	140	Red foci on caecum. Uterus distended with fluid.
	152	Dark foci on all lobes of lungs.
	156	Grey-green foci on all lobes of lungs. Swollen horns of uterus, 1.6g.

Six-Month Dietary Administration of Reacted Molasses to Albino Rats

APPENDIX 10

PATHOLOGY SUMMARY

PATHOLOGY REPORT OF NRC BALTIMORE
1-314 RAT STUDY.

The following tissues were received for histopathological examination:-

Lung	Stomach	
Heart	Duodenum	
Thymus	Ileum	
Lymph nodes (cervical and mesenteric)	Colon	
	Cerebral cortex	Central nervous system
Liver	Mid-brain	Central nervous system
Spleen	Cerebellum	Central nervous system
Pancreas	Medulla	Central nervous system
Kidney	Pituitary	
Urinary bladder	Eye	
Seminal vesicles	Any abnormal tissues	
Gonads		
Uterus		
Thyroids		
Adrenals		
Salivary gland		

The above-mentioned tissues were fixed in buffered formalin from two to four weeks, routinely processed in paraffin wax at 56°C M.P., sectioned at 5μ and stained with haematoxylin and eosin. Cryostat sections of liver and kidney fixed in formol calcium were cut at 12μ and stained for fat with Oil Red O.

PATHOLOGY REPORT OF HRC BALTIMORE
1-314 RAT STUDY

Summary and Conclusion

Summary

Detailed microscopical observations for individual animals are given in the appendix. The following comments are made in summary:-

Heart. Small foci of mononuclear cell infiltration were seen in the ventricular myocardium in two out of the six control males examined, in one female animal (rat 132♀ - Group 4) and in twelve out of the twenty males in Group 4. This increased incidence in males in Group 4 was associated with a minimal increase in the extent of these lesions although the nature was similar to that occurring in the controls. Focal myocarditis in association with myocardial degeneration is known to occur spontaneously in the rat and consequently the increased incidence seen in Group 4♂ was considered to be an exacerbation of this spontaneous condition probably associated with a non-specific stress factor, and was not of toxicological significance.

Lung. Peribronchiolar lymphoid hyperplasia and perivascular accumulations of lymphocytes were seen in the majority of animals examined and were associated with small foci of chronic inflammatory cell infiltration, aggregations of distended macrophages, patchy congestion, bronchiectasis and bronchopneumonia of occasional individuals from both control and treatment groups. These changes were considered to be those that commonly occur in the lungs of rats. They were non-specific and hence were disregarded from the point of view of the experiment.

Small foci of dystrophic mineralisation were seen in the media of the pulmonary artery in rat 85♀ and 89♀ (Control).

Liver. No group related changes were seen in the livers examined. Foci of mononuclear cell infiltration were seen in occasional animals from both control and treatment groups and foci of necrosis seen in rat 78♂ (Group 2) and 23♂ (Group 3).

Kidney. Foci of dystrophic mineralisation were seen at the cortico-medullary junction in one male and in fifteen females in Group 4. No similar lesions were seen in the controls. This entity is known to occur spontaneously in the kidney of the rat, particularly in females of certain strains. In this experiment therefore it was considered to represent a response to high mineral content of molasses and was considered to be of no toxicological significance.

Other changes seen in the kidneys of a proportion of animals included, mononuclear cell infiltration of the interstitial tissue, sometimes associated with minimal changes in small groups of tubules,

Summary (continued)

an extensive nephritis with peritonitis in rat 112♀ (Group 4) and small foci of dystrophic mineralisation beneath the epithelium of the renal pelvis in rats 16♂, 52♂ and 72♂ (Group 4). These changes were considered to be spontaneous in nature and of no toxicological significance.

Other histopathological entities observed but not considered to be significant included:-

Foci of extra-medullary haemopoiesis and groups of macrophages containing brown pigment in the spleen of both control and treatment animals,

A plug of eosinophilic material in the urinary bladder of rat 1♂ (Control),

Chronic cystitis in rat 112♀ (Group 4),

Hydrometra in rats 144♀ and 156♀ (Group 4),

Squamous metaplasia in occasional endometrial glands in rat 132♀ (Group 4)

Cystic distension of endometrial glands in rat 136♀ (Group 4),

Epithelial whorls in the thyroid of rats 84♀ and 132♀ (Group 4),

A haematoma in rat 76♂ (Group 4),

A distended lymphatic in the capsule of the spleen in rat 143♀ (Group 3),

Areas of haemangiomatous change in the adrenal cortex of rats 107♀ and 127♀ (Group 3) and 116♀ (Group 4),

Lipomata in rats 13♂ (Control) and 31♂ (Group 3),

Branchial cysts in the thymus of rats 97♀ (Control) and 92♀ (Group 4),

Follicular cysts on the ovary of rat 120♀ (Group 4),

A nodule of fat necrosis in rat 53♂ (Control),

Atrophic tubules in the testes of rats 53♂ (Control), 12♂, 28♂ 56♂ and 60♂ (Group 4),

Inflammatory cell infiltration of the uterine cervix in rat 113♀ (Control),

Peritoneal adhesions in rats 11♂ (Group 3), 112♀ (Group 4).

Conclusion

No histopathological change or variation from normal was seen in the tissues examined that was considered to be of toxicological significance.

HRC BALTIMORE 1-314 RAT STUDY

Group 1d - 50% Sucrose

Rat 1

Heart:	A single small focus of mononuclear cell infiltration is noted in the ventricular myocardium.
Lung:	Minimal peribronchiolar lymphoid hyperplasia is noted.
Liver:	Hepatic architecture within normal limits. With the Oil Red O no fat is seen.
Spleen:	Occasional foci of extra-medullary haemopoisis are seen in association with groups of macrophages containing brown pigment.
Urinary bladder:	A plug of eosinophilic material is seen in the lumen.

Rat 5

Lung:	A minimal degree of peribronchiolar lymphoid hyperplasia is seen in association with occasional perivascular accumulations of lymphocytes.
Liver:	Hepatic architecture within normal limits. With the Oil Red O no fat is seen.
Urinary bladder:	A plug of eosinophilic material is seen in the lumen.

Group 16 (continued)

Rat 9

Lung:	Minimal peribronchiolar lymphoid hyperplasia is noted.
Liver:	Hepatic architecture within normal limits. With the Oil Red O no fat is seen.

Rat 13

Lung:	Minimal peribronchiolar lymphoid hyperplasia is noted.
Liver:	Hepatic architecture within normal limits. With the Oil Red O no fat is seen.
Kidney:	Small foci of mononuclear cell infiltration are noted in the interstitial tissue of the cortex, and are associated with occasional distended tubules containing eosinophilic material.
Nodule in abdomen:	This is a nodule of fat tissue, probably a lipoma.

Group 16 (continued)

Rat 17

Heart:	Foci of chronic inflammatory cell infiltration amongst which fibroblasts and macrophages are noted are seen in the ventricular myocardium.
Lung:	Minimal peribronchiolar lymphoid hyperplasia is seen in association with occasional perivascular accumulations of lymphocytes.
Liver:	Foci of mononuclear cell infiltration are seen beneath the capsule in occasional portal tracts. With the Oil Red O no fat is seen.

Rat 21

Lung:	Congested. Minimal peribronchiolar lymphoid hyperplasia is noted.
Liver:	Hepatic architecture within normal limits. With the Oil Red O no fat is seen.

HRC BALTIMORE 1-314 RAT STUDY

Group 1♂ - 50% Sucrose - Abnormalities only

Rat 37

Lung:	Patchy congestion is seen in association with minimal peribronchiolar lymphoid hyperplasia. A small focus of dystrophic mineralisation is seen in the media of the pulmonary artery.

Rat 53

Nodule in epididymis:	A nodule of fat necrosis.
Testes:	A proportion of atrophic tubules are seen in both testes.

Rat 57

Liver:	Hepatic architecture within normal limits.

HRC BALTIMORE 1-314 RAT STUDY

Group 1¥ - 50% Sucrose

Rat 81

Lung:	Minimal peribronchiolar lymphoid hyperplasia is seen in association with occasional perivascular accumulations of lymphocytes.
Spleen:	Occasional foci of extra-medullary haemopoiesis are seen in association with small groups of macrophages containing brown pigment.
Liver:	Hepatic architecture within normal limits. With the Oil Red O no fat is seen.

Rat 85

Lung:	Minimal peribronchiolar lymphoid hyperplasia is seen. A focus of dystrophic mineralisation is noted in the media of the pulmonary artery in one lobe.
Liver:	Hepatic architecture within normal limits. With the Oil Red O no fat is seen.
Spleen:	Foci of extra-medullary haemopoeisis and occasional groups of macrophages containing brown pigment are noted.

Group 1¥ (continued)

Rat 89

Lung:	Minimal peribronchiolar lymphoid hyperplasia is seen. Foci of dystrophic mineralisation are present in the media of the pulmonary artery.
Liver:	Hepatic architecture within normal limits. With the Oil Red O no fat is seen.

Rat 93

Lung:	Minimal peribronchiolar lymphoid hyperplasia is noted.
Liver:	Hepatic architecture within normal limits. With the Oil Red O no fat is seen.

Rat 97

Thymus:	A branchial cyst is seen.
Lung:	Minimal peribronchiolar lymphoid hyperplasia is seen in association with occasional perivascular accumulations of lymphocytes.
Liver:	Hepatic architecture within normal limits. With the Oil Red O no fat is seen.
Spleen:	Small groups of macrophages containing brown pigment are noted.

Group 19 (continued)

Rat 101

Lung:	Minimal peribronchiolar lymphoid hyperplasia is noted.
Liver:	Hepatic architecture within normal limits. With the Oil Red O no fat is seen.

HRC BALTIMORE 1-314 RAT STUDY

Group 19 - 50% Sucrose - Abnormalities only

Rat 113

Lung:	Minimal peribronchiolar lymphoid hyperplasia is seen in association with occasional small perivascular accumulations of lymphocytes.
Mass at cervix:	There is evidence of a diffuse inflammatory change involving the endometrium and myometrium.

Rat 117

Lung:	A minimal degree of chronic interstitial pneumonitis is seen in association with occasional subpleural focal aggregations of distended macrophages and occasional perivascular accumulations of lymphocytes.

Rat 129

Cervical lymph node:	No abnormalities are detected.

HRC BALTIMORE 1-314 RAT STUDY

Group 1♀ (continued)

Rat 145

Cervical lymph node: No abnormalities are detected.

HRC BALTIMORE 1-314 RAT STUDY

Group 2♂ - 12.5% - Abnormalities only

Rat 34

Lung: Minimal peribronchiolar lymphoid hyperplasia is seen in association with occasional small perivascular accumulations of lymphocytes.

Rat 78

Liver: A small focus of necrotic cells is seen in one lobe and is associated with an area of fibrosis and chronic inflammatory cell infiltration. Patchy congestion is also noted.

HRC BALTIMORE 1-314 RAT STUDY

Group 2# - 12.5% - Abnormalities only

Rat 86

Uterus: No abnormalities are detected. The distension of the lumen is attributed to normal physiological changes.

Rat 98

Uterus: No abnormalities are detected. The distension of the lumen reported macroscopically is attributable to normal physiological changes.

Rat 102

Uterus: No abnormalities are detected. The distension of the lumen reported macroscopically is attributable to normal physiological changes.

Group 2♀ (continued)

Rat 110

Lung:	Patchy congestion is seen in association with minimal peribronchiolar lymphoid hyperplasia, occasional perivascular accumulations of lymphocytes and occasional small groups of macrophages containing brown pigment.
Adrenals:	No abnormality is detected.
Kidney:	Groups of tubules showing evidence of cystic distension with eosinophilic material are seen in the cortex and are associated with minimal mononuclear cell infiltration of the interstitial tissue.

Rat 118

Uterus:	No abnormalities are detected. The distension of the lumen reported macroscopically is attributable to normal physiological changes.

Rat 130

Small intestine:	No abnormality is detected in the multiple sections examined.

Group 2♀ (continued)

Rat 138

Lung:	Minimal peribronchiolar lymphoid hyperplasia is seen.
Uterus:	No abnormality is detected. The minimal distension reported macroscopically is attributable to normal physiological change.

Rat 142

Uterus:	No abnormality is detected. The minimal distension reported macroscopically is attributable to normal physiological change.

HRC BALTIMORE 1-314 RAT STUDY

Group 3d - 25% - Abnormalities only

Rat 11

Kidney: There is evidence of adhesions between the capsule of the kidney and the liver. Within the kidney occasional tubules characterised by their basophilic staining are seen in association with minimal mononuclear cell infiltration of the interstitial tissue.

Rat 23

Liver: Small foci of necrosis with associated inflammatory cell infiltration and minimal haemorrhage are seen scattered throughout the parenchyma.

Rat 31

Nodule in epididymis: This is an encapsulated lipoma.

Group 3♂ (continued)

Rat 43

Lung: Patchy congestion is seen in association with minimal peribronchiolar lymphoid hyperplasia.

Rat 47

Lung: Bronchiectasis is seen in association with peribronchiolar lymphoid hyperplasia, perivascular accumulations of lymphocytes and a bronchiectatic abscess in one lobe.

Rat 67

Kidney: Areas of chronic inflammatory cell infiltration are seen in the interstitial tissue beneath the capsule. These are associated with the presence of cellular exudate in occasional tubules and foci of dystrophic mineralisation in the medulla.

HRC BALTIMORE 1-314 RAT STUDY

Group 3♀ - 25% - Abnormalities only

Rat 107

Adrenal: A small area of haemangiomatous change is noted in the cortex.

Rat 127

Adrenal: A small area of haemangiomatous change is seen in the section examined.

Rat 143

Spleen: The cyst reported macroscopically is considered to be a distended lymphatic.

Rat 151

Uterus: No abnormality is detected. The dilatation of the lumen reported macroscopically is attributable to normal physiological change.

Group 4δ - 50% Compound

Rat 4

Lung: Minimal peribronchiolar lymphoid hyperplasia is seen in association with occasional perivascular accumulations of lymphocytes.

Liver: Hepatic architecture within normal limits. With the Oil Red O no fat is seen.

Rat 8

Lung: A minimal degree of chronic interstitial pneumonitis is seen scattered throughout the lung fields examined.

Liver: Hepatic architecture within normal limits. With the Oil Red O no fat is seen.

Rat 12

Heart: Minimal diffuse chronic inflammatory cell infiltration is noted in the ventricular myocardium. The predominant cells include fibroblasts and macrophages.

Lung: Minimal peribronchiolar lymphoid hyperplasia is noted.

Liver: Hepatic architecture within normal limits. With the Oil Red O no fat is seen.

Spleen: Occasional foci of extra-medullary haemopoiesis are noted.

Kidney: Minimal diffuse mononuclear cell infiltration is seen in small areas of the cortex.

Testes: A maturity of tubules in one testis are atrophic.

Group 46 (continued)

Rat 16

Heart:	Small foci of mononuclear cell infiltration are seen in the ventricular myocardium, at the base of papillary muscle.
Lung:	Minimal peribronchiolar lymphoid hyperplasia is seen.
Liver:	Hepatic architecture within normal limits. With the Oil Red O no fat is seen.
Kidney:	Small foci of dystrophic mineralisation are noted beneath the epithelium of the renal pelvis.

Rat 20

Heart:	Small foci of chronic inflammatory cell infiltration in which fibroblasts predominate are seen scattered throughout the ventricular myocardium.
Lung:	Occasional small perivascular accumulations of lymphocytes are noted.
Liver:	Hepatic architecture within normal limits. With the Oil Red O no fat is seen.

HRC BALTIMORE 1-314 RAT STUDY

Group 46 (continued)

Rat 24

Lung:	Minimal peribronchiolar lymphoid hyperplasia is seen in association with occasional perivascular accumulations of lymphocytes.
Liver:	Hepatic architecture within normal limits. With the Oil Red O no fat is seen.
Kidney:	A single small focus of dystrophic mineralisation is noted at the cortico-medullary junction.
Urinary bladder:	A plug of eosinophilic material is seen in the lumen.

Rat 28

Lung:	Minimal peribronchiolar lymphoid hyperplasia is seen in association with occasional perivascular accumulations of lymphocytes.
Liver:	Hepatic architecture within normal limits. With the Oil Red O no fat is seen.
Kidney:	Small foci of mononuclear cell infiltration are noted in the interstitial tissue of the cortex.
Test es:	A proportion of tubules are atrophic.

Group 4d (continued)

Rat 32

Heart:	Minimal diffuse fibrosis is seen in association with vacuolated and degenerate myocardial fibres in the ventricular myocardium.
Lung:	Minimal peribronchiolar lymphoid hyperplasia is noted.
Liver:	Hepatic architecture within normal limits. With the Oil Red O no fat is seen.
Kidney:	Occasional tubules showing evidence of cystic distension with eosinophilic material are seen in association with minimal mononuclear cell infiltration of the cortex.

Rat 36

Lung:	A minimal degree of chronic interstitial pneumonitis is seen in association with occasional perivascular and subpleural accumulations of lymphocytes.
Spleen:	Groups of macrophages containing brown pigment are noted.
Liver:	Hepatic architecture within normal limits. With the Oil Red O no fat is seen.
Kidney:	Occasional small foci of mononuclear cell infiltration are noted in the cortex.

HRC BALTIMORE 1-314 RAT STUDY

Group 46 (continued)

Rat 40

Lung:	Minimal peribronchiolar lymphoid hyperplasia is noted.
Liver:	Hepatic architecture within normal limits. With the Oil Red O no fat is seen.

Rat 44

Heart:	Minimal diffuse mononuclear cell infiltration is seen in the ventricular myocardium.
Lung:	Minimal peribronchiolar lymphoid hyperplasia is seen in association with occasional perivascular accumulations of lymphocytes.
Liver:	Hepatic architecture within normal limits. With the Oil Red O no fat is seen.
Kidney:	Occasional groups of distended tubules are seen in association with minimal chronic inflammatory cell infiltration of the interstitial tissue of the cortex.

Group 4d (continued)

Rat 48

Heart:	Minimal diffuse mononuclear cell infiltration is seen in the ventricular myocardium and is associated with groups of degenerate fibres.
Lung:	Minimal peribronchiolar lymphoid hyperplasia is present.
Spleen:	Macrophages containing brown pigment are noted.
Liver:	Hepatic architecture within normal limits. With the Oil Red O no fat is seen.
Kidney:	Occasional distended tubules containing eosinophilic material are noted in the cortex.

Rat 52

Heart:	Multiple small foci of chronic inflammatory cell infiltration amongst which macrophages and fibroblasts are prominent are seen in the ventricular myocardium.
Lung:	Minimal peribronchiolar lymphoid hyperplasia is noted.
Liver:	Hepatic architecture within normal limits. With the Oil Red O no fat is seen.
Kidney:	Occasional small foci of dystrophic mineralisation are noted beneath the epithelium of the renal pelvis.

Group 46 (continued)

Rat 56

Heart:	Areas of chronic inflammatory cell infiltration including fibroblasts are seen in association with focal myocardial degeneration.
Lung:	Minimal peribronchiolar lymphoid hyperplasia is seen in association with occasional perivascular accumulations of lymphocytes.
Spleen:	Foci of extra-medullary haemopoiesis are noted.
Liver:	Hepatic architecture within normal limits. With the Oil Red O no fat is seen.
Testes:	A proportion of tubules in both testes are seen to be atrophic.

Rat 60

Lung:	Minimal peribronchiolar lymphoid hyperplasia is noted.
Liver:	Hepatic architecture within normal limits. With the Oil Red O no fat is seen.
Testes:	A proportion of tubules in both testes are seen to be atrophic.

Group 4d (continued)

Rat 64

Heart:	Occasional small foci of mononuclear cell infiltration are noted in the ventricular myocardium.
Lung:	Minimal peribronchiolar lymphoid hyperplasia is noted.
Spleen:	Groups of macrophages containing brown pigment are noted.
Liver:	Hepatic architecture within normal limits. With the Oil Red O no fat is seen.

Rat 68

Heart:	Foci of chronic inflammatory cell infiltration are seen in the ventricular myocardium. In some areas there is evidence of fibrosis and macrophages containing brown pigment are seen.
Lung:	Minimal peribronchiolar lymphoid hyperplasia is noted.
Liver:	Hepatic architecture within normal limits. With the Oil Red O no fat is seen.

Group 4d (continued)

Rat 72

Heart:	Small foci of chronic inflammatory cell infiltration characterised by fibrosis and by the presence of macrophages are seen in the ventricular myocardium.
Lung:	Minimal peribronchiolar lymphoid hyperplasia is noted.
Spleen:	Groups of macrophages containing brown pigment are noted.
Liver:	Hepatic architecture within normal limits. With the Oil Red O no fat is seen.
Kidney:	Small foci of mononuclear cell infiltration are seen in the interstitial tissue of the cortex. Foci of dystrophic mineralisation are present beneath the epithelium of the renal pelvis in one kidney only.

Rat 76

Lung:	Minimal peribronchiolar lymphoid hyperplasia is seen in association with occasional perivascular accumulations of lymphocytes.
Liver:	Hepatic architecture within normal limits. With the Oil Red O no fat is seen.
Kidney:	A group of tubules showing evidence of minimal dilatation with eosinophilic material is seen in the cortex of one kidney.
Mass related to oesophagus:	This is a haematoma.

HRC BALTIMORE 1-314 RAT STUDY

Group 4c (continued)

Rat 80

Heart:	Small foci of mononuclear cell infiltration are noted in the ventricular myocardium.
Lung:	Minimal chronic interstitial pneumonitis is seen in the lung fields examined.
Spleen:	Macrophages containing brown pigment are noted.
Liver:	Hepatic architecture within normal limits. With the Oil Red O no fat is seen.

HRC BALTIMORE 1-314 RAT STUDY

Group 4♀ - 50% Compound

Rat 84

Lung:	Minimal chronic interstitial pneumonitis is seen scattered throughout the lung fields examined.
Spleen:	Groups of macrophages containing brown pigment are noted.
Liver:	Occasional small foci of mononuclear cell infiltration are noted in portal tracts. With the Oil Red O no fat is seen.
Thyroid:	An epithelial whorl is seen in one gland.

Rat 88

Lung:	Peribronchiolar lymphoid hyperplasia is seen in association with occasional perivascular accumulations of lymphocytes.
Liver:	Hepatic architecture within normal limits. With the Oil Red O no fat is seen.
Kidney:	Small foci of dystrophic mineralisation are seen at the cortico-medullary junction.

HRC BALTIMORE 1-314 RAT STUDY

Group 49 (continued)

Rat 92

Thymus:	Branchial cysts are seen.
Lung:	Minimal peribronchiolar lymphoid hyperplasia is seen in association with occasional small perivascular accumulations of lymphocytes.
Liver:	Hepatic architecture within normal limits. With the Oil Red O no fat is seen.
Spleen:	Occasional foci of extra-medullary haemopoiesis are seen in association with macrophages containing brown pigment.

