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CARBOHYDRATE UTILIZATION BY BABY PIGS

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INTRODUCTION

The carbohydrate ingested by the growing-finishing pig is mostly of the polysaccharide type. Since the young pig does not efficiently utilize polysaccharides, considerable amounts of the disaccharides, sucrose and lactose, and the monosaccharides, glucose and fructose, are used in baby pig diets. The disaccharides which are ingested are hydrolyzed to monosaccharides within the mucosal cells of the small intesine, from whence the monosaccharides are absorbed. Absorption of monosaccharides takes place readily only from the small intestine, and occurs predominantly by way of the portal blood (Wilson, 1962, p. 73). Limited absorption from the stomach and large intestine has been reported to occur under some conditions but it is quantitatively unimportant compared to absorption from the small intestine. Furthermore, it has now been adequately demonstrated that absorption of sugars from the small intestine occurs by both passive and active processes, the former being an energy-independent entrance of sugar into the cell, the latter an energy-dependent movement against a concentration difference (Crane, 1960). The nature of the active transport system has not yet been fully elucidated.

Glucose, which is actively absorbed, has been shown to be absorbed as the free sugar (Wiseman, 1964, pp. 32-33). In contrast, fructose has been shown to be passively absorbed from the intestine, and a marked individual and species difference in the ability to enzymatically convert fructose to glucose in the intestinal wall has also been reported (Verzár, 1936, pp. 142-144; Wiseman, 1964, pp. 27-29). Whether or not such a conversion occurs in the intestine of the pig has not been reported. The inability of fructose to resuscitate comatose pigs (Newton and Sampson, 1951) and

the failure of newborn pigs to grow and survive on fructose-based synthetic milk diets, indicates an extremely slow rate of metabolism and conversion of fructose to glucose in the liver or intestinal wall of the newborn pig.

The blood of the fetal lamb contains a high concentration of fructose and this disappears within a few hours of birth (Shelley and Dawes, 1962). Nevertheless, Andrews et al. (1960) have shown that the perfused liver of the fetal and newborn lamb is unable to metabolize fructose before the fifth day after birth. Shelley and Dawes (1962) observed that the amount of fructose excreted in the urine of the newborn lamb was more than sufficient to account for the apparent loss of fructose from the blood. Goodwin (1956) and Curtis et al. (1966) reported similar high concentrations of fructose in the blood of the newborn pig, followed by a rapid disappearance of fructose within the first 24 hours after birth. The urinary excretion of fructose by the piglet has not been determined. Curtis et al. (1964), on the basis of high levels in fetal blood and of the rapid disappearance of fructose from the piglet blood, suggested a unique role of fructose as an energy substrate.

These observations emphasize the need for additional experimentation to determine the fate of fructose in the fetal and newborn pig. The history of experimentation on the intestinal absorption and on the utilization of carbohydrates is characterized by a profusion of techniques (Wilson, 1962, pp. 20-39). It is the purpose of this dissertation to use such techniques as feeding trials, resuscitation from coma, intestinal loops, administration of carbohydrates by stomach tube and enzyme analyses to study the absorption and utilization of mono- and disaccharides by young pigs.

REVIEW OF LITERATURE

A Comparison of the Nutritive Value of Various Carbohydrates in the Diet of the Baby Pig

Studies of the enzyme activity of the digestive system of the piglet during its first weeks of life give some idea of the qualitative and quantitative enzyme potential *in vivo*. Such evidence as the presence of an enzyme in a tissue requires care in interpretation since the enzyme may not be secreted in direct proportion to its concentration in the tissue. Nevertheless, an evaluation of the changes in enzyme activity gives valuable qualitative and comparative information and provides a basis on which the results of feeding trials using lactose and sucrose may be explained. Most reports agree that the sucrase activity of extracts of intestinal tissue is low at birth, whereas the lactase activity is high (Bailey *et al.*, 1956; Walker, 1959; Hartman *et al.*, 1961; Dahlqvist, 1961). These authors also agree that sucrase activity increases with age, but there are some differences of opinion as to the subsequent changes in lactase activity. Bailey *et al.* (1956), suggests that the lactase activity increases up to two or three weeks of age and then declines rapidly. Walker (1959) and Hartman *et al.* (1961) maintain that it declines steadily from birth to three to five weeks and is then followed by a fairly constant level. There is considerable variation from animal to animal in tissue enzyme concentration at a given age, and therefore chronological age is probably a poor baseline against which to plot enzyme changes, since it may be the physiological age that is controlling the enzyme pattern of any given animal (Bailey *et al.*, 1956).