Rat 96

Lung:	Small foci of mononuclear cell infiltration are seen scattered throughout the parenchyma and are associated with minimal peribronchiolar lymphoid hyperplasia.
Spleen:	Macrophages containing brown pigment are noted.
Liver;	Hepatic architecture within normal limits. With the Oil Red O no fat is seen.
Kidney:	Occasional small foci of dystrophic mineralisation are seen at the cortico-medullary junction.
Uterus:	No abnormality is detected.

PRC BALTIMORE 1-314 RAT STUDY

Group 4♀ (continued)

Rat 100

Lung:	Minimal peribronchiolar lymphoid hyperplasia is seen.
Spleen:	Groups of macrophages containing brown pigment are noted.
Liver:	Hepatic architecture within normal limits. With the Oil Red O no fat is seen.
Kidney:	Foci of dystrophic mineralisation are seen at the cortico-medullary junction.

Rat 104

Lung:	Minimal degree of chronic interstitial pneumonitis is seen in association with minimal peribronchiolar lymphoid hyperplasia.
Liver:	Hepatic architecture within normal limits. With the Oil Red O no fat is seen.
Kidney:	Occasional small foci of dystrophic mineralisation are noted at the cortico-medullary junction.

Group 4♀ (continued)

Rat 108

Lung:	Occasional perivascular accumulations of lymphocytes are noted.
Liver:	Hepatic architecture within normal limits. With the Oil Red O no fat is seen.
Spleen:	Macrophages containing brown pigment are noted with foci of extra-medullary haemopoiesis.
Kidney:	Foci of dystrophic mineralisation are seen at the cortico-medullary junction.

Rat 112

Lung:	Minimal peribronchiolar lymphoid hyperplasia is noted.
Liver:	Hepatic architecture within normal limits. With the Oil Red O no fat is seen.
Kidney:	There is evidence of an extensive chronic nephritis involving both kidneys and adjacent structures in the peritoneum. Considerable dystrophic mineralisation is seen in the inflammatory reaction present but this lesion is considered to be isolated and spontaneous occurence but is not associated with the changes in the kidneys in the other animals in this group.
Urinary bladder:	The epithelium shows evidence of squamous metaplasia and hyperplasia and there is associated diffuse chronic inflammatory cell infiltration of the subepithelial connective tissue.
Spleen:	The adhesions reported macroscopically are not seen.

Group 4♀ (continued)

Rat 112 Contd...

Pancreas:	Peritoneal adhesions are noted.
Adrenals:	Diffuse chronic inflammatory cell infiltration is seen in the connective tissue adjacent to one gland.

Rat 116

Lung:	Minimal peribronchiolar lymphoid hyperplasia is noted.
Liver:	Hepatic architecture within normal limits. With the Oil Red O no fat is seen.
Kidney:	Small foci of dystrophic mineralisation are noted at the cortico-medullary junction.
Adrenals:	A small area of haemangiomatous change is noted in the cortex.

Rat 120

Lung:	A moderate degree of peribronchiolar lymphoid hyperplasia is seen in association with the development of bronchiectatic abscesses and minimal bronchopneumonic consolidation. The nodule reported macroscopically is seen to be a group of bronchiectatic abscesses.
Spleen:	Foci of extra-medullary haemopoiesis are noted.
Liver:	Hepatic architecture within normal limits. With the Oil Red O no fat is seen.
Kidney:	Foci of dystrophic mineralisation are noted at the cortico-medullary junction.
Uterus:	No abnormality is detected.
Ovaries:	Follicular cysts are present.

Group 4♀ (continued)

Rat 124

Lung:	Minimal peribronchiolar lymphoid hyperplasia is noted.
Liver:	Minimal mononuclear cell infiltration is noted in occasional portal tracts. With the Oil Red O no fat is seen.
Kidney:	Occasional small foci of dystrophic mineralisation are noted at the cortico-medullary junction.

Rat 128

Lung:	Minimal peribronchiolar lymphoid hyperplasia is noted.
Spleen:	Groups of macrophages containing brown pigment are seen in association with occasional foci of extra-medullary haemopoiesis.
Liver:	Hepatic architecture within normal limits. With the Oil Red O no fat is seen.
Kidney:	Foci of dystrophic mineralisation are seen at the cortico-medullary junction.
Thyroid:	Only one thyroid is present. The other gland was mislaid at post mortem.

Group 4♀ (continued)

Rat 132

Heart:	A small focus of mononuclear cell infiltration is noted in the ventricular myocardium.
Lung:	Occasional perivascular accumulations of lymphocytes are noted.
Liver:	Hepatic architecture within normal limits. With the Oil Red O no fat is seen.
Uterus:	Small foci of squamous metaplasia are noted in the epithelium of the endometrial glands.
Thyroids:	An epithelial whorl is seen in one gland.

Rat 136

Lung:	A minimal degree of chronic interstitial pneumonitis is seen scattered throughout the lung fields examined.
Liver:	Hepatic architecture within normal limits. With the Oil Red O no fat is seen.
Kidney:	Occasional small foci of dystrophic mineralisation are noted at the cortico-medullary junction.
Uterus:	Cystic distension of an endometrial gland is seen to distort one horn of the uterus.

Group 4♀ (continued)

Rat 140

Lung:	A minimal degree of chronic interstitial pneumonitis is seen scattered throughout the lung fields examined.
Spleen:	Macrophages containing brown pigment are noted.
Liver:	There is minimal mononuclear cell infiltration of occasional portal tracts. With the Oil Red O no fat is seen.
Kidney:	Occasional small foci of dystrophic mineralisation are noted at the cortico-medullary junction.
Uterus:	No abnormality is detected. The distension reported macroscopically is attributed to the normal physiological changes seen in this organ.
Caecum:	No abnormality is detected. A small foci reported macroscopically are not seen.

Rat 144

Lung:	Minimal peribronchiolar lymphoid hyperplasia is noted.
Liver:	Hepatic architecture within normal limits. With the Oil Red O no fat is seen.
Uterus:	There is evidence of minimal hydrometra with compression of the uterine wall.

HRC BALTIMORE 1-314 RAT STUDY

Group 42 (continued)

Rat 148

Lung:	Minimal peribronchiolar lymphoid hyperplasia is noted.
Liver:	Hepatic architecture within normal limits. With the Oil Red O no fat is seen.
Kidney:	Foci of dystrophic mineralisation are seen at the cortico-medullary junction.

Rat 152

Lung:	A minimal degree of chronic interstitial pneumonitis is seen scattered throughout the lung fields examined.
Liver:	Hepatic architecture within normal limits. With the Oil Red O no fat is seen.
Kidney:	Small foci of dystrophic mineralisation are seen deep to the epithelium of the renal pelvis in one kidney only.

HRC BALTIMORE 1-314 RAT STUDY

Group 4? (continued)

Rat 156

Lung:	Minimal peribronchiolar lymphoid hyperplasia is seen in association with occasional groups of distended macrophages and perivascular accumulations of lymphocytes. An area of collapse with associated bronchiectasis is seen in one lobe.
Liver:	Hepatic architecture within normal limits. With the Oil Red O no fat is seen.
Spleen:	Groups of macrophages containing brown pigment are seen in association with occasional foci of extra-medullary haemopoiesis.
Kidney:	Foci of dystrophic mineralisation are noted at the cortico-medullary junction.
Uterus:	Minimal hydrometra is seen.

Rat 160

Lung:	Minimal peribronchiolar lymphoid hyperplasia is seen in association with occasional perivascular accumulations of lymphocytes.
Liver:	Hepatic architecture within normal limits. With the Oil Red O no fat is seen.
Kidney:	Occasional foci of dystrophic mineralisation are noted at the cortico-medullary junction.

EXHIBIT 27

Six-Month Dietary Administration of Reacted Molasses to Beagle Dogs

CONFIDENTIAL

1-315
727-291

FINAL REPORT

SIX-MONTH DIETARY ADMINISTRATION OF REACTED MOLASSES TO BEAGLE DOGS

Mr. Hirotoshi Konishi
Managing Director
Hokkaido Sugar Co., Ltd.
Iwanami Fudosan Jinbocho Building
1,2 - Chome, Jinbocho Kanda
Chiyoda-Ku
Tokyo, Japan

Huntingdon Research Center
Post Office Box 6837
Baltimore, Maryland 21204
U. S. A.

Six-Month Dietary Administration of Reacted Molasses to Beagle Dogs

TABLE OF CONTENTS

		Pages
SUMMARY		i
EXPERIMENTAL PROCEDURE		1 - 3
RESULTS		4 - 5
TABLES		
1)	Mean Weekly Bodyweights	6 - 8
2)	Mean Weekly Food Consumption	9 - 11
3)	Group Mean Food Conversion Ratios	12
4)	Group Mean Water Consumption	13
5)	Group Mean Hematology	14 - 17
6)	Group Mean Biochemistry	18 - 21
7)	Mesophile Count and pH Record of Molasses	22
8)	Group Mean Organ Weights in Grams	23
9)	Group Mean Organ Weights as a Percent of Bodyweight	24
APPENDICES		
1)	Individual Bodyweights	25 - 27
2)	Individual Food Consumption	28 - 33
3)	Individual Water Consumption	34 - 37
4)	Individual Hematology	38 - 54
5)	Individual Biochemistry	55 - 62
6)	Individual Urinalysis	63 - 70
7)	Ophthalmoscopy	71 - 74
8)	Gross Observations at Necropsy	75
9)	Individual Organ Weights in Grams.	76 - 77
10)	Individual Organ Weights as a Percent of Bodyweight	78 - 79
11)	Pathology Summary	80 - 91

Six-Month Dietary Administration of Reacted Molasses to Beagle Dogs

SUMMARY

Thirty-two dogs were divided into 4 groups, each consisting of 4 males and 4 females. They were treated as follows for 26 weeks:

Group	Male	Female	% Reacted Molasses (Meliblase) in diet
1	4	4	0
2	4	4	12.5
3	4	4	25.00
4	4	4	50.00

This report covers the feeding phase of the 26-week study.

Other than soft feces at the 25% and 50% test levels, no adverse effects were noted which could be attributed to the compound tested. No compound-related changes were noted in the blood chemistry, hematology or urinalysis of the dogs tested. Pathologic-anatomical evaluation of tissues revealed no compound-related changes.

Submitted by:

Paul J. Upman

Paul J. Upman, B.A.
Senior Supervisor, Toxicology

Approved by:

Luis E. Van Petten

L. E. Van Petten, D.V.M.
Director
Pathology and Toxicology
June 15, 1973

EXPERIMENTAL PROCEDURE

Animal Management

Thirty-two purebred beagles were obtained from the White Eagle Breeding Corporation, Doylestown, Pennsylvania. The dogs were acclimated to the laboratory for two weeks prior to collecting the pretreatment data. After approximately four weeks, the animals were randomized into the following treatment groups:

Group	No. Dogs Male	No. Dogs Female	% Reacted Molasses (Melibiase) in diet
1	4	4	0
2	4	4	12.5
3	4	4	25.0
4	4	4	50.0

The dogs were individually housed with the appropriate diet and tap water freely available.

Diet Preparation

A special high-protein diet was formulated to insure a balanced diet at all levels and to insure that each group of dogs received the same basic ingredients:

% Ingredients	Group 1	Group 2	Group 3	Group 4
Water	10.0	7.5	5.0	-
Sucrose	35.0	26.2	17.5	-
Salt Mix	5.0	3.8	2.5	-
Molasses	-	12.5	25.0	50.0
High-protein feed	50.0	50.0	50.0	50.0
TOTAL DIET	100.0	100.0	100.0	100.0

Observations and Laboratory Estimates

a) Clinical signs - Physical appearance and behavior were observed daily, and any abnormalities noted were recorded.

b) Bodyweight - All dogs were weighed on a weekly basis. Group mean values were calculated.

c) Food consumption - Calculated on a weekly basis for individual animals and group mean values.

d) Water Consumption - Measured for 2 consecutive days: Prior to and after 4 and 13 weeks of treatment and at termination.

e) Ophthalmic examination - All eyes were dilated with 1% Mydriacyl and examined with an indirect ophthalmoscope prior to treatment and at 13 and 26 weeks.

f) Electrocardiogram studies - Performed on all dogs during the first week of treatment and at termina-termination. In addition, all high dose and control dogs were monitored at 4 and 13 weeks.

g) Hematology - Samples of blood were withdrawn by venipuncture from all animals in a fasting state for measurement of the following parameters prior to treatment and after weeks 4 and 13 and at termination:
   1) Sedimentation rate - Wintrobe-Landsburg method
   2) Hematocrit (Hct) - microhematocrit

3) Hemoglobin concentration (Hb) - by Cyanmethemoglobin of Cannon, 1958; Am. J. Clin Path., 30: 211.
4) Red cell count (RBC) - Coulter counter
5) White cell count (WBC) - Coulter counter
6) Differential white cell count - by direct visual count of smear after staining with Wright's stain. Counters were recorded under the following categories:

N	=	Neutrophils
L	=	Lymphocytes
E	=	Eosinophils
B	=	Basophils
M	=	Monocytes
S	=	Stabs

7) Platelet count - using standard hemocytometer with Neubauer ruling
8) Prothrombin time - by FIBROSYSTEM™, developed by BioQuest, Division of Becton, Dickinson and Company

h) Blood chemistry - Samples of blood were withdrawn by venipuncture from all animals, prior to treatment and after weeks 4 and 13 and at termination:

1) Serum glutamate - pyruvate transaminase (SGPT) - Reitman and Frankel: Am. J. Clin. Path., 28: 56, 1957; modified for the Beckman 560 Discrete Sample Analyzer
2) Urea nitrogen - Chaney and Marbach - Clin. Chem., 8: 130, 1962; a modification of the enzymatic (urease) method for use on the Beckman 560
3) Glucose - Ware and Marbach - Clin. Chem., 14: 584, 1968; a modification of the glucose-oxidase method for use with the Beckman 560
4) Alkaline phosphatase - Bessey, Lowry and Brock - J. Biol. Chem., 164: 321, 1946; hydrolysis of p-nitrophenyl phosphate as modified for the Beckman 560
5) Serum proteins and electrophoresis
6) Serum bilirubin - Jendrassik-Grof: Biochem. Z. 297: 81, 1938.
7) Serum electrolytes - sodium, potassium and chloride
8) Serum glutamic - oxalacetic transaminase (SGOT) - Reitman and Frankel. Am. J. Clin. Path., 28: 56, 1957; modified for the Beckman 560 Discrete Sample Analyzer

i) Urinalysis - Individual urine samples were collected over a 24-hour period from all animals in a fasting state prior to treatment and at weeks 5 and 13 and at termination:

1) Specific gravity (SG) - by refractometry using American Optical Company TS meter
2) pH - Beckman Research Model pH meter, to nearest 0.1 pH
3) Albumin - Kingsbury Method
4) Sugar - Clinitest®, graded 0, 0.25, 0.50, 0.75, 1 and 2 percent
5) Ketones - Labstix®
6) Occult blood - Labstix®, graded negative, small medium, large (-, S, M, L)
7) Microscopy - Clay Adams "Sedi-Cal" Procedure, deposits examined for:

epithelial cells	(E)
polymorphonuclear leucocytes	(P)
mononuclear leucocytes	(M)
erythrocytes	(R)
organisms	(O)
casts	(C)
abnormal constituents	(A)

Gradings of cell frequency in deposit were recorded as follows:

0	-	signifies none observed
1	-	signifies few in some fields
2	-	signifies few in all fields
3	-	signifies many in all fields
3+	-	signifies field obscured by crystals

j) Terminal studies - Following 26 weeks of treatment, all dogs were anesthetized with Diabutal I.V. and exsanguinated. Following a macroscopic examination, the following organs were removed, trimmed and weighed:

myocardium	kidneys	thyroids	prostate or
liver	adrenals	brain	uterus
spleen	gonads	pituitary	pancreas

k) Sections of the following tissues were fixed in 10% buffered formalin and examined in hematoxylin and eosin stained paraffin sections:

heart	thymus	cervical node
lungs	(where present)	sciatic nerve
liver	pancreas	mesenteric node
spleen	gall bladder	skeletal muscle
kidneys	stomach	skin
adrenals	esophagus	femur
gonads	small intestine	rib junction
urinary bladder	(3 sections)	eyes
prostate/uterus	large intestine	tongue

RESULTS

a) Clinical Signs

Summary of gross observations are listed below:

Control (50% Sugar)	-	no remarkable observations
12.5% Molasses	-	no remarkable observations
25.0% Molasses	-	all dogs experienced episodes of soft feces, but not on a regular basis
50.0% Molasses	-	all dogs had soft feces or diarrhea throughout the 26-week treatment period.

No other treatment-related observations were noted; and there were no deaths in any of the groups tested.

b) Bodyweight (Table 1, pages 5 - 7; Appendix 1, pages 22 - 24)

Males - rate of growth was acceptable for all male dogs; and terminal bodyweights were comparable in all groups.

Females - rate of growth of the 25% and 50% test dogs was slightly less than the control and 12.5% dogs but within acceptable limits.

c) Food Consumption (Table 2, pages 8 - 10; Appendix 2, pages 25 - 30)

Dogs receiving molasses in their diet tended to show increased food consumption when compared to the control animals. Food conversion ratios presented in Table 3, page 11, verify that the control dogs received a better utilization of their diet. This is not regarded as an adverse effect.

d) Water Consumption (Table 4, page 12; Appendix 3, pages 31 - 34)

No remarkable differences were noted in water consumption of test dogs as compared to the control animals.

e) Ophthalmic Examinations (Appendix 7, pages 68 - 71)

No treatment-related abnormalities were observed during or after the 26 weeks of treatment.

f) Electrocardiograms (Retained in HRC files)

Electrocardiograms were comparable for all dogs monitored and revealed no treatment-related abnormalities.

g) Hematology (Table 5, pages 13 - 16; Appendix 4, pages 35 - 51)

Group mean data was comparable throughout all groups for the parameters measured.

Several dogs, from test and control groups, demonstrated an elevated sedimentation rate which was not considered to be treatment-related.

h) Blood Chemistry (Table 6, pages 17 - 20; Appendix 5, pages 52 - 59)

Group mean data was comparable for all groups tested and no treatment-related abnormalities were noted.

i) Urinalysis (Appendix 6, pages 60 - 67)

No remarkable differences were noted which could be attributed to the treatment received.

j) Terminal Studies

1. A summary of gross observations at necropsy is presented in Appendix 8, page 75. Microscopic pathologic-anatomical evaluation of tissues from test and control animals is presented in Appendix 9, pages 76 to 77. No lesions or abnormalities attributable to the compound tested were observed.

2. Organ to Bodyweight Ratios - In both absolute and relative organ weights, no significant differences could be observed between test and controls.

k) Conclusions

Under the conditions of this experiment, incorporation of reacted molasses melibiase to the diet at dose levels of 12.5%, 25.00% and 50.00% for 26 weeks showed no adverse effects with respect to bodyweight changes, water consumption, ophthalmic examination, electrocardiograms, hematology, blood chemistries, urinalysis and gross and microscopic evaluation of tissues when compared to a negative control population run concurrently.

A dose-related occurrence of soft feces was observed in dogs receiving 25% and 50% melibiase throughout the 26-week period of study.

Six-Month Dietary Administration of Reacted Molasses to Beagle Dogs

TABLE 1

Mean Weekly Bodyweights (kg)

Group Sex	Week -1	0	1	2	3	4	5	6	7	8
1 ♂ Control	9.5	9.9	10.2	10.5	11.0	10.7	10.6	11.4	10.5	11.0
2 ♂ 12.25%	9.6	9.7	9.7	9.9	10.2	10.0	9.6	10.1	10.1	11.0
3 ♂ 25%	9.3	9.1	9.6	10.1	10.1	9.9	9.7	10.2	9.9	10.8
4 ♂ 50%	9.7	9.3	9.4	9.8	9.9	9.6	9.3	10.0	9.5	10.0
1 ♀ Control	8.5	8.1	8.4	8.7	8.7	8.4	8.5	8.8	8.6	9.0
2 ♀ 12.25%	8.7	8.5	8.7	9.0	8.8	8.6	8.3	9.1	8.9	9.5
3 ♀ 25%	9.1	9.1	9.2	9.3	9.4	9.2	9.1	9.3	9.0	9.3
4 ♀ 50%	8.3	8.1	8.2	8.1	8.4	8.2	7.9	8.0	7.9	8.3

TABLE 1
(continued)

Mean Weekly Bodyweights (kg)

Group Sex	Week 9	10	11	12	13	14	15	16	17
1 ♂ Control	10.5	10.5	10.8	10.9	10.6	10.6	10.7	10.6	10.8
2 ♂ 12.5%	10.3	10.4	11.0	11.1	10.9	11.1	11.2	11.2	11.5
3 ♂ 25%	10.2	10.2	10.6	10.8	10.8	10.9	11.0	11.1	11.2
4 ♂ 50%	9.6	9.7	10.0	10.3	10.1	10.3	10.6	10.5	10.5
1 ♀ Control	8.5	8.5	8.9	9.0	8.7	8.9	8.9	8.8	9.0
2 ♀ 12.5%	9.0	9.0	9.3	9.4	9.3	9.3	9.6	9.7	9.7
3 ♀ 25%	8.9	8.9	9.2	9.3	9.1	9.2	9.4	9.3	9.5
4 ♀ 50%	8.1	8.1	8.3	8.5	8.1	8.3	8.4	8.4	8.5

TABLE 1
(continued)

Mean Weekly Bodyweights (kg)

Group Sex	Week 18	19	20	21	22	23	24	25	26
1 ♂ Control	11.0	11.2	11.3	11.3	11.2	11.3	11.5	11.4	11.4
2 ♂ 12.5%	11.4	11.5	11.7	11.7	11.5	11.7	12.0	11.6	11.5
3 ♂ 25%	11.4	11.5	11.6	11.7	11.6	11.8	12.0	12.3	11.9
4 ♂ 50%	10.7	10.8	10.9	10.8	10.7	10.6	10.8	11.4	10.7
1 ♀ Control	9.4	9.2	9.3	9.3	9.3	9.4	9.5	9.6	9.3
2 ♀ 12.5%	9.8	9.8	10.0	10.0	9.9	9.9	10.4	9.9	9.9
3 ♀ 25%	9.8	9.6	9.7	9.7	9.6	9.7	9.9	9.8	9.7
4 ♀ 50%	8.7	8.6	8.7	8.6	8.7	8.7	8.9	9.2	8.8

TABLE 2

Mean Weekly Food Consumption
(g/dog/wk)

Group Sex	Week 1	2	3	4	5	6	7	8
1 ♂ Control	3284	2516	3280	2906	2314	2552	2530	3105
2 ♂ 12.5%	3331	2154	2960	2768	1894	2520	2644	3291
3 ♂ 25%	3129	2664	3122	2885	2201	2591	2575	3372
4 ♂ 50%	2481	3068	3711	2406	2039	2540	2587	3260
1 ♀ Control	2278	2613	2575	2374	2281	2203	2492	2841
2 ♀ 12.5%	3331	2154	2602	2398	1961	2547	2568	3165
3 ♀ 25%	3129	2664	3124	2824	2214	2544	2609	3096
4 ♀ 50%	2296	2370	3082	2526	1991	2488	2544	2886

TABLE 2
(continued)

Mean Weekly Food Consumption
(g/dog/wk)

Group Sex	Week 9	10	11	12	13	14	15	16	17
1 ♂ Control	2588	2501	2757	3360	2753	3381	3221	3316	3123
2 ♂ 12.5%	2516	2585	2828	3332	2924	3422	3294	3379	3383
3 ♂ 25%	2572	2544	2856	3500	3000	3500	3500	3375	3247
4 ♂ 50%	2575	2573	2859	3500	3000	3397	3500	3275	3388
1 ♀ Control	2506	2533	2753	3297	2721	3121	3016	3169	3008
2 ♀ 12.5%	2491	2484	2778	2582	2893	3441	3334	3367	3005
3 ♀ 25%	2528	2565	2817	3415	2712	3339	3258	2886	3110
4 ♀ 50%	2532	2614	2713	3295	2680	3370	3377	2955	3337

TABLE 2
(continued)

Mean Weekly Food Consumption
(g/dog/wk)

Group Sex	Week 18	19	20	21	22	23	24	25	26
1 ♂ Control	3267	3187	3176	3348	3416	3358	3318	3069	2754
2 ♂ 12.5%	2784	2814	3222	3356	3318	3395	3271	2391	2016
3 ♂ 25%	3386	3500	3500	3379	3450	3448	3369	4194	2626
4 ♂ 50%	3374	3226	3280	3362	3500	3393	3440	3358	2863
1 ♀ Control	3065	2848	3124	3062	3174	2853	2961	2639	2662
2 ♀ 12.5%	2680	2647	2950	3414	3172	2881	3101	2271	2082
3 ♀ 25%	3203	2526	3055	3297	3385	3291	3089	2556	2361
4 ♀ 50%	3080	2888	2776	3053	3351	3195	3206	3013	2098

Six-Month Dietary Administration of Reacted Molasses to Beagle Dogs

TABLE 3

Group Mean Food Conversion Rations $\left(\frac{\text{Body wt}}{\text{Food cons}}\right)$

Week	GROUP ♂ Control	12.5%	25.0%	50.0%	GROUP ♀ Control	12.5%	25.0%	50.0%
1	3.1	2.9	3.07	3.8	3.7	2.6	2.9	3.6
2	4.2	4.6	3.8	3.2	3.3	4.2	3.5	3.4
3	4.6	3.4	3.2	2.7	3.4	3.4	3.0	2.7
4	3.7	3.6	3.4	4.0	3.5	3.6	3.3	3.3
Mean	3.9	3.6	3.5	3.4	3.5	3.5	3.2	3.3
5	4.6	5.1	4.4	4.6	3.7	4.2	4.1	4.0
6	4.5	3.8	3.9	3.9	4.0	3.6	3.7	3.2
7	4.2	3.8	3.9	3.7	3.5	3.5	3.5	3.1
8	3.5	3.3	3.2	3.1	3.2	3.0	3.0	2.9
Mean	4.2	4.0	3.9	3.8	3.6	3.6	3.6	3.3
9	4.1	4.1	4.0	3.7	3.4	3.6	3.5	3.2
10	4.2	4.0	4.0	3.8	3.4	3.6	3.5	3.1
11	3.9	3.9	3.7	3.5	3.2	3.4	3.3	3.1
12	3.2	3.3	3.1	2.9	2.7	3.6	2.7	2.6
Mean	3.9	3.8	3.7	3.5	3.2	3.6	3.3	3.5
13	3.9	3.7	3.6	3.4	3.2	3.2	3.4	3.0
14	3.1	3.2	3.1	3.0	2.8	2.7	2.7	2.5
15	3.3	3.4	3.1	3.0	3.0	2.9	2.9	2.5
16	3.2	3.3	3.3	3.2	2.8	2.9	3.2	2.8
Mean	3.4	3.4	3.3	3.2	3.0	2.9	3.1	2.7

Six-Month Dietary Administration of Reacted Molasses to Beagle Dogs

TABLE 4

Group Mean Water Consumption (ml)

Group/Sex Level	Pretreat Day 1	Pretreat Day 2	Pretreat Average	Week 4 Day 1	Week 4 Day 2	Week 4 Average	Week 13 Day 1	Week 13 Day 2	Week 13 Average	Week 26 Day 1	Week 26 Day 2	Week 26 Average
1 ♂ Control	1500	1325	1413	2450	2150	2300	850	1013	931	1025	1825	1425
4 ♂ 50%	1313	1250	1281	2475	1925	2200	1075	1375	1225	900	1625	1263
1 ♀ Control	900	1038	969	1925	1400	1662	1000	1450	1225	700	1425	1063
4 ♀ 50%	1188	800	994	1950	1463	1707	1000	1050	1025	575	1275	925

Six-Month Dietary Administration of Reacted Molasses to Beagle Dogs

TABLE 5

Group Mean Hematology
(Pretreat)

Group Sex	Platelet Count cmm	ESR mm	Hct. %	Hb g%	RBC $x10^6$ cmm	Retic %	WBC $x10^3$ cmm	% Differential N	S	L	E	B	M	Prothrombin / sec.
1 ♂ Control	438,000	1.5	45	15.2	6.23	0.7	15.6	67.3	0.2	32.3	0.2	0	0.0	7.1
2 ♂ 12.5%	397,000	0.25	47	15.7	6.56	0.4	13.6	54.5	0.3	44.2	1.0	0	0.0	8.8
3 ♂ 25%	441,000	0.8	47	15.5	7.38	1.0	12.5	62.5	0.0	34.5	2.3	0	0.7	7.1
4 ♂ 50%	360,000	1.0	44	14.5	6.35	0.5	14.0	54.5	0.2	44.8	0.5	0	0.0	7.1
1 ♀ Control	385,000	0.0	47	15.7	6.48	0.5	12.5	64.8	0.2	33.3	1.5	0	0.2	7.2
2 ♀ 12.5%	399,000	1.3	48	15.6	6.43	0.7	13.8	67.0	0.7	30.3	2.0	0	0.0	8.0
3 ♀ 25%	349,000	2.0	49	16.0	6.83	0.5	12.1	65.0	1.0	33.8	0.2	0	0.0	7.0
4 ♀ 50%	318,000	0.5	51	16.6	7.54	0.4	10.8	61.3	0.5	37.8	0.2	0	0.2	7.1

TABLE 5
(continued)

Group Mean Hematology
(Week 4)

Group Sex	Platelet Count cmm	ESR mm	Hct. %	Hb g%	RBC $x10^6$ cmm	Retic %	WBC $x10^3$ cmm	% Differential N	S	L	E	B	M	Prothrombin / sec.
1 ♂ Control	392,000	0.5	45	15.4	5.55	0.5	13.8	62.5	0.0	35.8	1.2	0	0.5	7.4
2 ♂ 12.5%	292,000	1.5	45	15.7	5.21	0.3	13.0	60.8	0.0	39.2	0.0	0	0.0	10.3
3 ♂ 25%	321,000	1.3	47	16.1	5.75	0.4	11.3	64.5	0.0	34.2	1.0	0	0.3	7.4
4 ♂ 50%	391,000	2.0	45	15.7	5.85	0.4	11.9	55.0	0.0	44.8	0.0	0	0.2	6.9
1 ♀ Control	403,000	0.1	48	16.5	5.87	0.4	12.8	57.0	0.0	42.8	0.0	0	0.2	7.5
2 ♀ 12.5%	359,000	1.0	48	16.7	5.87	0.6	13.7	61.3	0.0	37.5	0.0	0	1.2	8.4
3 ♀ 25%	264,000	4.0	46	15.7	5.61	0.6	13.2	60.0	0.0	39.6	0.2	0	0.2	7.1
4 ♀ 50%	220,000	0.0	49	16.7	6.16	0.4	11.8	60.3	0.0	39.3	0.2	0	0.2	7.3

TABLE 5
(continued)

Group Mean Hematology
(Week 13)

Group Sex	Platelet Count cmm	ESR mm	Hct. %	Hb g%	RBC $x10^6$ cmm	Retic %	WBC $x10^3$ cmm	% Differential N	S	L	E	B	M	Prothrombin / sec.
1 ♂ Control	139,000	1.5	44	15.3	5.50	0.4	10.9	58.5	0.5	35.2	4.5	0	1.3	7.9
2 ♂ 12.5%	194,000	3.5	41	15.2	5.40	0.6	12.6	62.8	0.5	33.7	3.0	0	0.0	9.0
3 ♂ 25%	165,000	3.0	44	15.6	6.28	0.7	12.6	62.0	0.5	31.0	6.0	0	0.5	7.9
4 ♂ 50%	186,500	7.0	42	14.9	5.40	0.6	11.6	60.5	0.3	36.5	1.2	0	1.5	7.4
1 ♀ Control	150,750	2.5	46	15.7	5.96	0.4	12.0	69.0	0.2	27.3	1.5	0	1.2	7.6
2 ♀ 12.5%	230,000	1.5	47	16.1	6.48	0.8	12.1	70.8	0.5	25.5	2.2	0	1.0	8.5
3 ♀ 25%	217,000	7.0	44	15.7	6.09	0.4	11.8	65.0	0.3	32.2	2.5	0	0.0	7.5
4 ♀ 50%	151,750	2.0	44	15.6	5.34	1.4	11.1	63.5	0.2	33.6	2.0	0	0.7	7.4

TABLE 5
(continued)

Group Mean Hematology
(Week 26)

Group Sex	Platelet Count cmm	ESR mm	Hct. %	Hb g%	RBC x10^6 cmm	Retic %	WBC x10^3 cmm	% Differential N	S	L	E	B	M	Prothrombin / sec.
1 ♂ Control	216,250	2	46	15.2	6.24	0.3	11.5	64.3	0.0	31.0	4.5	0	0.2	7.6
2 ♂ 12.5%	230,000	1	46	15.1	6.22	0.4	11.2	57.5	0.2	40.3	1.3	0	0.7	9.5
3 ♂ 25%	216,250	2	48	15.9	6.59	0.7	11.9	68.2	0.0	27.5	3.3	0	1.0	7.5
4 ♂ 50%	243,500	3	45	14.5	6.55	0.4	11.1	65.5	0.0	33.5	0.5	0	0.5	7.4
1 ♀ Control	169,750	1	46	15.1	5.93	0.3	11.4	68.8	0.0	27.5	3.5	0	0.2	7.8
2 ♀ 12.5%	237,250	0	50	16.8	6.95	0.8	12.6	66.0	0.5	29.8	3.0	0	0.7	8.4
3 ♀ 25%	235,000	6	45	15.2	6.09	0.5	12.0	69.3	0.0	28.3	2.2	0	0.2	7.3
4 ♀ 50%	172,500	2	47	15.1	6.49	0.5	10.2	67.3	0.0	32.7	0.0	0	0.0	7.6

Six-Month Dietary Administration of Reacted Molasses to Beagle Dogs

TABLE 6

Group Mean Biochemistry
(Pretreat)

Group Sex	Urea Nitrogen mg%	Glucose mg%	Total Protein gm%	Protein Electrophoresis g% Alb	α1	α2	β	γ	Serum Alkaline Phosphatase BLB U/ml	SGOT RF U/ml	SGPT RF U/ml	Bilirubin mg%	Electrolytes meq/L Na^+	K^+	Cl^- (as Cl^-)
1 ♂ Control	12	83	4.7	2.5	0.2	0.4	0.5	0.9	2.5	26	20	0.43	125	3.7	84.6
2 ♂ 12.5%	11	77	5.8	3.1	0.2	0.5	0.7	1.2	2.0	25	20	0.45	133	3.8	97.8
3 ♂ 25%	10	88	4.9	2.6	0.3	0.4	0.6	1.1	1.9	27	18	0.42	128	3.9	93.3
4 ♂ 50%	11	73	4.4	2.5	0.3	0.3	0.5	1.0	2.2	25	19	0.42	127	3.6	88.9
1 ♀ Control	13	90	5.6	3.2	0.3	0.4	0.7	1.2	1.9	47	31	0.41	123	4.2	112.8
2 ♀ 12.5%	12	99	5.5	3.2	0.3	0.3	0.7	1.1	2.0	53	35	0.41	128	4.0	113.0
3 ♀ 25%	12	86	4.7	2.5	0.2	0.4	0.6	1.0	2.2	29	30	0.41	139	3.8	114.1
4 ♀ 50%	15	93	5.2	2.9	0.3	0.4	0.6	1.0	2.0	34	32	0.41	130	4.1	111.8

TABLE 6
(continued)

Group Mean Biochemistry
(Week 4)

Group Sex	Urea Nitrogen mg%	Glucose mg%	Total Protein gm%	Protein Electrophoresis g% Alb	α1	α2	β	γ	Serum Alkaline Phosphatase BLB U/ml	SGOT RF U/ml	SGPT RF U/ml	Bilirubin mg%	Electrolytes meq/L Na^+	K^+	Cl^- (as Cl^-)
1 ♂ Control	10	99	6.2	3.5	0.3	0.6	0.7	1.2	2.5	25	20	0.22	144	5.0	93.7
2 ♂ 12.5%	10	98	6.2	3.3	0.3	0.6	0.8	1.4	2.2	22	22	0.18	143	4.8	100.8
3 ♂ 25%	11	104	6.1	3.3	0.3	0.5	0.8	1.2	2.3	21	17	0.22	141	4.9	98.0
4 ♂ 50%	10	101	6.1	3.3	0.3	0.6	0.7	1.3	2.2	23	22	0.20	146	4.8	102.1
1 ♀ Control	9	84	4.9	2.8	0.3	0.4	0.5	0.9	2.6	30	22	0.20	150	4.9	93.9
2 ♀ 12.5%	10	102	6.1	3.5	0.3	0.5	0.7	1.1	2.2	23	19	0.19	148	5.0	98.4
3 ♀ 25%	10	87	4.8	2.7	0.3	0.4	0.5	1.0	2.7	22	19	0.20	152	5.0	106.7
4 ♀ 50%	13	85	5.2	3.1	0.3	0.4	0.6	0.9	1.9	30	22	0.21	149	5.0	103.1

TABLE 6
(continued)

Group Mean Biochemistry
(Week 13)

Group Sex	Urea Nitrogen mg%	Glucose mg%	Total Protein mg%	Protein Electrophoresis g% Alb	α1	α2	β	γ	Serum Alkaline Phosphatase BLB U/ml	SGOT RF U/ml	SGPT RF U/ml	Bilirubin mg%	Electrolytes meq/L Na+	K+	Cl⁻ (as Cl⁻)
1 ♂ Control	12	96	6.1	3.2	0.3	0.6	0.6	1.2	2.5	30	18	0.13	144	4.4	104.1
2 ♂ 12.5%	14	98	6.2	2.9	0.3	0.6	0.8	1.3	1.9	31	20	0.15	143	4.8	103.0
3 ♂ 25%	15	97	6.2	2.9	0.3	0.6	0.8	1.4	2.5	38	20	0.16	145	4.6	103.9
4 ♂ 50%	13	98	5.8	2.8	0.3	0.6	0.7	1.2	1.9	30	19	0.16	143	4.5	104.4
1 ♀ Control	19	102	5.8	3.1	0.3	0.5	0.6	1.1	3.7	30	21	0.16	143	4.4	103.2
2 ♀ 12.5%	14	102	6.1	3.4	0.3	0.5	0.7	1.0	2.5	28	20	0.16	144	4.5	104.6
3 ♀ 25%	14	102	6.0	3.1	0.3	0.5	0.6	1.1	3.3	29	19	0.16	141	4.7	104.5
4 ♀ 50%	19	104	5.9	3.3	0.3	0.5	0.6	1.1	1.8	31	20	0.17	141	4.5	104.8

TABLE 6
(continued)

Group Mean Biochemistry
(Week 26)

Group Sex	Urea Nitrogen mg%	Glucose mg%	Total Protein gm%	Protein Electrophoresis g% Alb	α^1	α^2	β	γ	Serum Alkaline Phosphatase BLB U/ml	SGOT RF U/ml	SGPT RF U/ml	Bili-rubin mg%	Electrolytes meq/L Na^+	K^+	Cl^- (as Cl^-)
1 ♂ Control	13	111	6.2	3.0	0.3	0.6	0.8	1.6	3.1	26	20	0.20	149	5.0	109
2 ♂ 12.5%	12	106	6.1	3.0	0.3	0.7	0.9	1.4	2.2	27	26	0.20	143	4.8	111
3 ♂ 25%	17	107	6.5	3.1	0.3	0.6	0.9	1.6	2.6	23	24	0.20	142	4.7	107
4 ♂ 50%	16	108	6.1	3.0	0.3	0.6	0.8	1.5	2.4	26	22	0.10	144	4.8	110
1 ♀ Control	15	106	5.9	3.0	0.4	0.6	0.7	1.4	2.7	24	21	0.10	147	4.9	105
2 ♀ 12.5%	11	114	6.2	3.1	0.3	0.7	0.7	1.4	2.3	21	26	0.10	150	4.7	106
3 ♀ 25%	13	111	5.7	2.6	0.4	0.6	0.7	1.5	2.1	18	21	0.50	146	4.8	105
4 ♀ 50%	16	111	6.1	2.4	0.30	0.6	0.6	1.4	2.2	23	23	0.20	145	4.4	103

TABLE 7

Mesophile Count and pH Record of Molasses

Date	Week	pH	Mesophile Count (per gram)	Batch
8-11-72	-2	6.85	acceptable limits (500)	1 240kg
8-19-72	0	6.90		1
11- 2-72	6	6.80		1
11-10-72	7	6.91	acceptable limits	2 75kg
11-28-72	9	6.70	acceptable limits (290)	3 240kg
1-17-73	17	6.70	acceptable limits (2,300)	4 240kg
2-20-73	23	6.90	acceptable limits	5 85.6kg
3-12-73	26	6.88		5

Six-Month Dietary Administration of Reacted Molasses to Beagle Dogs

TABLE 8

Group Mean Organ Weights in Grams

Group/ Sex	Heart	Liver	Spleen	Kidney	Adrenals	Gonads	Thyroids	Brain	Pituitary	Pancreas	Prostate/ Uterus
1 ♂ Control	92.25	380.45	25.33	71.55	1.30	22.28	1.13	84.20	.074	27.33	7.33
2 ♂ 12.5%	82.85	318.13	23.35	57.80	1.10	21.75	1.03	84.90	.057	30.85	7.00
3 ♂ 25%	93.10	368.73	24.50	60.98	1.28	21.63	1.08	80.45	.078	26.13	5.48
4 ♂ 50%	85.77	390.75	26.18	73.58	1.25	22.40	0.95	85.33	.080	29.25	6.25
1 ♀ Control	74.28	282.85	21.20	49.13	1.23	1.63	1.13	79.68	.074	23.15	8.33
2 ♀ 12.5%	78.83	287.90	23.60	51.60	1.23	1.25	0.75	77.20	.075	25.15	4.90
3 ♀ 25%	82.68	328.93	24.93	61.60	1.38	2.53	1.06	76.53	.070	24.68	12.40
4 ♀ 50%	70.73	328.85	23.50	51.48	1.30	1.68	0.98	74.88	.066	26.40	11.78

Six-Month Dietary Administration of Reacted Molasses to Beagle Dogs

TABLE 9

Group Mean Organ Weights as a Percent of Bodyweight

Group/ Sex	Heart	Liver	Spleen	Kidneys	Adrenals	Gonads	Thyroids	Brain	Pituitary	Pancreas	Prostate/ Uterus
1 ♂ Control	0.82	3.37	0.23	0.64	0.0118	0.20	0.0098	0.750	0.0006	0.24	0.07
2 ♂ 12.5%	0.74	2.84	0.20	0.52	0.0098	0.20	0.0091	0.761	0.0006	0.28	0.07
3 ♂ 25%	0.80	3.36	0.21	0.53	0.0108	0.19	0.0092	0.698	0.0005	0.22	0.05
4 ♂ 50%	0.78	3.56	0.29	0.67	0.0115	0.21	0.0086	0.784	0.0006	0.27	0.06
1 ♀ Control	0.82	3.11	0.23	0.55	0.0135	0.02	0.0122	0.897	0.0008	0.25	0.10
2 ♀ 12.5%	0.84	3.05	0.25	0.55	0.0120	0.01	0.0078	0.824	0.0007	0.27	0.05
3 ♀ 25%	0.86	3.45	0.26	0.64	0.0144	0.03	0.0113	0.804	0.0007	0.26	0.13
4 ♀ 50%	0.82	3.75	0.27	0.59	0.0151	0.20	0.0112	0.859	0.0007	0.30	0.13

APPENDIX 1

Individual Bodyweight Data (kg)

Group Sex	Dog No.	Week -1	0	1	2	3	4	5	6	7	8
1 ♂ Control	980	9.3	8.5	9.0	9.0	9.6	9.5	9.6	10.4	9.1	9.9
	970	10.5	10.6	10.7	11.2	11.9	11.3	11.5	12.3	11.0	11.8
	972	9.7	9.7	9.5	9.5	10.0	9.6	9.1	10.0	9.8	9.6
	969	11.1	10.8	11.5	12.4	12.4	12.2	12.2	13.0	12.1	12.5
2 ♂ 12.5%	981	9.8	9.7	9.6	9.9	10.3	9.6	9.1	9.8	9.8	10.9
	974	10.5	10.5	10.8	11.2	11.0	11.1	10.4	10.7	10.7	11.6
	973	9.6	9.7	9.7	9.9	10.5	10.0	9.5	9.9	10.0	11.1
	984	8.5	8.8	8.6	8.9	8.9	9.1	9.3	9.8	9.7	10.2
3 ♂ 25%	985	9.0	9.0	9.3	9.5	9.9	9.7	9.2	9.7	9.8	10.4
	976	10.5	10.4	10.6	11.5	11.3	11.1	11.3	11.3	10.8	11.8
	977	9.2	9.1	9.8	10.0	10.1	9.8	9.2	9.7	9.5	10.6
	975	8.4	8.0	8.5	9.5	9.1	9.0	9.1	10.1	9.3	10.3
4 ♂ 50%	982	10.3	10.4	10.0	10.1	11.0	10.1	10.1	10.8	9.9	10.7
	979	10.4	10.2	10.2	11.1	10.7	10.2	9.5	9.9	9.8	10.2
	978	9.1	8.6	8.8	9.2	9.0	9.0	8.5	8.9	8.8	8.7
	986	8.8	8.1	8.5	8.9	9.0	9.0	9.2	10.5	9.3	10.5
1 ♀ Control	988	8.4	7.8	8.0	8.8	9.2	8.4	8.5	9.2	8.2	8.9
	990	9.4	9.4	9.4	9.8	9.8	9.6	9.2	9.9	9.8	10.5
	994	9.0	8.6	8.6	8.7	8.5	7.8	8.1	8.2	8.3	8.1
	993	7.0	6.7	7.4	7.6	7.4	7.8	8.0	7.8	8.0	8.3
2 ♀ 12.5%	989	8.3	8.6	8.2	8.4	8.1	8.3	7.3	9.0	9.0	9.7
	992	9.5	9.1	9.7	10.0	10.0	9.6	9.8	10.1	9.7	10.5
	997	8.7	8.0	8.3	8.7	8.6	8.5	8.0	8.5	8.3	9.0
	1000	8.2	8.1	8.4	8.7	8.5	8.1	8.0	8.6	8.6	8.6
3 ♀ 25%	987	10.5	10.9	10.6	11.1	10.7	10.7	10.1	10.7	10.7	10.6
	999	9.3	9.2	9.1	9.1	9.3	9.1	8.5	7.6	7.2	7.8
	998	8.6	8.6	8.7	8.5	8.7	8.3	8.9	9.5	9.4	9.6
	1002	8.1	7.8	8.2	8.4	8.9	8.7	8.7	9.3	8.6	9.3
4 ♀ 50%	995	8.1	7.4	7.6	7.8	8.2	7.8	7.9	8.1	7.6	8.4
	1003	9.4	9.3	9.6	9.1	9.0	9.1	9.1	9.3	9.1	9.0
	996	8.5	8.0	8.2	8.0	8.4	8.3	7.7	7.5	7.8	7.9
	1004	7.3	7.7	7.5	7.6	8.0	7.5	7.0	7.2	7.0	7.9