Becker et al., (1954b) fed diets containing 56 percent glucose to one-day-old pigs and obtained very satisfactory performance in terms of weight gain and survival. Survival on invert sugar was equally as good as on the glucose diets, but weight gain and feed efficiency of the piglets was significantly depressed. In the same experiment sucrose and fructose fed at the same levels as glucose resulted in severe scouring, weight loss and very high mortality. From these results Becker et al. (1954b) concluded that baby pigs cannot utilize fructose or sucrose during the first week of life. These authors further suggested that the failure to utilize sucrose was probably due to the inability to hydrolyze the glycosidic bond of this disaccharide. Two of the seven pigs fed fructose and one of seven pigs fed sucrose did survive the trial. This difference in the ability to survive on sucrose or fructose may indicate differences in physiological age. Becker et al. (1954a) confirmed that sucrose at levels of 56 percent of the diet for one-day-old pigs resulted in diarrhea and 100 percent mortality. With pigs from 7 to 35 days of age, sucrose at this level was more satisfactory. Scouring and high mortality (40 percent), occurred initially in the sucrose fed pigs, but the survivors gained weight at a rate similar to those fed diets containing similar amounts of glucose, lactose, dextrin or corn starch. They concluded that by seven days of age about 60 percent of the pigs had developed the capacity to utilize sucrose in the diet. No deaths were recorded on the lactose based diet. These results with the disaccharides lactose and sucrose fit in well with the knowledge of the digestive enzyme system of the young pig.

Using one-day-old pigs, McCrea and Tribe (1956) compared diets containing 40 percent sucrose and 25 percent starch with similar diets in which lactose replaced sucrose and obtained similar weight gains on the two diets, but 50 percent sucrose in the diet resulted in severe diarrhea and high mortality. They suggested that there may be a critical level for the content of sucrose and maize starch. When diets containing 20 percent sucrose were fed to pigs weaned at 36 to 72 hours of age, the rate of hydrolysis of sucrose, as judged by presence of fructose in the blood, increased with age but was low until the pigs reached 17 days of age (Kidder et al., 1963a). These authors noted wide differences within age in the ability to hydrolyze sucrose. They observed no differences in health or weight gain between pigs on the sucrose or the glucose diets. Dollar et al. (1957) orally dosed pigs of various ages with sucrose solutions and they concluded from the resultant blood sugar curves that the pigs were unable to utilize sucrose until approximately 10 days of age.

In contrast, Wintrobe (1939) successfully reared pigs from two days of age on diets containing 62.5 percent sucrose in spite of much diarrhea. He reported that lactose, dextrin or whey were not superior to sucrose as regards growth or in preventing diarrhea. Hogan and Anderson (1948) and Anderson and Hogan (1950) also observed no mortality when two-day-old pigs were fed diets containing as high as 70 percent sucrose. The sucrose diets gave results which were not significantly different from diets containing equal levels of glucose. Johnson (1949) on the other hand reported that the feeding of sucrose to baby pigs resulted in acute

diarrhea, rapid weight loss, kidney damage and death within 48 hours. The addition of sucrase to these diets showed no beneficial effect on survival or performance. When glucose replaced sucrose in these diets growth was satisfactory, with little or no diarrhea or kidney damage. Pigs fed lactose also grew well at the start of the trials but later developed diarrhea and grew more slowly than those receiving glucose. Fructose was as unsatisfactory as sucrose when included in the diet as the carbohydrate source. Johnson (1949) neglects to mention the age of the pigs with which he worked and he does not include either the composition of the diets used or the number of pigs used per treatment. Manner and McCrea (1962) and Johnson et al. (1948) also obtained very satisfactory results when glucose was the carbohydrate source in the diets of one to three day old pigs.