APPENDIX 1
(continued)

Individual Bodyweight Data (kg)

Group Sex	Dog No.	Week 9	10	11	12	13	14	15	16	17
1 ♂	980	9.2	9.3	9.3	9.5	9.0	9.1	9.3	9.3	9.6
Control	970	11.1	11.1	11.3	11.6	11.2	11.1	11.2	11.1	11.3
	972	9.7	9.9	10.6	10.5	10.2	10.2	10.3	10.2	10.1
	969	11.8	11.8	12.0	12.1	11.8	11.8	11.9	11.9	12.1
2 ♂	981	10.1	10.1	10.8	10.9	10.6	10.7	10.9	11.0	11.1
12.5%	974	10.8	10.9	11.4	11.8	11.9	11.9	12.1	12.1	12.2
	973	10.2	10.5	11.1	11.3	10.9	11.3	11.5	11.5	11.8
	984	10.0	10.1	10.6	10.6	10.2	10.4	10.3	10.3	10.8
3 ♂	985	10.4	10.2	10.7	11.1	11.3	11.4	11.7	11.9	11.6
25%	976	11.1	11.2	11.8	11.8	11.7	12.0	11.9	11.9	12.3
	977	9.6	9.6	9.7	10.0	10.4	10.1	10.4	10.2	10.1
	975	9.7	9.6	10.1	10.2	9.7	10.0	10.2	10.3	10.6
4 ♂	982	10.0	10.0	10.4	10.2	9.7	9.9	10.0	10.4	10.0
50%	979	9.8	9.9	10.3	10.7	10.7	10.7	11.0	11.0	11.1
	978	8.9	8.9	9.3	9.6	9.8	9.9	10.6	9.8	9.8
	986	9.7	9.9	10.1	10.5	10.1	10.5	10.7	10.7	11.0
1 ♀	988	8.2	8.2	8.3	8.5	8.0	8.0	8.3	8.2	8.5
Control	990	10.2	10.3	10.8	10.9	10.8	10.9	10.7	11.0	11.0
	994	7.6	7.3	8.1	8.3	7.9	8.4	8.2	7.8	8.1
	993	8.0	8.3	8.3	8.3	7.0	8.2	8.3	8.2	8.4
2 ♀	989	9.0	9.0	9.5	9.6	9.7	9.6	10.0	10.5	10.3
12.5%	992	9.6	9.6	9.8	9.9	9.5	9.8	10.0	9.7	10.0
	997	8.5	8.5	8.7	9.2	8.8	8.9	9.2	9.1	9.1
	1000	8.7	8.7	9.0	9.0	9.1	9.2	9.4	9.4	9.2
3 ♀	987	10.7	10.7	11.2	11.4	11.2	11.2	11.3	11.2	11.0
25%	999	7.1	7.1	7.3	7.4	7.5	7.4	7.6	7.8	8.2
	998	9.0	8.9	9.1	9.3	8.7	9.0	9.2	8.8	9.3
	1002	8.6	8.7	9.3	9.2	9.0	9.1	9.3	9.3	9.3
4 ♀										
50%	995	7.9	8.0	8.1	8.2	7.7	7.8	7.9	7.9	7.9
	1003	8.8	8.7	8.7	8.9	8.2	8.4	8.4	8.3	8.6
	996	8.1	8.4	8.7	9.0	8.6	9.1	9.2	9.0	9.1
	1004	7.4	7.4	7.8	8.0	8.0	7.8	8.2	8.2	8.2

APPENDIX 1
(continued)

Individual Bodyweight Data (kg)

Group Sex	Dog No.	Week 18	19	20	21	22	23	24	25	26
1 ♂ Control	980	10.0	10.1	10.5	10.4	10.3	10.5	10.9	10.6	10.8
	970	11.6	11.7	11.8	11.9	11.7	11.9	11.9	11.7	11.8
	972	10.2	10.2	10.3	10.3	10.3	10.2	10.6	10.3	10.3
	969	12.4	12.6	12.9	12.8	12.6	12.6	12.7	12.8	12.5
2 ♂ 12.5%	981	11.0	10.9	11.2	11.1	10.9	11.1	11.2	10.6	10.6
	974	12.7	12.8	12.8	12.8	12.8	13.0	13.6	13.2	13.0
	973	11.1	11.5	11.8	11.7	11.7	11.9	12.4	11.7	11.6
	984	10.7	10.6	10.8	11.0	10.7	10.9	10.8	10.7	10.7
3 ♂ 25%	985	11.8	12.1	12.0	12.3	12.2	12.5	13.1	12.8	12.4
	976	12.6	12.7	12.9	12.8	12.7	13.0	13.0	13.8	13.2
	977	10.4	10.3	10.2	10.7	10.3	10.5	10.6	11.2	10.8
	975	10.9	10.9	11.2	11.1	11.1	11.0	11.2	11.2	11.3
4 ♂ 50	982	10.1	10.1	10.1	9.9	9.8	9.8	9.9	10.5	9.8
	979	11.4	11.6	11.7	11.6	11.6	11.6	11.7	12.3	11.6
	978	10.1	10.1	9.9	10.1	10.1	10.1	10.3	10.4	10.1
	986	11.2	11.5	11.7	11.6	11.3	11.0	11.3	12.2	11.3
1 ♀ Control	988	9.5	9.2	9.1	9.3	9.3	9.0	9.5	9.4	9.3
	990	11.4	11.1	11.3	11.4	11.6	11.6	11.6	11.4	11.3
	994	8.4	8.2	8.5	8.1	8.1	8.2	8.1	8.3	8.1
	993	8.4	8.2	8.2	8.2	8.3	8.6	8.6	8.8	8.6
2 ♀ 12.5%	989	10.3	10.0	10.3	10.6	10.6	10.3	10.0	9.8	9.8
	992	10.1	10.4	10.6	10.2	10.2	10.5	10.7	10.2	10.5
	997	9.4	9.3	9.5	9.6	9.3	9.5	10.4	10.0	10.0
	1000	9.2	9.4	9.4	9.7	9.5	9.4	10.3	9.5	9.4
3 ♀ 25%	987	11.7	11.2	11.2	11.2	11.0	11.0	11.5	11.2	11.2
	999	8.5	8.5	8.7	8.8	8.7	8.8	8.8	8.6	8.5
	998	9.3	9.1	9.3	9.4	9.4	9.4	9.5	9.5	9.6
	1002	9.5	9.4	9.6	9.5	9.3	9.5	9.8	10.0	9.6
4 ♀ 50%	995	8.1	8.1	8.2	8.2	8.0	8.3	8.4	8.9	8.3
	1003	8.7	8.5	8.6	8.5	8.3	8.5	8.4	9.2	8.8
	996	9.3	9.3	9.4	9.4	10.0	9.6	10.0	9.8	9.5
	1004	8.7	8.5	8.7	8.4	8.3	8.4	8.6	8.9	8.4

Six-Month Dietary Administration of Reacted Molasses to Beagle Dogs

APPENDIX 2

Individual Food Consumption (gm/wk)

Dog No.	Dose Level	WEEK 1	2	3	4	5	6	7	8	9
980	1 ♂	3503	2564	3380	3313	2637	2470	2487	3302	2699
970	Control	3728	3279	3413	3310	2550	2567	2627	3408	2727
972		2680	2597	2381	1638	1506	2607	2382	2385	2276
969		3223	4141	3946	3362	2562	2562	2627	3324	2651
981	2 ♂	3370	2528	3268	2529	1686	2621	2700	3446	2574
974	12.5%	3205	3183	2628	3216	1651	2530	2563	3341	2482
973		3326	2601	2947	2528	1663	2514	2692	3320	2453
984		3423	2777	2796	2798	2577	2413	2622	3057	2556
985	3 ♂	2862	3152	3306	2685	1710	2542	2530	3463	2594
976	25%	3239	3354	3500	3604	2679	2638	2397	3305	2613
977		3040	3078	2942	2628	1753	2670	2698	3360	2530
975		3366	3125	2741	2621	2662	2515	2674	3359	2562
982	4 ♂	2430	3032	4728	2392	2426	2511	2650	3437	2559
979	50%	2475	3016	3647	2250	1533	2523	2574	3455	2467
978		2505	3045	2454	2517	1676	2513	2516	2583	2630
986		2514	3180	4015	2465	2519	2612	2606	3564	2643

APPENDIX 2
(continued)

Individual Food Consumption (gm/wk)

Dog No.	Dose Level	WEEK 10	11	12	13	14	15	16	17
980	1 ♂	2681	2977	3500	3000	3500	3500	3500	3500
970	Control	2560	2842	3500	3000	3500	3500	3500	3500
972		1976	2332	2939	2012	3023	2382	2763	1993
969		2787	2876	3500	3000	3500	3500	3500	3500
981	2 ♂	2558	2742	3500	3000	3500	3500	3500	3500
974	12.5%	2613	2825	3500	3000	3500	3500	3500	3500
973		2617	2879	3500	3000	3500	3500	3500	3500
984		2551	2864	2829	2697	3187	2675	3016	3030
985	3 ♂	2546	2837	3500	3000	3500	3500	3000	2487
976	25%	2551	2874	3500	3000	3500	3500	3500	3500
977		2550	2844	3500	3000	3500	3500	3500	3500
975		2528	2869	3500	3000	3500	3500	3500	3500
982	4 ♂	2602	2880	3500	3000	3500	3500	3500	3500
979	50%	2609	2872	3500	3000	3500	3500	3500	3500
978		2544	2792	3500	3000	3087	3500	2599	3053
986		2537	2890	3500	3000	3500	3500	3500	3500

APPENDIX 2
(continued)

Individual Food Consumption (gm/wk)

Dog No.	Dose Level	WEEK 18	19	20	21	22	23	24	25	26
980	1 ♂	3500	3500	3500	3500	3500	3500	3500	2615	2658
970	Control	3500	3500	3500	3500	3500	3500	3500	3500	3500
972		2568	2246	2205	2893	3163	2933	2771	2659	2691
969		3500	3500	3500	3500	3500	3500	3500	3500	2648
981	2 ♂	2717	2582	2790	3313	3125	3500	2982	2121	1996
974	12.5%	3500	3500	3500	3500	3500	3500	3500	2704	1952
973		2512	2871	3500	3500	3500	3500	3500	2855	1908
984		2405	2303	3101	3111	3145	3079	3102	1885	2207
985	3 ♂	3139	3500	3500	3500	3299	3500	3500	7224	1975
976	25%	3404	3500	3500	3460	3500	3389	3500	3500	3000
977		3500	3500	3500	3418	3500	3500	3500	3500	3000
975		3500	3500	3500	3139	3500	3403	2977	2552	2528
982	4 ♂	3500	3500	3500	3500	3500	3500	3500	3500	3000
979	50%	3500	3500	3500	3500	3500	3500	3500	3500	2720
978		2993	2405	2618	2949	3500	3072	3411	2932	2732
986		3500	3500	3500	3500	3500	3500	3347	3500	3000

APPENDIX 2
(continued)

Individual Food Consumption (gm/wk)

Dog No.	Dose Level	WEEK 1	2	3	4	5	6	7	8	9
988	1 ♀	2418	3084	3244	2642	2629	2359	2633	3356	2733
990	Control	2216	2734	2620	2636	1754	2473	3623	3154	2489
994		2072	2200	2161	1709	2470	1987	2178	2381	2299
993		2406	2435	2275	2506	2270	1992	2532	2473	2502
989	2 ♀	2610	2621	2625	2623	1748	2580	2590	3328	2365
992	12.5%	2462	2702	2862	2529	2529	2320	2613	3499	2476
997		2376	2403	2543	2617	1746	2679	2617	3294	2529
1000		2281	2304	2377	1822	1821	2608	2452	2537	2594
987	3 ♀	2483	2448	2631	2544	1709	2618	2547	2618	2552
999	25%	2584	2581	2972	2586	1748	2586	2638	3169	2505
998		2495	2741	3732	2644	2729	2500	2636	3244	2537
1002		2523	3335	3161	3521	2670	2472	2614	3353	2518
995	4 ♀	2732	2735	3363	2586	2657	2374	2580	3231	2602
1003	50%	2084	2103	2510	2691	2063	2350	2513	2460	2240
996		1987	1640	2601	2389	1623	2542	2503	2518	2556
1004		2379	3001	3852	2438	1620	2687	2580	3336	2730

APPENDIX 2
(continued)

Individual Food Consumption (gm/wk)

Dog No.	Dose Level	WEEK 10	11	12	13	14	15	16	17
988	1 ♀	2679	2874	3500	2881	3500	3500	3500	3500
990	Control	2793	2932	3008	2697	2855	2805	3029	2863
994		2544	2935	3500	2686	3118	2973	3500	3500
993		2114	2271	3178	2619	3009	2785	2646	3500
989	2 ♀	2421	2839	3500	3000	3500	3500	3360	2832
992	12.5%	2549	2875	3500	3000	3500	3500	3500	3500
997		2584	2787	3500	3000	3500	3500	3500	3500
1000		2382	2612	2976	2570	3263	2837	3108	2187
987	3 ♀	2579	2720	3158	2162	2855	2693	2326	2145
999	25%	2551	2872	3500	3000	3500	3500	3333	3500
998		2556	2841	3500	2685	3500	3337	2386	3294
1002		2574	2833	3500	3000	3500	3500	3500	3500
995	4 ♀	2694	2931	3500	2703	3500	3500	3500	3355
1003	50%	2491	2221	2823	2330	3199	3008	2402	3252
997		2644	2826	3357	2686	3282	3500	2499	3242
1004		2627	2872	3500	3000	3500	3500	3420	3500

APPENDIX 2
(continued)

Individual Food Consumption (gm/wk)

Dog No.	Dose Level	WEEK 18	19	20	21	22	23	24	25	26
988	1 ♀	3500	2932	3318	3500	3331	2698	2638	2642	6182
990	Control	2440	2424	3050	2714	3389	2785	2948	1871	2086
994		3500	3500	3500	3500	3500	3443	3500	3500	3000
993		2820	2534	2628	2532	2476	2484	2759	2541	2079
989	2 ♀	2420	2269	3324	3500	3281	2427	2694	1730	1971
992	12.5%	3500	3500	2922	3500	3121	3500	3236	2205	2416
997		2594	2565	2937	3500	3500	3500	3263	2654	1940
1000		2206	2254	2515	3155	2786	2097	3211	2495	1999
987	3 ♀	3079	1736	2153	3466	3041	3021	3125	2285	1826
999	25%	3094	2897	3398	3069	3500	3500	3385	2366	2491
998		3138	2578	3170	3154	3500	3143	2552	2672	2562
1002		3500	3298	3500	3500	3500	3500	3294	2899	2565
995	4 ♀	3500	3500	3500	3373	3500	3500	3500	3074	3000
1003	50%	2711	2736	2391	2809	3500	3106	2718	2613	2453
996		2609	2594	2545	2778	2907	2673	3401	2866	2637
1004		3500	2721	2669	3251	3500	3500	3204	3500	3000

APPENDIX 3

Individual Water Consumption

(Pretreat)

Group Sex	Dog No.	Day 1 ml	Day 2 ml
1 ♂ Control	980	1800	900
	970	1400	1800
	972	1000	800
	969	1800	1800
4 ♂ 50%	982	1800	900
	979	1100	1800
	978	550	1400
	986	1800	900
1 ♀ Control	988	200	1300
	990	1800	800
	994	400	1050
	993	1200	1000
4 ♀ 50%	995	1600	500
	1003	900	900
	996	850	1000
	1004	1400	800

APPENDIX 3
(continued)

Individual Water Consumption

(Week 4)

Group Sex	Dog No.	Day 1 ml	Day 2 ml
1 ♂ Control	980	2700	2700
	970	3600	2700
	972	800	500
	969	2700	2700
4 ♂ 50%	982	2700	1900
	979	2700	2700
	978	1800	400
	986	2700	2700
1 ♀ Control	988	2700	2700
	990	2700	1600
	994	1000	400
	993	1300	900
4 ♀ 50%	995	1800	1800
	1003	1500	900
	996	1800	1050
	1004	2700	2100

APPENDIX 3
(continued)

Individual Water Consumption

(Week 13)

Group Sex	Dog No.	Day 1 ml	Day 2 ml
1 ♂ Control	980	900	1000
	970	800	1100
	972	700	600
	969	1000	1350
4 ♂ 50%	982	1000	1200
	979	1500	1700
	978	900	1350
	986	900	1250
1 ♀ Control	988	900	1300
	990	1500	2100
	994	900	1300
	993	700	1100
4 ♀ 50%	995	700	500
	1003	1400	1200
	996	750	1200
	1004	1150	1300

APPENDIX 3
(continued)

Individual Water Consumption

(Week 26)

Group Sex	Dog No.	Day 1 ml	Day 2 ml
1 ♂ Control	980	800	2000
	970	1300	2100
	972	1000	800
	969	1000	2400
4 ♂ 50%	982	1100	2300
	979	1000	1500
	978	500	900
	986	1000	1800
1 ♀ Control	988	400	1600
	990	1000	1500
	994	900	1200
	993	500	1400
4 ♀ 50%	995	400	900
	1003	800	1500
	996	400	1300
	1004	700	1400

APPENDIX 4

Individual Hematology

Hematology

Proj. No: 1-315 Group: 1 Sex: ♂

Interval: Pretreat WK-1 Species: Dog

Date: 9-20-72 Level: Control

Animal Numbers	980	970	972	969			Total Group	Total Mean
Parameters								
Hct	46	48	40	47				45
ESR/mm 1 hr.	2	0	2	2				1.5
PCV %								
Hgb g %	15.6	15.8	13.4	15.6				15.2
RBC/cmm	5.71	6.40	5.97	6.84				6.23
Platelets	459,000	522,000	405,000	361,000				456,000
Retics %	0.8	1.2	0.3	0.6				0.7
MCHC %								
MCV cu								
WBC/cmm	22.1	12.8	14.4	12.9				15.6
DIFFERENTIAL %								
N	73	54	64	78				
L	27	46	34	22				32.3
S			1					0.2
E			1					0.2
M								
B								
X 10^3/cmm								
N								
L								
E								
Prothrombin/Sec.	7.0	8.0	6.5	7.0				7.1
Thromboplastin/Sec.								
Fibrinogen Assay								

Hematology

Proj. No: 1-315 Group: 1 Sex: ♂

Interval: 1 Month Species: Dog

Date: 10-25-72 Level: Control

Animal Numbers	980	970	972	969			Total Group	Total Mean
Parameters								
Hct	43	44	45	46				45
ESR/mm 1 hr.	2	0	0	0				0.5
PCV %								
Hgb g %	14.4	15.0	15.8	16.2				15.4
RBC/cmm	5.55	6.15	5.12	5.40				5.55
Platelets	420,000	495,000	384,000	273,000				392,000
Retics %	0.4	0.4	0.3	0.7				0.5
MCHC %								
MCV cu								
WBC/cmm	12.2	12.7	15.0	15.2				13.8
DIFFERENTIAL %								
N	76	62	60	52				62.5
L	23	32	40	48				35.8
S								
E	1	4						1.2
M		2						0.5
B								
X 10^3/cmm								
N								
L								
E								
Prothrombin/Sec.	7.0	8.0	7.5	7.0				7.4
Thromboplastin/Sec.								
Fibrinogen Assay								

Hematology

Proj. No: 1-315
Interval: 3 months
Date:

Group: 1 Sex: ♂
Species: Dog
Level: Control

Animal Numbers	980	970	972	969			Total Group	Total Mean
Parameters								
Hct	41	45	42	48				44
ESR/cm 1 hr.	4	1	1	0				1.5
PCV %								
Hgb g %	14.7	15.6	14.7	16.2				15.3
RBC/cmm	5.24	5.52	5.09	6.18				5.52
Platelets	170,000	119,000	131,000	134,000				131,000
Retics %	0.5	0.5	0.2	0.4				0.4
MCHC %								
MCV cu								
WBC/cmm	8.3	13.6	12.9	9.8				10.9
DIFFERENTIAL %								
N	67	53	56	58				58.5
L	26	37	40	38				35.2
S	1	1	0	0				0.5
E	5	8	1	4				4.5
M	1	1	3	0				1.3
B	0	0	0	0				0
X 10^3/cmm								
N								
L								
E								
Prothrombin/Sec.	7.5	8.9	7.5	7.5				7.9
Thromboplastin/Sec.								
Fibrinogen Assay								

Hematology

Proj. No: 1-315
Interval: 6 months
Date:

Group: 1 Sex: ♂
Species: Dog
Level: Control

Animal Numbers	980	970	972	969			Total Group	Total Mean
Parameters								
Hct	46	46	41	51			184	46
ESR/cm 1 hr.	2	0	4	0			6	2
PCV %								
Hgb g %	15.0	15.0	13.9	16.7			60.6	15.2
RBC/cmm	6.66	6.17	5.42	6.73			24.98	6.24
Platelets	296,000	219,000	225,000	180,000			965,000	241,250
Retics %	0.3	0.4	0.3	0.2			1.2	0.3
MCHC %								
MCV cu								
WBC/cmm	11.4	10.9	12.5	11.3			46.1	11.5
DIFFERENTIAL %								
N	66	56	69	68			257	64.3
L	30	34	31	29			124	31.0
S	0	0	0	0			0	0
E	4	10	1	3			18	4.5
M	0	0	1	0			1	0.2
B	0	0	0	0			0	0
X 10^3/cmm								
N								
L								
E								
Prothrombin/Sec.	7.5	8.0	7.5	7.5			30.5	7.6
Thromboplastin/Sec.								
Fibrinogen Assay								

Hematology

Proj. No: 1-315
Interval: PreTreat WK-1
Date: 9/20/72

Group: 2 Sex: ♂
Species: Dog
Level: 12.5%

Animal Numbers	981	974	973	984			Total Group	Total Mean
Parameters								
Hct	47	48	47	46				47
ESR/cm 1 hr.	1	0	0	0				0.2
PCV %								
Hgb g %	15.8	16.2	15.8	15.0				15.7
RBC/cmm	6.03	7.01	6.65	6.55				6.16
Platelets	350	305	378	558				392
Retics %	0.5	0.2	0.3	0.4				0.4
MCHC %								
MCV cu								
WBC/cmm	14.6	14.0	10.6	15.0				13.6
DIFFERENTIAL %								
N	50	60	50	58				54.6
L	49	40	49	39				44.2
S	1							0.3
E			1	3				1.0
M								
B								
X 10^3/cmm								
N								
L								
E								
Prothrombin/Sec.	10.5	10.0	7.5	7.0				8.8
Thromboplastin/Sec.								
Fibrinogen Assay								

Hematology

Proj. No: 1-315
Interval: 1 month
Date: 10-25-72

Group: 2 Sex: ♂
Species: Dog
Level: 12.5%

Animal Numbers	981	974	973	984			Total Group	Total Mean
Parameters								
Hct	47	44	45	45				45
ESR/cm 1 hr.	0	2	4	0				1.5
PCV %								
Hgb g %	16.5	5.0	15.8	15.3				15.7
RBC/cmm	5.21	5.25	5.51	6.20				5.21
Platelets	286	270	286	378				292
Retics %	0.4	0.4	0.2	0.3				0.3
MCHC %								
MCV cu								
WBC/cmm	14.0	15.0	9.9	12.9				13.0
DIFFERENTIAL %								
N	67	54	71	51				60.8
L	33	46	29	49				39.2
S								
E								
M								
B								
X 10^3/cmm								
N								
L								
E								
Prothrombin/Sec.	13.5	13.0	7.5	7.0				10.3
Thromboplastin/Sec.								
Fibrinogen Assay								

Hematology

Proj. No: 1-315
Interval: 3 months
Date:

Group: 2 Sex: ♂
Species: Dog
Level: 12.5%

Animal Numbers	981	974	973	984			Total Group	Total Mean
Parameters								
Hct	42	42	40	40				41
ESR/mm 1 hr.	2	4	8	0				3.5
PCV %								
Hgb g %	15.3	15.3	15.0	15.3				15.2
RBC/cmm	5.52	5.70	5.19	5.19				5.40
Platelets	117,000	147,000	240,000	254,000				154,000
Retics %	0.3	0.5	0.4	1.1				0.6
MCHC %								
MCV cu								
WBC/cmm	11.8	11.7	15.9	11.1				12.6
DIFFERENTIAL %								
N	58	72	62	59				62.2
L	34	28	33	40				33.7
S	1	0	1	0				0.5
E	7	0	4	1				3.0
M	0	0	0	0				0
B	0	0	0	0				0
X 10^3/cmm								
N								
L								
E								
Prothrombin/Sec.	12.0	12.0	8.0	7.5				9.9
Thromboplastin/Sec.								
Fibrinogen Assay								

Hematology

Proj. No: 1-315
Interval: 6 months
Date:

Group: 2 Sex: ♂
Species: Dog
Level: 12.5%

Animal Numbers	981	974	973	984			Total Group	Total Mean
Parameters								
Hct	45	46	46	45			182	46
ESR/mm 1 hr.	2	0	3	0			5	1
PCV %								
Hgb g %	14.7	15.3	15.0	15.6			60.6	15.1
RBC/cmm	5.39	6.44	6.69	6.39			24.91	6.22
Platelets	355,000	91,000	55,000	175,000			676,000	239,000
Retics %	0.4	0.3	0.3	0.7			1.7	0.4
MCHC %								
MCV cu								
WBC/cmm	11.5	13.0	9.1	11.0			44.6	11.2
DIFFERENTIAL %								
N	66	62	45	54			230	57.5
L	32	38	49	42			161	40.3
S	0	0	0	1			1	0.2
E	0	0	2	3			5	1.3
M	2	0	1	0			3	0.2
B	0	0	0	0			0	0
X 10^3/cmm								
N								
L								
E								
Prothrombin/Sec.	12.0	11.5	7.5	7.0			38.0	9.5
Thromboplastin/Sec.								
Fibrinogen Assay								

Hematology

Proj. No: 1-315
Interval: Pretreat I wk-1
Date: 9/20/72

Group: 3 Sex: ♂
Species: Dog
Level: 25%

Animal Numbers	985	976	977	975			Total Group	Total Mean
Parameters								
Hct	48	45	49	47				47
ESR/mm 1 hr.	0	0	1	2				0.8
PCV %								
Hgb g %	15.3	14.4	16.5	15.6				15.5
RBC/cmm	7.78	7.02	7.82	6.91				7.38
Platelets	455,000	505,000	360,000	445,000				441,000
Retics %	1.1	0.7	1.1	0.5				1.0
MCHC %								
MCV cu								
WBC/cmm	11.6	13.2	11.7	13.4				12.5
DIFFERENTIAL %								
N	60	68	65	57				62.5
L	38	28	32	40				34.5
S								
E		4	3	2				
M	2			1				0.7
B								
X 10^3/cmm								
N								
L								
E								
Prothrombin/Sec.	7.0	7.5	6.5	7.5				7.1
Thromboplastin/Sec.								
Fibrinogen Assay								

Hematology

Proj. No: 1-315
Interval: 1 Month
Date: 10-25-72

Group: 3 Sex: ♂
Species: Dog
Level: 25%

Animal Numbers	985	976	977	975			Total Group	Total Mean
Parameters								
Hct	50	45	46	47				47
ESR/mm 1 hr.	0	0	3	0				1.3
PCV %								
Hgb g %	17.0	15.3	15.8	16.2				16.1
RBC/cmm	6.78	5.16	5.58	5.48				5.75
Platelets	344,000	[illegible]	221,000	321,000				[illegible]
Retics %	0.3	0.5	0.4	0.3				0.4
MCHC %								
MCV cu								
WBC/cmm	13.1	9.6	12.7	9.6				11.3
DIFFERENTIAL %								
N	61	66	79	52				64.5
L	38	33	20	46				34.2
S								
E		1	1	2				1.0
M	1							.3
B								
X 10^3/cmm								
N								
L								
E								
Prothrombin/Sec.	8.5	7.0	7.0	7.0				7.4
Thromboplastin/Sec.								
Fibrinogen Assay								

Hematology

Proj. No: 1-315
Interval: 3 Months
Date:

Group: 3 Sex: ♂
Species: Dog
Level: 25%

Animal Numbers	985	976	977	975			Total Group	Total Mean
Parameters								
Hct	48	41	42	46				44
ESR/mm 1 hr.	0	6	6	0				3.0
PCV %								
Hgb g %	16.7	15.3	14.7	15.6				15.6
RBC/cmm	6.52	5.67	6.83	6.08				6.28
Platelets	140,000	145,000	221,000	139,000				165,000
Retics %	1.1	0.7	0.7	0.3				0.7
MCHC %								
MCV cu								
WBC/cmm	15.5	10.7	12.9	11.1				12.6
DIFFERENTIAL %								
N	67	63	59	59				62.0
L	32	29	32	31				31.0
S	0	2	0	0				0.5
E	1	5	9	9				6.0
M	0	1	0	1				0.5
B	0	0	0	0				0
X 10^3/cmm								
N								
L								
E								
Prothrombin/Sec.	8.0	7.0	7.5	8.5				7.8
Thromboplastin/Sec.								
Fibrinogen Assay								

Hematology

Proj. No: 1-315
Interval: 6 months
Date:

Group: 3 Sex: ♂
Species: Dog
Level: 25%

Animal Numbers	985	976	977	975			Total Group	Total Mean
Parameters								
Hct	50	45	47	51			193	48
ESR/mm 1 hr.	0	2	5	0			7	2
PCV %								
Hgb g %	16.5	14.7	15.6	16.7			63.5	15.9
RBC/cmm	6.96	6.23	6.20	7.00			26.39	6.59
Platelets	209,000	233,000	254,000	158,000			854,000	[illegible]
Retics %	1.0	0.7	0.6	0.5			2.8	0.7
MCHC %								
MCV cu								
WBC/cmm	13.3	11.2	10.8	12.3			47.6	11.9
DIFFERENTIAL %								
N	82	58	71	62			273	68.2
L	18	34	26	32			110	27.5
S	0	0	0	0			0	0
E	0	4	3	6			13	3.3
M	0	4	0	0			4	1.0
B	0	0	0	0			0	0
X 10^3/cmm								
N								
L								
E								
Prothrombin/Sec.	8.0	7.0	6.9	8.0			29.9	7.5
Thromboplastin/Sec.								
Fibrinogen Assay								

Hematology

Proj. No: 1-315 Group: 4 Sex: ♂

Interval: Pretreat I, wk-1 Species: Dog

Date: 9/20/72 Level: 50%

Animal Numbers	982	979	978	986			Total Group	Total Mean
Parameters								
Hct	46	44	45	39				44
ESR/mm 1 hr.	2	2	0	0				1
PCV %								
Hgb g %	15.8	14.2	14.7	13.3				14.5
RBC/cmm	6.71	6.14	6.73	5.80				6.35
Platelets	314,000	307,000	222,000	516,000				36.00
Retics %	0.5	0.7	0.3	0.3				0.5
MCHC %								
MCV cu								
WBC/cmm	16.5	14.0	8.8	16.8				14.0
DIFFERENTIAL %								
N	59	61	42	56				54.5
L	40	39	58	42				44.8
S				1				2.2
E	1			1				0.5
M								
B								
X 10^3/cmm								
N								
L								
E								
Prothrombin/Sec.	7.5	7.0	7.0	7.0				7.1
Thromboplastin/Sec.								
Fibrinogen Assay								

Hematology

Proj. No: 1-315 Group: 4 Sex: ♂

Interval: 1 Month Species: Dog

Date: 10-25-72 Level: 50%

Animal Numbers	982	979	978	986			Total Group	Total Mean
Parameters								
Hct	45	45	47	42				45
ESR/mm 1 hr.	0	3	1	3				2.0
PCV %								
Hgb g %	15.8	15.8	14.7	14.7				15.7
RBC/cmm	5.18	6.58	6.35	4.82				5.85
Platelets	301,000	461,000	274,000	543,000				[illegible]
Retics %	0.4	0.4	0.7	0.2				0.4
MCHC %								
MCV cu								
WBC/cmm	13.0	11.3	12.6	10.9				11.9
DIFFERENTIAL %								
N	56	57	59	48				55
L	44	43	40	52				44.8
S								
E								
M			1					0.2
B								
X 10^3/cmm								
N								
L								
E								
Prothrombin/Sec.	7.0	7.0	7.0	6.5				6.9
Thromboplastin/Sec.								
Fibrinogen Assay								

Hematology

Proj. No: 1-315
Interval: 3 months
Date:

Group: 4 Sex: ♂
Species: Dog
Level: 50%

Animal Numbers	982	979	978	986			Total Group	Total Mean
Parameters								
Hct	43	39	43	42				42
ESR/mm 1 hr.	12 [illegible]	10 [illegible]	2	4				7.0
PCV %								
Hgb g %	15.8	14.4	15.0	14.7				14.9
RBC/cmm	5.05	5.43	5.47	5.68				5.40
Platelets	241,000	206,000	164,000	135,000				176,500
Retics %	0.6	0.9	0.4	0.4				0.6
MCHC %								
MCV cu								
WBC/cmm	10.5	12.7	11.2	11.9				11.6
DIFFERENTIAL %								
N	58	72	58	54				60.5
L	40	27	39	40				36.5
S	0	0	1	0				0.3
E	1	0	0	4				1.2
M	1	1	2	2				1.5
B	0	0	0	0				0
X 10^3/cmm								
N								
L								
E								
Prothrombin/Sec.	7.0	7.5	7.5	7.5				7.4
Thromboplastin/Sec.								
Fibrinogen Assay								

[ ] repeated value

Hematology

Proj. No: 1-315
Interval: 6 months
Date:

Group: 4 Sex: ♂
Species: Dog
Level: 50%

Animal Numbers	982	979	978	986			Total Group	Total Mean
Parameters								
Hct	43	45	48	43			179	45
ESR/mm 1 hr.	3	8	0	2			13	3
PCV %								
Hgb g %	13.9	14.4	15.8	13.9			58.0	14.5
RBC/cmm	6.23	6.71	6.77	6.50			26.21	6.55
Platelets	199,000	151,000	415,000	219,000			974,000	243,500
Retics %	0.3	0.3	0.2	0.7			1.5	0.4
MCHC %								
MCV cu								
WBC/cmm	9.6	10.3	10.7	13.6			44.2	11.1
DIFFERENTIAL %								
N	66	68	65	63			262	65.5
L	33	32	34	35			134	33.5
S	0	0	0	0			0	0
E	0	0	1	1			2	0.5
M	1	0	0	1			2	0.5
B	0	0	0	0			0	0
X 10^3/cmm								
N								
L								
E								
Prothrombin/Sec.	7.0	7.5	7.4	7.5			29.4	7.4
Thromboplastin/Sec.								
Fibrinogen Assay								

Hematology

Proj. No: 1-315 Group: 1 Sex: ♀

Interval: Pretreat WK-1 Species: Dog

Date: 9-20-72 Level: Control

Animal Numbers	988	990	994	993			Total Group	Total Mean
Parameters								
Hct	46	49	46	48				47
ESR/mm 1 hr.	0	0	0	0				0
PCV %								
Hgb g %	15.3	16.2	15.0	16.2				15.7
RBC/cmm	7.38	6.62	6.07	5.78				6.48
Platelets	522,000	340,000	223,000	348,000				385,000
Retics %	0.6	0.6	0.2	0.6				0.5
MCHC %								
MCV cu								
WBC/cmm	12.1	12.4	11.8	13.5				12.5
DIFFERENTIAL %								
N	59	62	78	60				64.8
L	40	33	22	38				33.3
S				1				0.2
E	1	4		1				1.5
M		1						0.2
B								
X 10^3/cmm								
N								
L								
E								
Prothrombin/Sec.	8.0	6.5	7.5	7.0				7.2
Thromboplastin/Sec.								
Fibrinogen Assay								

Hematology

Proj. No: 1-315 Group: 1 Sex: ♀

Interval: 1 Month Species: Dog

Date: 11-25-76 Level: Control

Animal Numbers	988	990	994	993			Total Group	Total Mean
Parameters								
Hct	46	53	42	50				48
ESR/mm 1 hr.	0	0	2	0				0.1
PCV %								
Hgb g %	15.8	18.0	14.7	17.4				16.5
RBC/cmm	5.03	6.35	4.90	6.71				5.27
Platelets	478,000	465,000	276,000	409,000				403,000
Retics %	0.3	0.3	0.3	0.9				0.4
MCHC %								
MCV cu								
WBC/cmm	13.1	14.3	12.1	11.7				12.8
DIFFERENTIAL %								
N	61	52	61	52				54.0
L	39	46	39	47				42.2
S								
E								
M				1				0.2
B								
X 10^3/cmm								
N								
L								
E								
Prothrombin/Sec.	7.5	7.5	7.0	8.0				7.5
Thromboplastin/Sec.								
Fibrinogen Assay								

Hematology

Proj. No: 1-315
Interval: 3 Months
Date:

Group: 1 Sex: ♀
Species: Dog
Level: Control

Animal Numbers	988	990	994	993			Total Group	Total Mean
Parameters								
Hct	41	49	43	49				46
ESR/mm 1 hr.	6	0	4	0				2.5
PCV %								
Hgb g %	14.4	16.7	15.3	16.5				15.7
RBC/cmm	5.10	7.43	5.16	6.18				5.96
Platelets	156,000	122,000	195,000	136,000				152,750
Retics %	0.2	0.4	0.4	0.5				0.4
MCHC %								
MCV cu								
WBC/cmm	12.2	11.6	13.1	11.2				12.0
DIFFERENTIAL %								
N	76	65	65	73				69.8
L	19	33	32	26				27.3
S	0	0	1	0				0.2
E	2	2	2	0				1.5
M	4	0	0	1				1.2
B	0	0	0	0				0
X 10^3/cmm								
N								
L								
E								
Prothrombin/Sec.	8.0	7.0	8.0	7.5				7.6
Thromboplastin/Sec.								
Fibrinogen Assay								

Hematology

Proj. No: 1-315
Interval: 6 months
Date:

Group: 1 Sex: ♀
Species: Dog
Level: Control

Animal Numbers	988	990	994	993			Total Group	Total Mean
Parameters								
Hct	44	51	38	50			183	46
ESR/mm 1 hr.	1	0	4	0			5	1
PCV %								
Hgb g %	14.2	16.7	12.8	16.5			60.2	15.1
RBC/cmm	5.59	6.68	5.08	6.40			23.75	5.93
Platelets	143,000	156,000	218,000	162,000			679,000	169,750
Retics %	0.4	0.3	0.2	0.3			1.2	0.3
MCHC %								
MCV cu								
WBC/cmm	13.9	10.3	8.2	13.0			45.4	11.4
DIFFERENTIAL %								
N	66	65	70	76			275	68.8
L	30	33	30	17			110	27.5
S	0	0	0	0			0	0
E	4	4	0	6			14	3.5
M	0	0	0	1			1	0.2
B	0	0	0	0			0	0
X 10^3/cmm								
N								
L								
E								
Prothrombin/Sec.	8.0	7.5	7.5	8.0			31.0	7.8
Thromboplastin/Sec.								
Fibrinogen Assay								

Hematology

Proj. No: 1-315 Group: 2 Sex: ♀

Interval: Pretreat, Wk-1 Species: Dog

Date: 9-20-72 Level: 12.5%

Animal Numbers	989	992	997	1000			Total Group	Total Mean
Parameters								
Hct	45	45	47	54				48
ESR/cm 1 hr.	4	0	1	0				1.3
PCV %								
Hgb g %	14.4	14.7	15.6	17.6				15.6
RBC/cmm	6.51	5.08	6.41	7.73				6.43
Platelets	372,000	[illegible]	411,000	463,000				319,000
Retics %	0.4	1.0	0.5	0.9				0.7
MCHC %								
MCV cu								
WBC/cmm	13.2	16.3	12.3	13.4				13.8
DIFFERENTIAL %								
N	64	70	65	69				67
L	32	29	31	29				30.3
S		1		2				0.8
E	4		4					2.0
M								
B								
X 10^3/cmm								
N								
L								
E								
Prothrombin/Sec.	7.0	7.0	8.0	10.0				8.0
Thromboplastin/Sec.								
Fibrinogen Assay								

Hematology

Proj. No: 1-315 Group: 2 Sex: ♂

Interval: 1 Month Species: Dog

Date: 10-25-72 Level: 12.5%

Animal Numbers	989	992	997	1000			Total Group	Total Mean
Parameters								
Hct	46	45	49	53				48
ESR/cm 1 hr.	4	1	0	0				1.0
PCV %								
Hgb g %	16.2	15.8	16.7	17.0				16.7
RBC/cmm	5.87	5.34	5.22	6.86				5.77
Platelets	371,000	[illegible]	432,000	315,000				358,000
Retics %	0.7	0.8	0.5	0.8				0.6
MCHC %								
MCV cu								
WBC/cmm	13.3	12.8	14.8	14.0				13.7
DIFFERENTIAL %								
N	71	60	61	53				61.5
L	27	40	39	44				32.5
S				0				
E								
M	2			3				1.2
B								
X 10^3/cmm								
N								
L								
E								
Prothrombin/Sec.	7.0	7.0	7.5	12.0				8.4
Thromboplastin/Sec.								
Fibrinogen Assay								

Hematology

Proj. No: 1-315
Interval: 3 Months
Date:

Group: 2 Sex: ♀
Species: Dog
Level: 12.5%

Animal Numbers	989	992	997	1000			Total Group	Total Mean
Parameters								
Hct	43	47	45	51				47
ESR/mm 1 hr.	2	0	4	0				1.5
PCV %								
Hgb g %	15.0	16.2	15.6	17.4				16.1
RBC/cmm	5.07	6.19	7.29	7.39				6.48
Platelets	342,000	164,000	362,000	192,000				230,000
Retics %	0.7	0.4	0.5	1.6				0.8
MCHC %								
MCV cu								
WBC/cmm	8.9	11.3	16.5	16.7				12.1
DIFFERENTIAL %								
N	73	64	78	68				70.2
L	26	34	17	25				25.5
S	0	0	2	0				0.5
E	0	0	2	7				2.2
M	1	2	1	0				1.0
B	0	0	0	0				0
X 10^3/cmm								
N								
L								
E								
Prothrombin/Sec.	7.0	7.5	8.0	11.5				8.5
Thromboplastin/Sec.								
Fibrinogen Assay								

Hematology

Proj. No: 1-315
Interval: 6 months
Date:

Group: 2 Sex: ♀
Species: Dog
Level: 12.5%

Animal Numbers	989	992	997	1000			Total Group	Total Mean
Parameters								
Hct	47	52	49	53			201	50
ESR/mm 1 hr.	0	0	0	0			0	0
PCV %								
Hgb g %	15.8	17.0	16.5	18.0			67.3	16.8
RBC/cmm	6.27	7.24	6.69	7.63			27.83	6.95
Platelets	229,000	199,000	212,000	229,000			949,000	237,250
Retics %	0.6	0.4	0.6	1.6			3.2	0.8
MCHC %								
MCV cu								
WBC/cmm	8.7	13.0	14.2	14.3			50.2	12.6
DIFFERENTIAL %								
N	65	69	69	61			264	66.0
L	29	31	27	32			119	29.8
S	2	0	0	0			2	0.5
E	3	0	3	6			12	3.0
M	1	0	1	1			3	0.7
B	0	0	0	0			0	0
X 10^3/cmm								
N								
L								
E								
Prothrombin/Sec.	7.0	7.5	8.0	11.0			33.5	8.4
Thromboplastin/Sec.								
Fibrinogen Assay								

Hematology

Proj. No: 1-315 Group: 3 Sex: ♀

Interval: Pretest, wk-1 Species: Dog

Date: 9/20/72 Level: 2.5%

Animal Numbers	987	999	998	1002			Total Group	Total Mean
Parameters								
Hct	55	46	46	48				49
ESR/1st 1 hr.	0	1	2	5				2
PCV %								
Hgb g %	17.4	15.0	15.3	16.2				15.9
RBC/cmm	7.43	6.50	6.28	6.61				6.93
Platelets	320,000	399,000	362,000	356,000				34[illegible]
Retics %	0.6	0.7	0.6	0.3				0.6
MCHC %								
MCV cu								
WBC/cmm	10.9	10.9	11.7	14.9				12.1
DIFFERENTIAL %								
N	65	76	57	62				65
L	33	24	43	35				33.8
S	2			2				1.0
E				1				0.2
M								
B								
X 10^3/cmm								
N								
L								
E								
Prothrombin/Sec.	7.0	6.5	7.5	7.0				7.0
Thromboplastin/Sec.								
Fibrinogen Assay								

Hematology

Proj. No: 1-315 Group: 3 Sex: ♂

Interval: 1 week Species: Dog

Date: 10-25-72 Level: 2.5%

Animal Numbers	987	999	998	1002			Total Group	Total Mean
Parameters								
Hct	51	42	44	45				46
ESR/1st 1 hr.	1	9	4	0				4
PCV %								
Hgb g %	17.6	14.7	15.0	15.6				15.7
RBC/cmm	6.47	6.15	5.24	4.81				5.6
Platelets	197,000	173,000	321,000	277,000				[illegible]
Retics %	0.9	0.6	0.5	0.3				0.6
MCHC %								
MCV cu								
WBC/cmm	12.4	18.1	10.1	12.0				13.2
DIFFERENTIAL %								
N	66	66	55	53				60
L	34	32	45	47				39.6
S								
E		1						2
M		1						2
B								
X 10^3/cmm								
N								
L								
E								
Prothrombin/Sec.	7.5	7.0	7.0	7.0				7.1
Thromboplastin/Sec.								
Fibrinogen Assay								

Hematology

Proj. No: 1-315 Group: 3 Sex: ♀

Interval: 3 months Species: Dog

Date: Level: 25%

Animal Numbers	987	999	998	1002			Total Group	Total Mean
Parameters								
Hct	50	39	44	44				44
ESR/mm 1 hr.	0	20 (?)	6	2				7.0
PCV %								
Hgb g %	17.6	14.4	15.0	15.6				15.7
RBC/cmm	7.60	6.00	5.66	5.10				6.09
Platelets	174,000	361,000	158,000	175,000				217,000
Retics %	0.6	0.2	0.5	0.2				0.4
MCHC %								
MCV cu								
WBC/cmm	14.7	12.4	7.8	12.4				11.8
DIFFERENTIAL %								
N	80	63	63	54				65.0
L	18	31	37	43				32.2
S	1	0	0	0				0.3
E	1	6	0	3				2.5
M	0	0	0	0				0
B	0	0	0	0				0
X 10^3/cmm								
N								
L								
E								
Prothrombin/Sec.	7.5	7.0	7.5	8.0				7.5
Thromboplastin/Sec.								
Fibrinogen Assay								

Hematology

Proj. No: 1-315 Group: 3 Sex: ♀

Interval: 6 months Species: Dog

Date: Level: 25%

Animal Numbers	987	999	998	1002			Total Group	Total Mean
Parameters								
Hct	47	40	45	49			181	45
ESR/mm 1 hr.	10	12	2	0			24	6
PCV %								
Hgb g %	15.8	13.9	14.7	16.2			60.6	15.2
RBC/cmm	6.02	5.21	6.53	6.61			24.37	6.09
Platelets	209,000	276,000	253,000	204,000			942,000	235,000
Retics %	0.4	0.7	0.5	0.3			1.9	0.5
MCHC %								
MCV cu								
WBC/cmm	13.6	9.7	12.3	12.2			47.8	12.0
DIFFERENTIAL %								
N	75	64	69	69			277	69.3
L	23	29	31	30			113	28.3
S	0	0	0	0			0	0
E	2	6	0	1			9	2.2
M	0	1	0	0			1	0.2
B	0	0	0	0			0	0
X 10^3/cmm								
N								
L								
E								
Prothrombin/Sec.	8.0	7.0	7.0	7.0			29	7.3
Thromboplastin/Sec.								
Fibrinogen Assay								

Hematology

Proj. No: 1-315 Group: 4 Sex: ♀

Interval: Pretreat, WK-1 Species: Dog

Date: 9/20/72 Level: 50%

Animal Numbers	995	1003	996	1004			Total Group	Total Mean
Parameters								
Hct	52	45	50	55				51
ESR/cm 1 hr.	1	1	0	0				0.5
PCV %								
Hgb g %	17.0	15.0	16.7	17.6				16.6
RBC/cmm	7.32	6.90	7.27	8.67				7.54
Platelets	348,000	322,000	345,000	294,000				312,000
Retics %	0.7	0.2	0.4	0.3				0.4
MCHC %								
MCV cu								
WBC/cmm	13.4	14.1	7.0	8.8				10.8
DIFFERENTIAL %								
N	71	63	43	68				61.3
L	27	35	57	32				37.8
S	2							0.5
E		1						.2
M		1						.2
B								
X 10^3/cmm								
N								
L								
E								
Prothrombin/Sec.	6.7	6.5	8.0	7.0				7.1
Thromboplastin/Sec.								
Fibrinogen Assay								

Hematology

Proj. No: 1-315 Group: 4 Sex: ♀

Interval: 1 MONTH Species: Dog

Date: 9-25-72 Level: 50%

Animal Numbers	995	1003	996	1004			Total Group	Total Mean
Parameters								
Hct	50	48	50	47				49
ESR/cm 1 hr.	0	0	0	0				0
PCV %								
Hgb g %	17.0	16.5	17.0	16.2				16.7
RBC/cmm	6.25	5.54	6.07	5.77				6.16
Platelets	201,000	271,000	206,000	209,000				22[illegible]
Retics %	0.5	0.2	0.3	0.6				0.4
MCHC %								
MCV cu								
WBC/cmm	12.6	13.9	8.9	11.6				11.8
DIFFERENTIAL %								
N	58	51	59	73				60.3
L	42	47	41	27				39.3
S								
E		1						0.2
M		1						0.2
B								
X 10^3/cmm								
N								
L								
E								
Prothrombin/Sec.	7.0	7.0	7.5	7.5				7.3
Thromboplastin/Sec.								
Fibrinogen Assay								

Hematology

Proj. No: 1-315
Interval: 3 months
Date:

Group: 4 Sex: ♀
Species: Dog
Level: 50%

Animal Numbers	995	1003	996	1004			Total Group	Total Mean
Parameters								
Hct	43	44	47	43				44
ESR/mm 1 hr.	4	4	0	0				2.0
PCV %								
Hgb g %	15.8	15.6	15.6	15.3				15.6
RBC/cmm	5.53	5.18	5.53	5.14				5.34
Platelets	160,000	154,000	173,000	133,000				155,250
Retics %	0.7	1.5	1.5	1.7				1.4
MCHC %								
MCV cu								
MGC/cmm	9.9	14.0	8.6	11.8				11.1
DIFFERENTIAL %								
N	72	60	58	64				63.5
L	22	35	39	32				33.6
S	0	1	0	0				0.2
E	0	3	3	2				2.0
M	0	1	0	2				0.7
B	0	0	0	0				0
X 10^3/cmm								
N								
L								
E								
Prothrombin/Sec.	7.0	7.0	8.0	7.5				7.4
Thromboplastin/Sec.								
Fibrinogen Assay								

Hematology

Proj. No: 1-315
Interval: 6 months
Date:

Group: 4 Sex: ♀
Species: Dog
Level: 50%

Animal Numbers	995	1003	996	1004			Total Group	Total Mean
Parameters								
Hct	48	43	45	50			186	47
ESR/mm 1 hr.	0	8	0	0			8	2
PCV %								
Hgb g %	15.3	14.0	14.7	14.5			60.5	15.1
RBC/cmm	6.92	6.06	6.01	6.95			25.97	6.49
Platelets	167,000	181,000	192,000	160,000			680,000	170,000
Retics %	0.5	0.3	0.3	0.7			1.8	0.5
MCHC %								
MCV cu								
MGC/cmm	9.8	11.5	7.8	11.7			40.8	10.2
DIFFERENTIAL %								
N	69	64	67	75			269	67.3
L	31	36	33	25			131	32.7
S	0	0	0	0			0	0
E	0	0	0	0			0	0
M	0	0	0	0			0	0
B	0	0	0	0			0	0
X 10^3/cmm								
N								
L								
E								
Prothrombin/Sec.	7.0	7.0	8.5	8.0			30.5	7.6
Thromboplastin/Sec.								
Fibrinogen Assay								

Six-Month Dietary Administration of Reacted Molasses to Beagle Dogs

APPENDIX 5

Individual Biochemistry
(Pretreat)

Dog No.	Group Sex	Urea Nitrogen mg%	Glucose mg%	Total Protein gm%	Protein Electrophoresis g% Alb	α 1	α 2	β	γ	Serum Alkaline Phosphatase BLB U/ml	SGOT RF U/ml	SGPT RF U/ml	Bili-rubin mg%	Electrolytes meq/L Na^+	K^+	Cl^- (as Cl^-)
980	1♂	13	75	5.0	2.5	0.1	0.4	0.4	0.6	2.8	24	17	0.44	128	4.0	90.1
970	Control	10	111	4.9	2.7	0.2	0.4	0.6	1.0	2.1	25	16	0.42	128	3.6	82.5
972		10	68	4.6	2.5	0.2	0.5	0.5	0.9	2.7	28	20	0.42	122	3.8	83.7
969		13	77	4.1	2.1	0.3	0.3	0.5	0.9	2.4	27	26	0.43	120	3.3	82.0
981	2♂	10	83	8.9	4.9	0.3	0.7	1.2	1.8	1.8	20	19	0.51	148	3.9	118.1
974	12.5%	15	74	5.3	2.9	0.2	0.4	0.6	1.1	1.7	29	23	0.38	136	3.9	107.3
973		8	83	4.7	2.4	0.2	0.4	0.5	1.1	2.6	24	21	0.44	123	3.6	82.3
984		9	68	4.2	2.2	0.2	0.4	0.5	0.8	1.9	25	17	0.46	125	3.8	83.3
985	3♂	9	83	4.8	2.4	0.3	0.5	0.6	1.1	2.0	35	19	0.44	126	3.9	87.6
976	25%	9	78	4.1	2.0	0.2	0.3	0.6	1.0	1.9	26	17	0.43	124	3.7	84.6
977		11	110	5.5	2.9	0.3	0.4	0.7	1.2	1.9	23	15	0.43	135	4.1	105.6
975		10	80	5.2	2.9	0.2	0.3	0.6	1.2	1.9	22	20	0.39	127	4.0	95.2
982	4♂	12	68	4.4	2.4	0.3	0.4	0.5	0.8	2.5	22	23	0.43	125	3.5	85.6
979	50%	8	74	4.0	2.5	0.2	0.3	0.5	0.9	2.2	22	19	0.44	122	4.0	84.0
978		10	77	4.4	2.6	0.3	0.2	0.5	0.9	2.1	29	19	0.40	132	3.2	90.1
986		12	71	4.9	2.5	0.2	0.4	0.6	1.2	1.9	25	15	0.40	130	3.8	96.0

APPENDIX 5
(continued)

Individual Biochemistry
(Pretreat)

Dog No.	Group Sex	Urea Nitrogen mg%	Glucose mg%	Total Protein gm%	Protein Electrophoresis g% Alb	α1	α2	β	γ	Serum Alkaline Phosphatase BLB U/ml	SGOT RF U/ml	SGPT RF U/ml	Bilirubin mg%	Electrolytes meq/L Na^+	K^+	Cl^- (as Cl^-)
988	1 ♀	12	89	5.7	2.9	0.3	0.5	0.8	1.3	1.8	41	29	0.41	122	4.4	111.9
990	Control	12	95	5.7	3.3	0.3	0.3	0.7	1.3	1.9	41	32	0.42	120	4.2	113.6
994		15	74	5.3	3.3	0.2	0.3	0.5	1.0	1.6	36	21	0.43	126	3.9	112.1
993		12	101	5.8	3.4	0.4	0.4	0.6	1.0	2.3	71	40	0.39	123	4.1	113.6
989	2 ♀	12	83	5.0	2.9	0.3	0.3	0.6	0.9	1.9	41	27	0.41	120	4.2	113.2
992	12.5%	12	89	5.2	3.2	0.2	0.3	0.6	0.9	2.3	36	34	0.42	126	4.1	108.8
997		15	75	5.7	3.2	0.3	0.2	0.8	1.2	2.1	97	44	0.39	129	3.5	114.5
1000		10	148	6.0	3.3	0.3	0.4	0.7	1.3	1.6	36	36	0.43	135	4.3	115.3
987	3 ♀	13	83	6.0	3.0	0.3	0.4	0.9	1.4	1.9	20	36	0.38	150	3.5	115.3
999	25%	12	81	5.6	3.4	0.2	0.4	0.6	1.0	2.0	36	27	0.41	135	4.4	114.3
998		11	89	5.7	2.8	0.3	0.5	0.6	1.5	1.9	41	36	0.44	144	3.7	113.4
1002		12	90	1.3	0.8	0.1	0.1	0.1	0.2	2.9	20	21	0.41	127	3.5	113.4
995	4 ♀	13	104	5.5	3.1	0.3	0.3	0.6	1.1	1.4	31	25	0.41	125	4.2	115.3
1003	50%	17	93	4.9	2.9	0.2	0.4	0.5	0.8	2.1	51	21	0.41	132	4.1	107.7
996		11	84	5.4	3.1	0.3	0.4	0.6	1.0	2.4	82	55	0.44	128	4.1	114.1
1004		17	89	4.8	2.5	0.3	0.3	0.6	1.1	1.9	56	27	0.39	133	3.9	110.2

APPENDIX 5
(continued)

Individual Biochemistry
(Week 4)

Dog No.	Group Sex	Urea Nitrogen mg%	Glucose mg%	Total Protein gm%	Protein Electrophoresis g% Alb	α1	α2	β	γ	Serum Alkaline Phosphatase BLB U/ml	SGOT RF U/ml	SGPT RF U/ml	Bilirubin mg%	Electrolytes meq/L Na^+	K^+	Cl^- (as Cl^-)
980	1 ♂	10	106	6.4	3.5	0.3	0.6	0.8	1.1	2.8	23	15	0.27	144	4.9	91.9
970	Control	12	76	6.4	3.5	0.4	0.6	0.6	1.3	2.0	24	16	0.22	144	5.2	86.9
972		7	100	5.6	3.2	0.3	0.6	0.6	1.0	2.7	22	21	0.17	144	5.1	90.6
969		9	113	6.4	3.7	0.3	0.5	0.6	1.2	2.4	31	27	0.24	143	4.7	105.3
981	2 ♂	11	101	6.3	3.4	0.3	0.6	0.7	1.3	1.8	19	15	0.17	144	4.7	90.2
974	12.5%	10	95	5.7	3.0	0.3	0.5	0.9	1.6	2.6	28	32	0.15	144	5.1	102.9
973		10	99	6.3	3.2	0.3	0.5	0.7	1.6	2.2	18	23	0.17	140	4.6	108.4
984		9	97	6.3	3.7	0.3	0.7	0.7	1.0	2.0	24	16	0.23	142	4.8	101.8
985	3 ♂	8	106	5.8	3.2	0.2	0.6	0.6	1.1	1.9	20	12	0.17	143	4.8	104.5
976	25%	13	99	6.1	3.1	0.3	0.5	1.0	1.2	2.4	21	14	0.24	139	5.1	85.5
977		11	97	6.2	3.5	0.3	0.5	0.7	1.1	2.7	22	21	0.21	144	4.7	99.2
975		13	115	6.4	3.4	0.3	0.5	0.7	1.4	2.1	22	19	0.26	137	4.8	102.9
982	4 ♂	13	85	6.3	3.6	0.3	0.6	0.6	1.1	2.5	26	22	0.24	148	4.8	101.0
979	50%	8	101	5.8	3.0	0.3	0.5	0.7	1.3	2.2	18	25	0.17	143	5.0	98.6
978		9	104	5.8	3.3	0.3	0.5	0.6	1.1	2.1	27	25	0.16	151	4.5	108.9
986		11	113	6.3	3.2	0.3	0.6	0.7	1.5	1.8	21	17	0.25	140	4.8	99.8

APPENDIX 5
(continued)

Individual Biochemistry
(Week 4)

Dog No.	Group Sex	Urea Nitrogen mg%	Glucose mg%	Total Protein gm%	Protein Electrophoresis g% Alb	α1	α2	β	γ	Serum Alkaline Phosphatase BLB U/ml	SGOT RF U/ml	SGPT RF U/ml	Bilirubin mg%	Electrolytes meq/L Na^+	K^+	Cl^- (as Cl^-)
988	1 ♀	8	80	5.4	2.9	0.3	0.5	0.6	1.0	2.2	28	22	0.22	151	5.1	94.1
990	Control	12	98	6.2	3.6	0.3	0.4	0.7	1.2	2.8	31	30	0.16	147	4.6	92.3
994		7	59	3.4	1.9	0.2	0.3	0.3	0.7	2.1	28	17	0.20	155	4.5	90.6
993		10	99	4.5	2.6	0.3	0.4	0.4	0.8	3.4	32	19	0.25	147	5.4	98.6
989	2 ♀	14	96	5.7	3.4	0.3	0.4	0.6	0.9	2.2	24	15	0.19	143	4.6	85.9
992	12.5%	8	83	5.7	3.5	0.3	0.5	0.6	0.8	2.3	24	21	0.22	148	5.2	107.1
997		11	112	6.6	3.7	0.3	0.5	0.9	1.2	2.7	21	26	0.19	154	5.3	98.9
1000		8	118	6.3	3.5	0.3	0.6	0.7	1.3	1.7	21	14	0.19	146	4.9	101.6
987	3 ♀	12	99	6.0	3.3	0.3	0.4	0.7	1.3	3.9	21	25	0.17	147	4.5	107.9
999	25%	10	108	5.6	3.3	0.2	0.5	0.6	1.0	2.1	26	18	0.19	141	5.1	92.3
998		8	72	4.1	2.2	0.3	0.4	0.4	0.9	2.1	19	18	0.22	170	5.5	117.1
1002		8	67	3.5	2.0	0.2	0.3	0.4	0.6	2.8	21	15	0.22	150	4.8	109.6
995	4 ♀	7	63	4.2	2.5	0.3	0.4	0.3	0.7	1.7	31	24	0.23	155	4.8	103.5
1003	50%	8	60	3.7	2.0	0.2	0.4	0.4	0.7	1.5	25	12	0.23	151	5.3	105.1
996		15	97	6.1	3.4	0.4	0.5	0.8	1.0	1.9	29	26	0.20	145	4.7	103.9
1004		23	119	6.7	4.4	0.3	0.2	0.7	1.2	2.5	34	25	0.20	146	5.3	99.8

APPENDIX 5
(continued)

Individual Biochemistry
(Week 13)

Dog No.	Group Sex	Urea Nitrogen mg%	Glucose mg%	Total Protein gm%	Protein Electrophoresis g% Alb	α1	α2	β	γ	Serum Alkaline Phosphatase BLB U/ml	SGOT RF U/ml	SGPT RF U/ml	Bili-rubin mg%	Electrolytes meq/L Na+	K+	Cl⁻ (as Cl⁻)
980	1 ♂	12	96	6.1	3.2	0.3	0.7	0.8	1.2	3.6	25	16	0.19	141	4.3	102.9
970	Control	13	96	5.8	3.1	0.3	0.6	0.6	1.3	1.7	33	16	0.11	145	4.2	98.6
972		10	95	5.9	3.0	0.4	0.6	0.6	1.3	2.2	39	19	0.10	146	4.3	105.0
969		13	95	6.2	3.5	0.3	0.5	0.7	1.2	2.6	22	22	0.13	144	4.7	110.0
981	2 ♂	15	99	6.1	3.2	0.4	0.6	0.9	1.1	2.1	24	17	0.16	142	4.3	100.8
974	12.5%	20	96	6.1	2.7	0.4	0.7	0.8	1.5	2.0	35	20	0.12	147	4.3	105.0
973		11	98	6.5	3.0	0.3	0.6	0.9	1.7	1.4	33	19	0.12	143	6.0	104.1
984		10	100	5.9	3.0	0.3	0.7	0.7	1.2	2.2	32	23	0.19	140	4.5	101.9
985	3 ♂	14	100	6.2	2.8	0.3	0.8	0.8	1.5	2.0	30	18	0.17	145	4.5	107.0
976	25%	13	96	6.1	2.7	0.3	0.7	0.9	1.4	2.0	35	17	0.16	142	4.4	103.7
977		16	97	6.2	3.3	0.3	0.6	0.8	1.2	3.6	39	19	0.16	144	4.7	105.6
975		18	95	6.3	2.9	0.4	0.6	0.8	1.7	2.4	46	24	0.13	149	4.6	99.4
982	4 ♂	15	99	5.5	2.8	0.5	0.7	0.7	1.1	2.4	24	19	0.17	143	4.6	102.5
979	50%	13	98	6.4	2.9	0.4	0.7	0.9	1.5	1.9	43	22	0.13	143	4.5	101.9
978		13	97	5.7	2.9	0.2	0.6	0.8	1.2	1.5	25	20	0.15	145	4.1	103.7
986		12	99	5.7	2.8	0.3	0.6	0.6	1.3	1.7	28	14	0.19	139	4.7	109.3

APPENDIX 5
(continued)

Individual Biochemistry
(Week 13)

Dog No.	Group Sex	Urea Nitrogen mg%	Glucose mg%	Total Protein gm%	Protein Electrophoresis g% Alb	α1	α2	β	γ	Serum Alkaline Phosphatase BLB U/ml	SGOT RF U/ml	SGPT RF U/ml	Bilirubin mg%	Electrolytes meq/L Na+	K+	Cl⁻ (as Cl⁻)
988	1 ♀	15	101	5.6	2.9	0.2	0.6	0.7	1.2	4.3	27	18	0.17	140	4.3	100.8
990	Control	20	100	6.1	3.6	0.3	0.5	0.7	0.9	3.4	35	34	0.19	150	4.6	107.3
994		21	105	5.5	3.0	0.3	0.5	0.6	1.1	2.5	28	15	0.10	138	4.4	102.1
993		18	102	6.0	3.0	0.4	0.6	0.7	1.3	4.6	30	18	0.17	144	4.4	102.7
989	2 ♀	14	99	5.8	3.3	0.3	0.6	0.7	0.8	2.2	32	18	0.21	139	4.5	108.7
992	12.5%	12	102	5.7	3.5	0.4	0.4	0.6	0.8	3.1	29	20	0.15	145	4.8	103.7
997		15	103	6.3	3.4	0.2	0.6	0.8	1.2	2.9	20	20	0.14	145	4.3	103.9
1000		13	103	6.6	3.4	0.3	0.6	0.8	1.5	1.7	29	20	0.13	147	4.4	101.9
987	3 ♀	13	94	6.1	3.2	0.3	0.5	0.7	1.3	4.7	29	22	0.20	148	4.5	107.7
999	25%	14	104	5.5	2.9	0.4	0.6	0.7	0.9	1.8	31	18	0.17	144	4.9	102.3
998		16	104	6.5	3.4	0.4	0.5	0.7	1.4	3.0	29	22	0.16	124	4.2	103.5
1002		14	104	5.8	3.0	0.4	0.6	0.6	1.1	3.7	28	14	0.10	149	5.0	106.0
995	4 ♀	18	102	5.7	3.0	0.4	0.5	0.6	1.1	2.5	30	24	0.13	139	4.5	102.9
1003	50%	17	104	5.7	3.3	0.3	0.5	0.6	1.0	1.5	27	14	0.15	139	4.7	107.9
996		22	104	6.0	3.3	0.4	0.5	0.7	1.1	1.6	35	27	0.23	145	4.8	104.1
1004		19	104	6.0	3.6	0.4	0.5	0.6	1.3	1.6	30	16	0.16	140	4.0	104.1

APPENDIX 5
(continued)

Individual Biochemistry
(Week 26)

Dog No.	Group Sex	Urea Nitrogen mg%	Glucose mg%	Total Protein gm%	Protein Electrophoresis g% Alb	α^1	α^2	β	γ	Serum Alkaline Phosphatase BLB U/ml	SGOT RF U/ml	SGPT RF U/ml	Bilirubin mg%	Electrolytes meq/L Na	K^+	Cl^- (as Cl^-)
980	1 ♂	13	123	6.8	3.0	0.3	0.7	1.0	1.8	3.1	16	18	0.20	143	4.8	113
970	Control	17	109	5.8	2.8	0.3	0.5	0.7	1.5	1.5	24	20	0.30	151	4.9	103
972		6	111	5.8	3.0	0.3	0.7	0.5	1.4	3.7	35	20	0.10	148	5.4	108
969		14	102	6.3	3.1	0.3	0.6	0.8	1.6	3.9	28	23	0.10	152	4.7	110
981	2 ♂	12	118	6.3	2.7	0.3	0.6	1.2	1.5	1.7	20	23	0.10	144	3.7	103
974	12.5%	10	99	5.9	2.8	0.2	0.6	0.8	1.5	2.6	31	31	0.10	143	5.0	109
973		13	112	6.1	2.9	0.3	0.7	0.8	1.5	1.6	35	25	0.10	136	5.8	106
984		13	95	6.0	3.4	0.2	0.7	0.7	1.0	2.8	20	23	0.40	148	4.8	126
985	3 ♂	12	112	6.2	3.3	0.2	0.5	0.8	1.2	2.5	20	18	0.10	147	4.6	106
976	25%	23	108	6.6	2.8	0.2	0.6	1.1	1.9	2.6	16	23	0.30	141	4.9	116
977		15	112	6.4	3.2	0.3	0.6	0.8	1.3	3.9	28	25	0.30	143	4.7	104
975		17	94	6.6	3.0	0.3	0.6	0.8	1.8	1.5	28	28	0.10	138	4.4	100
982	4 ♂	21	101	5.8	3.0	0.2	0.5	0.9	1.2	3.5	20	25	0.10	147	4.6	108
979	50%	14	106	6.4	2.9	0.3	0.6	0.9	1.7	2.0	35	18	0.10	141	5.1	106
978		11	114	6.1	3.1	0.3	0.5	0.8	1.4	2.3	31	23	0.10	145	4.8	106
986		16	112	6.2	3.1	0.2	0.6	0.7	1.6	1.9	16	20	0.10	144	4.5	118

APPENDIX 5
(continued)

Individual Biochemistry
(Week 26)

Dog No.	Group Sex	Urea Nitrogen mg%	Glucose mg%	Total Protein gm%	Protein Electrophoresis g% Alb	α^1	α^2	β	γ	Serum Alkaline Phosphatase BLB U/ml	SGOT RF U/ml	SGPT RF U/ml	Bili-rubin mg%	Electrolytes meq/L Na^+	K^+	Cl^- (as Cl^-)
988	1 ♀	13	106	6.0	2.9	0.4	0.5	0.7	1.4	2.4	20	18	0.20	155	5.0	104
990	Control	12	106	5.8	3.0	0.3	0.6	0.6	1.3	2.2	31	25	0.10	144	4.5	107
994		15	113	5.5	3.0	0.3	0.5	0.6	1.2	2.4	20	18	0.10	141	5.0	103
993		19	100	6.3	2.9	0.4	0.7	0.7	1.6	3.6	24	23	0.10	149	4.9	104
989	2 ♀	11	107	5.6	2.9	0.3	0.6	0.6	1.2	2.4	35	18	0.10	151	4.8	106
992	12.5%	16	106	5.9	3.2	0.4	0.5	0.6	1.2	2.5	24	33	0.10	151	4.6	105
997		9	110	6.4	3.3	0.2	0.8	0.8	1.3	2.4	16	28	0.10	150	4.8	108
1000		9	132	6.7	3.3	0.3	0.7	0.7	1.8	1.7	10	23	0.30	147	4.7	105
987	3 ♀	13	105	5.6	2.7	0.4	0.6	0.6	1.3	2.8	16	31	0.20	147	4.6	108
999	25%	12	118	5.0	2.1	0.4	0.6	0.6	1.4	1.4	28	18	0.10	146	4.9	105
998		13	106	6.3	3.1	0.3	0.6	0.7	1.6	2.6	16	20	1.40	152	5.3	103
1002		12	113	6.0	2.6	0.4	0.7	0.7	1.6	1.7	11	13	0.10	140	4.3	104
995	4 ♀	19	97	6.1	3.6	0.3	0.5	0.5	1.2	2.5	28	28	0.20	138	4.0	102
1003	50%	16	107	6.2	3.0	0.4	0.8	0.6	1.5	1.9	11	13	0.10	151	5.2	104
996		13	110	6.1	3.1	0.3	0.7	0.6	1.5	2.0	20	25	0.20	147	3.9	107
1004		17	129	5.8	3.0	0.3	0.5	0.6	1.4	2.3	31	25	0.20	143	4.4	100

APPENDIX 6

Individual Urinalysis Data (Pretreat)

Dog No.	Group	SG	pH	Color & turbidity	Protein mg%	Sugar	Occult Blood	Vol. ml/ 24 hr.	Microscopy E	F	M	R	O	C	A
980	1♂	1.044	6.4	2-	*	-	-	65	2	0	1	0	0	0	3
970	Control	1.036	6.1	2-	*	0.25%	-	121	3	0	2	0	0	0	2
972		1.040	6.1	2-	*	0.25%	-	68	3	0	2	0	0	0	2
969		1.040	6.5	2-	*	-	-	50	1	0	1	0	0	0	3
981	2♂	1.013	6.6	1-	*	-	-	200	1	0	0	0	0	0	1
974	12.5%	1.004	6.8	1-	*	-	-	500	1	0	0	0	0	0	1
973		1.020	6.9	1-	*	0.25%	-	160	1	0	1	0	0	0	1
984		1.060	5.9	3-	*	0.25%	-	50	2	0	2	0	0	0	2
985	3♂	1.025	7.0	2-	*	0.25%	-	190	1	0	1	0	0	0	0
976	25%	1.062	6.2	3-	*	0.50%	-	25	2	0	3	0	0	0	2
977		1.017	6.8	1-	*	-	-	200	1	0	0	0	0	0	1
975		1.044	6.3	2+	*	-	-	50	1	0	0	0	0	0	2
982	4♂	1.054	5.8	2-	*	0.25%	-	84	3	0	3	0	0	0	2
979	50%	1.032	6.5	2-	*	-	-	60	1	0	1	0	0	0	2
978		1.048	6.5	2-	*	-	-	61	1	0	0	0	0	0	2
986		1.046	6.1	2-	*	-	-	45	1	0	1	0	0	0	1

* Denotes less than 5mg%

APPENDIX 6
(continued)

Individual Urinalysis Data (Pretreat)

Dog No.	Group	SG	pH	Color & turbidity	Protein mg%	Sugar	Occult Blood	Vol. ml/ 24 hr.	Microscopy E	P	M	R	O	C	A
988	1♀	1.042	6.1	2+	*	-	-	60	1	0	0	0	0	0	2
990	Control	1.009	6.9	1-	*	-	-	460	1	0	0	0	0	0	1
994		1.052	6.0	2-	*	0.25%	-	50	1	0	2	0	0	0	1
993		1.033	6.0	2-	*	-	-	40	1	0	0	0	0	0	1
989	2♀	1.008	6.4	1-	*	-	-	480	1	0	0	0	0	0	0
992	12.5%	1.029	6.5	2-	*	-	-	121	2	0	1	0	0	0	2
997		1.020	6.1	2-	*	-	-	200	1	0	0	0	0	0	1
1000		1.020	6.6	1-	*	-	-	190	1	0	0	0	0	0	0
987	3♀	1.010	6.9	1+	*	-	-	440	2	0	1	0	0	0	1
999	25%	1.020	6.7	1-	*	-	M	170	2	0	1	0	0	0	0
998		1.038	6.9	2-	*	-	-	90	1	0	1	0	0	0	3
1002		1.056	6.4	2-	*	0.25%	-	77	1	0	1	0	0	0	2
995	4♀	1.058	6.1	2-	*	0.25%	-	101	2	1	1	1	0	0	2
1003	50%	1.046	6.0	2-	*	-	-	100	1	0	2	0	0	0	1
996		1.029	6.4	2++	*	-	-	92	1	0	0	0	0	0	1
1004		1.008	6.2	1-	*	-	M	175	2	0	0	0	0	0	1

* Denotes less than 5mg%

APPENDIX 6
(continued)

Individual Urinalysis Data (Week 4)

Dog No.	Group	SG	pH	Color & turbidity	Protein mg%	Sugar	Occult Blood	Vol. ml/ 24 hr.	Microscopy E	P	M	R	O	C	A
980	1♂	1.050	6.0	3-	*	0.25%	-	45	2	0	2	0	0	0	1
970	Control	1.040	6.8	2+	10mg%	0.25%	-	25	3	0	2	0	0	0	2
972		1.018	5.7	1-	*	-	-	250	2	0	1	0	0	0	0
969		1.050	6.2	3-	*	0.50%	-	35	2	0	2	0	0	0	2
981	2♂	1.024	6.1	2-	*	0.25%	-	110	2	0	1	1	0	0	1
974	12.5%	1.028	5.8	2-	*	0.25%	-	110	1	0	1	0	0	0	0
973		1.024	6.0	2-	*	0.25%	-	140	2	0	0	0	0	0	0
984		1.026	6.2	2-	5mg%	-	-	70	2	0	3	0	0	0	2
985	3♂	1.026	6.9	2-	*	0.25%	-	90	1	0	1	0	0	0	2
976	25%	1.032	6.5	3-	*	0.25%	-	220	2	0	1	0	0	0	0
977		1.023	7.1	2-	*	-	-	150	2	0	1	0	0	0	3+
975		1.040	6.4	3-	*	0.25%	-	40	1	0	1	0	0	0	2
982	4♂	1.034	6.3	2-	*	0.25%	-	52	3	0	2	0	0	0	1
979	50%	1.038	7.0	3-	*	0.25%	-	32	2	0	2	0	0	0	3+
978		1.025	7.7	2-	*	-	-	110	2	0	0	0	0	0	3+
986		1.038	6.4	2+	10mg%	-	-	40	2	0	2	0	0	0	1

* Denotes less than 5mg%

APPENDIX 6
(continued)

Individual Urinalysis Data (Week 4)

Dog No.	Group	SG	pH	Color & turbidity	Protein mg%	Sugar	Occult Blood	Vol. ml/ 24 hr.	Microscopy E	P	M	R	O	C	A
988	1♀	1.036	6.1	2-	5mg%	0.25%	-	76	1	0	2	0	0	0	1
990	Control	1.020	5.8	2-	*	-	-	210	2	0	2	0	0	0	1
994		1.028	6.8	3-	*	0.25%	-	150	2	0	1	0	0	0	1
993		1.028	6.4	2-	*	0.25%	-	77	1	0	2	0	0	0	0
989	2♀	1.025	6.1	2-	*	0.25%	-	120	1	0	1	0	0	0	0
**992	12.5%	1.020	6.9	2+++	75mg%	-	L	69	1	0	1	0	0	0	1
997		1.027	6.4	2-	*	-	-	320	2	0	1	0	0	0	1
1000		1.032	6.2	2-	*	0.25%	-	130	2	0	1	0	0	0	0
987	3♀	1.024	6.4	2-	*	0.25%	-	140	1	0	2	0	0	0	0
999	25%	1.019	6.1	1-	*	-	-	230	1	0	1	0	0	0	0
998		1.040	6.8	2-	*	0.25%	-	80	1	0	0	0	0	0	3+
1002		1.026	7.0	2-	*	0.25%	-	45	1	0	1	0	0	0	3+
995	4♀	1.030	6.3	2-	*	0.25%	-	68	1	0	1	0	0	0	1
1003	50%	1.032	6.1	1-	*	0.25%	-	130	1	0	2	0	0	0	1
996		1.040	6.7	2-	*	0.25%	-	100	1	0	1	0	0	0	3
1004		1.036	7.2	2-	*	0.25%	-	48	1	0	1	0	0	0	3

* Denotes less than 5mg%
** Repeat sample

992		1.019	7.1	1-	*	-	-	115	2	0	2	1	0	0	3

APPENDIX 6
(continued)

Individual Urinalysis Data (Week 13)

Dog No.	Group	SG	pH	Color & turbidity	Protein mg%	Sugar	Occult Blood	Vol. ml/24 hr.	Microscopy E	P	M	R	O	C	A
988	1 ♂	1.018	6.2	1-	*	-	-	800	1	0	0	0	2	0	1
970	Control	1.019	6.0	1+	*	-	-	690	2	0	0	0	2	0	2
972		1.040	6.7	2+	5mg%	-	-	35	0	0	0	0	3	0	2
969		1.017	6.0	1-	*	-	-	780	1	0	0	0	1	0	1
981	2 ♂	1.034	8.3	2+	5mg%	.25%	-	59	1	0	0	0	3	0	1
974	12.5%	1.033	7.2	2+	5mg%	-	-	39	0	0	2	0	2	0	2
973		1.026	7.4	2-	*	-	-	117	1	0	2	0	3	0	1
984		1.023	6.6	1+	*	-	-	150	0	1	1	0	2	0	2
985	3 ♂	1.032	7.1	2-	*	.25%	-	110	1	0	0	0	2	0	0
976	25%	1.023	6.8	1-	*	-	-	580	2	1	1	0	3	0	2
977		1.034	6.8	2-	*	-	-	137	1	0	2	0	2	0	2
975		1.017	6.8	1-	*	-	-	820	1	1	1	0	3+	0	2
982	4 ♂	1.024	7.8	1+	*	-	-	590	2	1	0	0	3	0	2
979	50%	1.025	7.9	2+	*	-	-	78	1	0	0	0	3	0	2
978		1.027	7.2	2-	*	-	-	131	0	0	0	0	3	0	2
986		1.018	6.6	1+	*	-	-	890	0	0	0	0	3+	0	3

* Denotes less than 5mg%

APPENDIX 6
(continued)

Individual Urinalysis Data (Week 13)

Dog No.	Group	SG	pH	Color & turbidity	Protein mg%	Sugar	Occult Blood	Vol. ml / 24 hr.	Microscopy E	P	M	R	O	C	A
988	1 ♀	1.013	7.2	1-	*	-	-	790	1	0	0	0	2	0	2
990	Control	1.027	6.6	1-	*	-	-	220	0	0	2	0	2	0	1
994		1.023	6.5	1-	*	-	-	340	1	0	0	0	2	0	2
993		1.022	7.8	1-	*	-	-	150	1	1	0	0	1	0	1
989	2 ♀	1.017	8.7	1+	*	-	-	38	1	0	0	0	3	0	1
992	12.5%	1.023	5.8	1-	*	-	-	84	1	0	1	0	2	0	2
997		1.038	6.7	2-	*	.25%	-	52	0	0	0	0	2	0	2
1000		1.034	6.5	2-	*	.25%	-	78	1	0	0	2	2	0	2
987	3 ♀	1.026	7.7	1+	*	-	-	146	2	0	0	0	3	0	1
999	25%	1.042	6.7	2-	*	.25%	-	56	0	0	0	0	2	1	1
998		1.025	6.5	1-	*	-	-	220	1	1	0	2	2	0	2
1002		1.032	8.3	1-	*	-	-	350	1	0	0	0	2	0	2
995	4 ♀	1.022	6.5	3+	(10) 10mg%	-	-	400	0	0	0	0	3+	0	3+
1003	50%	1.025	8.3	1-	*	-	-	200	2	0	0	0	2	0	2
996		1.032	7.3	2-	*	-	-	102	0	0	0	0	2	0	2
1004		1.035	7.3	2-	*	-	-	90	1	0	0	0	3	0	2

* Denotes less than 5mg%
( ) This value was repeated

APPENDIX 6
(continued)

Individual Urinalysis Data (Week 26)

Dog No.	Group	SG	pH	Color & turbidity	Protein mg%	Sugar	Occult Blood	Vol. ml / 24 hr.	Microscopy E	P	M	R	O	C	A
980	1 ♂	1.023	6.0	1-	*	-	-	380	3	0	2	0	1	0	2
970	Control	1.024	6.0	1+	*	-	-	580	2	0	0	0	2	0	3
972		1.027	5.0	2+	*	-	-	540	2	0	1	0	2	0	2
969		1.031	6.0	1-	*	-	-	220	2	0	3	0	2	0	2
981	2 ♂	1.029	6.0	1+	*	.25%	-	190	2	0	3	0	3	0	2
974	12.5%	1.025	6.5	1+	*	-	-	300	1	0	2	0	3	0	2
973		1.035	7.0	2-	*	.25%	-	150	1	0	3	0	2	0	2
984		1.042	7.0	2+	(30) 30	-	-	51	1	0	3	1	2	0	2
985	3 ♂	1.025	7.0	1-	*	-	-	700	2	0	1	0	3	0	2
976	25%	1.027	6.5	1-	*	-	-	500	2	0	2	0	1	0	2
977		1.033	6.0	2-	*	-	-	580	2	0	1	0	1	0	2
975		1.032	6.5	1-	*	-	-	360	1	0	2	0	2	0	3
982	4 ♂	1.017	7.0	1+	*	-	-	1250	1	0	2	0	2	0	2
979	50%	1.021	7.0	2-	*	-	-	1000	2	0	2	0	3	0	3
978		1.035	7.0	3+	*	.25%	-	330	1	0	1	0	2	0	3
986		1.019	7.0	1+	*	-	-	1000	2	0	1	0	1	0	3

* Denotes less than 5mg%
( ) This value was repeated

APPENDIX 6
(continued)

Individual Urinalysis Data (Week 26)

Dog No.	Group	SG	pH	Color & turbidity	Protein mg%	Sugar	Occult Blood	Vol. ml / 24 hr.	Microscopy E	P	M	R	O	C	A
988	1 ♀	1.021	6.5	1-	*	.25%	-	740	2	0	1	0	0	0	1
990	Control	1.023	6.0	1-	*	-	-	570	3	0	2	0	2	0	2
994		1.021	6.0	1-	*	-	-	800	2	0	1	0	0	0	2
993		1.018	6.0	1-	*	-	-	590	3	0	2	0	1	0	2
989	2 ♀	1.023	6.5	1-	*	-	-	510	2	0	2	0	2	0	3
992	12.5%	1.025	5.5	1-	*	-	-	420	1	0	2	0	0	0	2
997		1.019	6.0	1-	*	-	S	740	3	0	3	1	2	0	2
1000		1.022	6.5	1-	*	-	-	630	2	0	1	0	1	0	2
987	3 ♀	1.027	7.0	1-	*	.25%	-	280	3	0	3	0	2	0	3
999	25%	1.035	6.5	2-	*	.25%	-	560	2	0	2	0	3	0	2
998		1.026	6.5	1-	*	-	M	450	3	0	3	1	2	0	2
1002		1.025	7.0	1-	*	-	-	440	2	0	2	0	2	0	2
995	4 ♀	1.034	7.0	2++	*	-	-	470	1	0	0	0	1	0	3
1003	50%	1.019	7.0	1-	*	-	-	430	3	0	3	0	3	0	3
996		1.025	7.5	1-	*	-	-	890	3	0	2	0	2	0	2
1004		1.023	7.0	3++	*	-	S	1180	1	0	0	1	0	0	3

* Denotes less than 5mg%

APPENDIX 7

Ophthalmoscopy

LIONEL F. RUBIN, V.M.D.
415 MONTGOMERY AVENUE
MERION STATION, PENNSYLVANIA 19066

MOHAWK 4-5018

October 3, 1972

Huntingdon Research Corporation
Box 6851
Baltimore, Maryland 21204

Attn: Mr. Uttman

Pretest ophthalmoscopic examination of 36 dogs for project 1-315 was performed September 22, 1972. Dog 991* has a focal posterior cataract associated with a hyaloid remnant. This will probably not progress. Dog 1001* has hypertrophy and prolapse of the glands of the left third eyelid.

Lionel F. Rubin, V.M.D.

LFR/gj

* Dogs 991 and 1001 not used on this study.

LIONEL F. RUBIN, V.M.D.
415 MONTGOMERY AVENUE
MERION STATION, PENNSYLVANIA 19066

MOHAWK 4-5016

January 4, 1973

Huntingdon Research Laboratories
Box 6857
Baltimore, Maryland 21204

Attn: Dr. G. Owen

Ophthalmoscopic examination of project 1-315 dogs was performed December 19, 1972. No abnormalities were seen.

Lionel F. Rubin, V.M.D.

LFR/gj

LIONEL F. RUBIN, V.M.D.
418 MONTGOMERY AVENUE
MERION STATION, PENNSYLVANIA 19066

MOHAWK 4-5016

March 7, 1973

Huntingdon Research Corporation
Box 6851
Baltimore, Maryland 21204

Attn: Mr. Upman

Ophthalmoscopic examination of project 1-315 dogs was performed March 6, 1973. Dog 994 has focal posterior suture tip lens opacities, which are seen commonly in untreated dogs. No other abnormalities were seen.

Lionel F. Rubin, V.M.D.

LFR/gj

APPENDIX 8

Gross Observations at Necropsy

Group/Sex Level	Dog No.	Observations
1 ♂ Control	969	Spleen - black raised areas on margin
	970	Spleen - black raised areas on margin
	972	Spleen - black raised areas on margin
	980	Small intestine - few roundworms
2 ♂ 12.5%	973	Spleen - black raised areas on margin
	974	Spleen - black raised areas on margin
	981	Spleen - black raised areas on margin
	984	None noted
3 ♂ 25%	975	Spleen - black area on margin
		Lung - discolored spot on right lobe
		Small intestine - one roundworm noted
	976	Epididymis - apparently missing on one testis
	977	Spleen - black area on margin
		Small intestine - few roundworms
		Kidney - small indentation on outer cortex
	985	Small intestine - one roundworm
4 ♂ 50%	986	Spleen - black area on margin
	982	Spleen - black area on margin
	979	Spleen - black area on margin
		Small intestine - few roundworms
	978	Spleen - black areas on margin
1 ♀ Control	990	Spleen - black raised areas on margin
	993	Spleen - black raised areas on margin
	994	None noted
	988	Spleen - light and dark areas
2 ♀ 12.5%	987	Spleen - black raised areas on margin
		Small intestine - few roundworms
	992	Pituitary - small watery cyst
		Spleen - black areas on margin
	997	Small intestine - one roundworm noted
	1000	Small intestine - one roundworm noted
3 ♀ 25%	987	None noted
	998	Pituitary - apparent cyst
		Ovaries - differ in size (0.8 vs 0.2 gm)
	999	Spleen - black areas on margin
		Small intestine - few roundworms
	1002	Small intestine - few roundworms
4 ♀ 50%	1003	Spleen - black areas on margin
		Small intestine - few roundworms
	995	Spleen - black areas on margin
	1004	Spleen - black areas on margin
	996	Small intestine - few roundworms

APPENDIX 9

Individual Organ Weights in Grams - Males

Group 1 - Control

#	bwt kg	hrt	liv	spl	kid	adr	gon	thy	brn	pit	panc	pros
969	12.9	99.2	414.8	26.6	70.8	1.1	27.0	1.2	91.8	.085	28.7	6.0
970	11.7	92.7	417.8	29.1	84.5	1.4	24.2	1.8	84.7	.077	28.6	10.0
972	9.7	83.4	306.0	22.4	57.8	1.6	13.3	0.7	75.7	.067	25.0	3.2
980	10.8	93.7	383.2	23.2	73.1	1.1	24.6	0.8	84.6	.069	27.0	10.1

Group 2 - 12.5%

#	bwt kg	hrt	liv	spl	kid	adr	gon	thy	brn	pit	panc	pros
973	11.5	80.7	361.0	26.5	51.3	1.2	23.1	0.6	92.8	.028	32.7	5.2
974	12.7	87.8	334.7	28.1	60.0	1.1	25.0	1.3	81.7	.071	33.6	8.5
981	10.2	85.6	286.9	20.5	48.5	1.0	17.1	1.3	77.9	.064	29.2	6.2
984	10.5	77.3	289.9	18.3	71.4	1.1	21.8	0.9	87.2	.065	27.9	8.1

Group 3 - 25%

#	bwt kg	hrt	liv	spl	kid	adr	gon	thy	brn	pit	panc	pros
975	10.7	77.7	353.4	23.0	60.0	1.3	21.5	0.7	84.0	.072	24.6	2.8
976	13.5	07.3	492.6	23.6	62.9	1.6	24.6	1.4	77.7	.113	38.0	9.5
977	10.5	91.4	331.5	23.0	58.9	0.9	20.2	1.3	80.0	.069	20.0	2.9
985	12.0	96.0	398.4	28.4	62.1	1.3	20.2	0.9	80.1	.060	21.9	6.7

Group 4 - 50%

#	bwt kg	hrt	liv	spl	kid	adr	gon	thy	brn	pit	panc	pros
986	11.5	93.5	444.4	30.9	89.5	1.4	20.1	1.2	95.6	.101	32.3	8.5
982	10.6	88.5	405.8	19.7	62.0	1.0	25.3	0.9	86.0	.073	28.1	5.5
979	11.9	87.9	415.6	32.1	84.0	1.2	23.8	1.0	80.9	.080	32.1	6.3
978	9.7	73.2	297.2	22.0	58.8	1.4	20.4	0.7	78.8	.069	24.5	4.7

APPENDIX 9

Individual Organ Weights in Grams - Females

Group 1 - Control

#	bwt kg	hrt	liv	spl	kid	adr	gon	thy	brn	pit	panc	utr
990	11.2	87.9	351.4	29.5	51.9	1.3	2.0	1.6	78.1	.072	28.7	2.9
993	8.6	71.9	267.1	14.9	42.1	1.3	1.6	0.9	76.8	.079	24.3	17.4
994	7.9	66.3	230.0	19.0	50.5	0.8	2.0	0.8	93.7	.069	16.9	7.1
988	8.6	71.0	282.9	21.4	52.0	1.5	0.9	1.2	70.1	.076	22.7	5.9

Group 2 - 12.5%

#	bwt kg	hrt	liv	spl	kid	adr	gon	thy	brn	pit	panc	utr
989	9.2	75.3	280.0	30.6	52.3	1.2	1.2	0.8	77.8	.059	24.2	1.0
992	10.4	90.3	326.3	23.3	54.0	0.9	0.3	1.1	71.7	.076	22.8	1.9
997	9.2	74.2	308.8	23.0	50.3	1.2	2.1	0.8	77.6	.111	30.4	14.4
1000	8.9	75.5	236.5	17.5	49.8	1.2	1.4	0.3	81.7	.057	23.2	2.3

Group 3 - 25%

#	bwt kg	hrt	liv	spl	kid	adr	gon	thy	brn	pit	panc	utr
987	10.4	88.4	250.5	29.8	58.2	1.6	1.5	1.1	72.1	.071	22.0	10.1
998	9.7	86.4	386.4	20.0	63.5	1.0	1.0	1.3	72.4	.078	25.5	26.5
999	8.7	72.2	321.8	21.2	53.1	1.8	1.1	1.4	76.7	.062	25.0	4.8
1002	9.6	83.7	357.0	28.7	71.6	1.1	6.5	0.5	86.1	.071	26.2	8.2

Group 4 - 50%

#	bwt kg	hrt	liv	spl	kid	adr	gon	thy	brn	pit	panc	utr
1003	8.6	74.1	326.1	20.7	55.4	1.6	1.7	0.8	80.5	.060	27.1	18.0
996	9.6	69.0	380.0	32.9	52.9	0.6	2.3	0.8	73.0	.076	28.1	23.4
995	8.4	68.8	350.0	19.9	52.3	1.1	1.2	1.2	66.0	.070	26.2	2.7
1004	8.4	71.0	259.3	20.5	45.3	1.9	1.5	1.1	80.0	.058	24.2	3.0

APPENDIX 10

Individual Organ Weights as a Percent of Bodyweight - Males

Group 1 - Control

#	bwt kg	hrt	liv	spl	kid	adr	gon	thy	brn	pit	panc	pros
969	12.90	0.77	3.22	.21	.55	.0085	0.21	.0093	.712	.0007	0.22	0.05
970	11.70	0.79	3.57	.25	.72	.0120	0.21	.0154	.724	.0007	0.24	0.09
972	9.70	0.86	3.15	.23	.60	.0165	0.14	.0072	.780	.0007	0.26	0.03
980	10.80	0.87	3.55	.21	.68	.0102	0.23	.0074	.783	.0006	0.25	0.09

Group 2 - 12.5%

#	bwt kg	hrt	liv	spl	kid	adr	gon	thy	brn	pit	panc	pros
973	11.50	0.70	3.14	.23	.45	.0104	0.20	.0052	.807	.0002	0.28	0.05
974	12.70	0.69	2.64	.22	.47	.0087	0.20	.0102	.643	.0006	0.26	0.07
981	10.20	0.84	2.81	.20	.48	.0098	0.17	.0127	.764	.0006	0.29	0.06
984	10.50	0.74	2.76	.17	.68	.0105	0.21	.0086	.830	.0006	0.27	0.08

Group 3 - 25%

#	bwt kg	hrt	liv	spl	kid	adr	gon	thy	brn	pit	panc	pros
975	10.70	0.73	3.30	.21	.56	.0121	0.20	.0065	.785	.0007	0.23	0.03
976	13.50	0.79	3.65	.17	.47	.0119	0.18	.0104	.576	.0008	0.28	0.07
977	10.50	0.87	3.16	.22	.56	.0086	0.19	.0124	.762	.0007	0.19	0.03
985	12.00	0.80	3.32	.24	.52	.0108	0.17	.0075	.668	.0005	0.18	0.06

Group 4 - 50%

#	bwt kg	hrt	liv	spl	kid	adr	gon	thy	brn	pit	panc	pros
986	11.50	0.81	3.86	.27	.78	.0122	0.17	.0104	.831	.0009	0.28	0.07
982	10.60	0.83	3.83	.19	.58	.0094	0.24	.0085	.811	.0007	0.27	0.05
979	11.90	0.74	3.49	.27	.71	.0101	0.20	.0084	.680	.0007	0.27	0.05
978	9.70	0.75	3.06	.23	.61	.0144	0.21	.0072	.812	.0007	0.25	0.05

APPENDIX 10

Individual Organ Weights as a Percent of Bodyweight - Females

Group 1 - Control

#	bwt kg	hrt	liv	spl	kid	adr	gon	thy	brn	pit	panc	utr
990	11.20	0.78	3.14	.26	.46	.0116	0.02	.0143	.697	.0006	0.26	0.03
993	8.60	0.84	3.11	.17	.49	.0151	0.02	.0105	.893	.0009	0.28	0.20
994	7.90	0.84	2.91	.24	.64	.0101	0.03	.0101	1.186	.0009	0.21	0.09
988	8.60	0.83	3.29	.25	.60	.0174	0.01	.0140	.815	.0009	0.26	0.07

Group 2 - 12.5%

#	bwt kg	hrt	liv	spl	kid	adr	gon	thy	brn	pit	panc	utr
989	9.20	0.82	3.04	.33	.57	.0130	0.01	.0087	.846	.0006	0.26	0.01
992	10.40	0.87	3.14	.22	.52	.0087	0.00	.0106	.689	.0007	0.22	0.02
997	9.20	0.81	3.36	.25	.55	.0130	0.02	.0087	.843	.0012	0.33	0.16
1000	8.90	0.85	2.66	.20	.56	.0135	0.02	.0034	.918	.0006	0.26	0.03

Group 3 - 25%

#	bwt kg	hrt	liv	spl	kid	adr	gon	thy	brn	pit	panc	utr
987	10.40	0.85	2.41	.29	.56	.0154	0.01	.0106	.693	.0007	0.21	0.10
998	9.70	0.89	3.98	.21	.65	.0103	0.01	.0134	.746	.0008	0.26	0.27
999	8.70	0.83	3.70	.24	.61	.0207	0.01	.0161	.882	.0007	0.29	0.06
1002	9.60	0.87	3.72	.30	.75	.0115	0.07	.0052	.897	.0007	0.27	0.09

Group 4 - 50%

#	bwt kg	hrt	liv	spl	kid	adr	gon	thy	brn	pit	panc	utr
1003	8.60	0.86	3.79	.24	.64	.0186	0.02	.0093	.936	.0007	0.32	0.21
996	9.60	0.72	3.96	.34	.55	.0062	0.02	.0083	.760	.0008	0.29	0.24
995	8.40	0.82	4.17	.24	.62	.0131	0.01	.0143	.786	.0008	0.31	0.03
1004	8.40	0.85	3.09	.24	.54	.0226	0.02	.0131	.952	.0007	0.29	0.04

Six-Month Dietary Administration of Reacted Molasses to Beagle Dogs

APPENDIX 11

Pathology Summary

HRC 1-315

PATHOLOGY SUMMARY

Selected sections of tissue from male and female beagle dogs maintained on a diet containing 12.25, 25, and 50% molasses, for a six-months period of time were examined microscopically. A similar set of tissues from male and female beagle dogs maintained on a diet containing 50% sucrose, were also examined microscopically and served as controls.

No diet-related microscopic alterations were seen in any of the tissues examined from the dogs maintained on the experimental diet. A few scattered instances of spontaneous disease were seen in some of the tissues from both the test and control dogs. In general, the incidence and severity of these incidental lesions were slight.

The histological appearance of the thyroid glands from both the control and test dogs was within the limits of normal histological variation. In general, the thyroid follicular size was moderate and the epithelial cells lining the follicles were low cuboidal. A minimal to slight amount of parafollicular tissue was generally present in the thyroid gland. One instance of slight lymphoid thyroiditis was seen in Group 4 Male No. 979. This microscopic alteration was characterized by the presence of lymphoid follicles in the thyroid gland and a slight diffuse infiltration of lymphocytes and mononuclear macrophages into the interstitium of the gland.

Cysts were occasionally seen in the pars distalis of the pituitary in both the control and test dogs. The incidence of these pituitary cysts was comparable between the control and test animals. Occasionally, a slight

to moderate amount of vacuolation was present in the cells of the zona glomerulosa in the adrenal. This vacuolation was seen in the adrenals from both the control and test animals. Pigment deposition and occasionally extramedullary hematopoiesis were seen in the spleens from both the test and control dogs. Frequently a moderate amount of congestion was present under the capsule at the edge of the spleen. This congestion was seen in both the control and test animals and probably reflected the method of sacrifice. A few siderotic nodules were present in the spleens from both the test and control animals.

A minimal amount of focal mineralization was present in the collecting tubules of the kidneys from both the control and test animals. Occasionally a minimal, focal, nonsuppurative interstitial nephritis was present in both the control and test kidneys.

Testicular activity was generally high in both the control and test males. Focal unilateral aspermatogenesis was seen in the testes from Control Male No. 970 and Group 3 Male No. 976. A moderate focal orchitis was seen in the testis from Group 4 Male 982.

Ovarian activity was normal in both the control and test dogs. The histological appearance of the ovaries and uteri from both the control and test dogs reflected normal estrus cycles.

In conclusion, the incorporation of molasses into the diet of dogs at 12.25, 25, and 50% of the total diet, for six months, did not result in histological alterations in the tissues examined on this study. The incidence and severity of incidental microscopic changes were low in both the test and control animals.

Tony Busey

WILLIAM M. BUSEY, D.V.M., Ph.D.
Pathologist

KEY FOR INCIDENCE TABLE

P = Present

N = No Section

A = Autolysis

X = Not Remarkable

1 = Minimal

2 = Slight

3 = Moderate

4 = Moderately Severe/High

5 = Severe/High

HRC 1-315

DETAILED HISTOPATHOLOGY INCIDENCE TABLE

MALES	GROUP 1				GROUP 2				GROUP 3				GROUP 4			
ANIMAL NO.	969	970	972	980	973	974	981	984	975	976	977	985	978	979	982	986
BRAIN	X	X	X	X	X		X		X	X	X	X	X	X	X	X
Internal Hydrocephalus						2		2								
SPINAL CORD	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
EYE	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
PITUITARY	X		X	X			X	X	X		X	X			X	X
Cyst in Pars Distalis		P			?	P				P			P			
Focal Lymphoid Infiltration														2		
ADRENAL			X		X	X	X	X	X	X				X		
Vacuolation of Zona Glomerulosa	1	3		3							3	4	2		3	2
Vacuolation of Zona Fasciculata																
THYROID																
Epithelial Height	2	2	2	2	2	2	2	2	3	2	2	2	2	2	2	2
Follicle Size	2	3	2	2	2	2	3	3	2	2	3	3	2	2	2	3
Parafollicular Tissue	1	1	2	1	2	2	1	2	3	2		2	2	3	2	1
Lymphoid Thyroiditis														2		
TRACHEA	X	X	X	X	X	X	X		X	X	X	X	X	X		X
Squamous Metaplasia								3							2	
ESOPHAGUS	X	X	X	X	X	X	X	X	X	X	X	X	X	X	N	X
THYMUS	X		X		X		X	X	X	X	X	N	X	X	X	X
Cysts		P		P		P										
Involution																

HRC 1-315

DETAILED HISTOPATHOLOGY INCIDENCE TABLE

MALES	GROUP 1				GROUP 2				GROUP 3				GROUP 4			
ANIMAL NO.	969	970	972	980	973	974	981	984	975	976	977	985	978	979	982	986
AORTA	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
HEART	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
LUNG	X	X	X	X	X	X	X	X	X	X	X	X	X		X	X
Pulmonary Edema														2		
Alveolitis														3		
Bronchiolitis														2		
Bronchopneumonia																
Nematodiasis																
SPLEEN																
Extramedullary Hematopoiesis			1									2	1	1	1	
Pigmentation	1	2	2	1	2	2	2	1	1		1		2	2	2	1
Peripheral Congestion	3				3	3	4			3	3	3	3	3	3	3
Siderotic Nodule				P			P									
GALL BLADDER	X	X	X	X	X	X	X	X	X	X	X	X	N	X	X	X
Nonsuppurative cystitis																
LIVER	X		X	X	X	X	X	X	X		X	X	X	X	X	X
Bile Duct Proliferation		1														
Pericholangitis																
Granuloma										P						

HRC 1-315

DETAILED HISTOPATHOLOGY INCIDENCE TABLE

MALES	GROUP 1				GROUP 2				GROUP 3				GROUP 4			
ANIMAL NO.	969	970	972	980	973	974	981	984	975	976	977	985	978	979	982	986
KIDNEY		X				X		X			X					
Interstitial Nephritis					1									1		1
Mineralization	1		1	1	1		1		1	1		1	1		1	1
Pyelitis														1		
Tubular Dilatation																
STOMACH	X	X	X	X		X	X	X		X	X	X	X		X	X
Gastritis					2				1					2		
PANCREAS	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
SMALL INTESTINE	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
LARGE INTESTINE	X	X	X	X	X	X	X	X	X	X	X	X	X		X	X
Colitis														2		
MESENTERIC LYMPH NODE	X	X	X	X	X	X	X	X	X	X	X		X		X	X
Granuloma												P		P		
URINARY BLADDER	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
PROSTATE	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
TESTIS																
Spermatogenic Activity	4	4	4	3	4	3	4	4	4		4	4	4	4	1	3
Epididymitis	1															
Aspermatogenesis		3								3						
Orchitis															3	

HRC 1-315

DETAILED HISTOPATHOLOGY INCIDENCE TABLE

MALES	GROUP 1				GROUP 2				GROUP 3				GROUP 4			
ANIMAL NO.	969	970	972	980	973	974	981	984	975	976	977	985	978	979	982	986
MAMMARY GLAND	X	N	N	X	X	X	X	X	X	X	X	X	X	X	X	X
SKIN	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Suppurative Dermatitis																
PERIPHERAL NERVE	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
SKELETAL MUSCLE	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
SALIVARY GLAND		X	X	X	X	X	X		X		X	X	X	X	X	X
Nonsuppurative Adenitis	1							1		1						
BONE	N	X	X	N	X	X	X	X	X	X	X	X	X	X	X	X
BONE MARROW																
Cellularity	4	4	4	4	4	4	4	4	4	3	4	4	4	4	4	4
TONGUE	X	X	X	X	X	X	X	X	X	X	X	X	X	X	N	X
Glossitis																

HRC 1-315

DETAILED HISTOPATHOLOGY INCIDENCE TABLE

FEMALES	GROUP 1				GROUP 2				GROUP 3				GROUP 4			
ANIMAL NO.	988	990	993	994	989	992	997	1000	987	998	999	1002	995	996	1003	1004
BRAIN	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Internal Hydrocephalus																
SPINAL CORD	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
EYE	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
PITUITARY	X	X		X	X	X		X	X			X	X	X	X	X
Cyst in Pars Distalis			P				P			P	P					
Focal Lymphoid Infiltration																
ADRENAL	X		X			X	X		X		X	X	X	X	X	
Vacuolation of Zona Glomerulosa		2		1	1			2		1						
Vacuolation of Zona Fasciculata																2
THYROID																
Epithelial Height	2	2	2	3	2	2	2	2	2	3	2	3	2	3	2	2
Follicle Size	2	3	2	2	3	2	2	2	2	2	2	2	3	2	3	3
Parafollicular Tissue	3	1	1	3	3	2	2	2	1	2	2	2	1	2	1	1
Lymphoid Thyroiditis																
TRACHEA	X	X	X	X	X	X	X	X	X	X	X	N	X	X	X	X
Squamous Metaplasia																
ESOPHAGUS	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
THYMUS	X			X		X	X	X	X	X	X		X	X	X	X
Cysts												P				
Involution		3	3		3											

HRC 1-315

DETAILED HISTOPATHOLOGY INCIDENCE TABLE

FEMALES	GROUP 1				GROUP 2				GROUP 3				GROUP 4			
ANIMAL NO.	988	990	993	994	989	992	997	1000	987	998	999	1002	995	996	1003	1004
AORTA	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
HEART	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
LUNG	X	X	X	X	X	X	X	X	X	X		X	X			X
Pulmonary Edema																
Alveolitis																
Bronchiolitis											2				2	
Bronchopneumonia														4		
Nematodiasis														P		
SPLEEN																
Extramedullary Hematopoiesis	1	1	1	2			1	1	2	1						1
Pigmentation	2	1	1	3	1	1	1	1	3	2	3	1	2	2	2	2
Peripheral Congestion			2			3					3		3		3	4
Siderotic Nodule						P									P	
GALL BLADDER	X	X	X	X		X	X	X	X	X	X	X	X	N	X	X
Nonsuppurative cystitis					3											
LIVER	X					X	X		X	X	X	X	X		X	X
Bile Duct Proliferation				2												
Pericholangitis		1	1		1			1						1		
Granuloma					P											

HRC 1-315

DETAILED HISTOPATHOLOGY INCIDENCE TABLE

FEMALES	GROUP 1				GROUP 2				GROUP 3				GROUP 4			
ANIMAL NO.	988	990	993	994	989	992	997	1000	987	998	999	1002	995	996	1003	1004
KIDNEY					X		X								X	
Interstitial Nephritis			1										1			
Mineralization		1	1	1		1		1	1	1	1	1	1	1		1
Pyelitis						3										
Tubular Dilatation	1															
STOMACH	X	X		X	X	X	X	X	X	X	X	X	X	X	X	X
Gastritis			3													
PANCREAS	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
SMALL INTESTINE	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
LARGE INTESTINE	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Colitis																
MESENTERIC LYMPH NODE	X	X	X	X	X	X	X	X	X	X		X	X	X	X	X
Granuloma											P					
URINARY BLADDER	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
UTERUS	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
OVARY	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X

HRC 1-315

DETAILED HISTOPATHOLOGY INCIDENCE TABLE

FEMALES	GROUP 1				GROUP 2				GROUP 3				GROUP 4			
ANIMAL NO.	988	990	993	994	989	992	997	1000	987	998	999	1002	995	996	1003	1004
MAMMARY GLAND	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
SKIN	X	X	X	X	X	X	X		X	X	X	X	X	X	X	X
Suppurative Dermatitis								4								
PERIPHERAL NERVE	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
SKELETAL MUSCLE	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
SALIVARY GLAND	X	X	X	X		X		X	X		X	X	X	X	X	X
Nonsuppurative Adenitis					4		2			1						
BONE	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
BONE MARROW																
Cellularity	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4
TONGUE	X	X	X	X		X	X	X	X	X		X	X	X	X	X
Glossitis					2						2					