Hudman (1956) on the basis of his experimentation with pigs from one to five weeks of age, reported that lactose was the carbohydrate of choice in the presence of soybean oil meal, whereas sucrose gave best results when casein was the protein source. Many other experiments confirm that sucrose and lactose are satisfactory carbohydrate sources in the diets of young pigs of one to five weeks of age (Lewis et al., 1953, 1955; Diaz et al., 1956; Smith and Lucas, 1956). Evidence in support of Johnson's (1949) observation that lactose fed to young pigs causes scouring and slower growth rates than glucose diets is afforded by the report of Bustad et al. (1948). These authors showed that piglets weaned at three to five days of age developed diarrhea after about six days on a synthetic diet in which lactose was the carbohydrate

source. The use of penicillin or sulfa drugs was of no benefit in controlling the diarrhea obtained or in prolonging life, all three pigs eventually died after an average of 21 days on the basal diet. Fischer and Sutton (1949) reviewed the reports of numerous investigators on the occurrance of diarrhea in other species when fed various levels of lactose in their diets. These authors also report species, strain and individual differences in susceptability to lactose induced laxation. They suggest that lactose diarrhea is probably the result of slow lactose hydrolysis and its resulting hydragogic effect. If this is so, then the species, strain, age and individual differences in susceptability and severity of lactose induced diarrhea may be due to differences in intestinal lactase activity.

The reports cited show that there is still considerable disagreement as to the nutritive value of lactose and sucrose in the diet of the newborn pig. In contrast, the consensus does support the view that glucose is a satisfactory source of carbohydrate in synthetic milk diets for the baby pig. The nutritive value of fructose as the carbohydrate source in synthetic milk diets for baby pigs has been evaluated with only nine piglets. It should be noted that neither the composition of the diet nor the age of the piglets was reported for two of these pigs and that two of the remaining seven survived the period of the test. Such observations give indications of the nutritive value of fructose but can hardly be considered as conclusive.

The Effects of Starvation on the Blood Sugar Concentration

Sampson et al. (1942) demonstrated that acute hypoglycemia could be produced by starving pigs shortly after birth. These piglets showed a

progressive reduction in metabolism during the starvation period. This progressive decline in metabolism was evidenced by a steady decline in activity, weakness, pallor, hypothermia, bradycardia, convulsions and finally death within 48 to 72 hours. These authors also reported that pigs showing clinical symptoms of hypoglycemia manifested a definite recovery following repeated injections of glucose, provided the blood sugar concentration had not fallen below 40 mg. per 100 ml. of blood at the time of treatment. They suggested that, if the level of blood sugar was below 40 mg. per 100 ml., treatment with glucose was ineffective. Pigs 120 to 140 hours of age were relatively refractory to the development of severe hypoglycemia when subjected to a fast of one week. At the end of the weeks fast the blood glucose levels of these pigs still averaged 85 mg. per 100 ml. of blood. Hanawalt and Sampson (1947) weaned and fasted piglets at various ages from birth to 10 days of age. Their results showed that as the period of suckling was increased from 0 to 10 days, the time to coma increased from 30 to approximately 400 hours. They concluded that the newborn pig is highly susceptible to acute hypoglycemia during the first few days of life, possibly because of low glycogen stores at birth and an inability to synthesize glycogen and glucose from other available materials. Presumptive evidence of an undeveloped capacity for gluconeogenesis in the newborn pig is also provided by the observations of Newland et al. (1952), Morrill (1952), Goodwin (1955, 1957), Elneil and McCance (1965) and Curtis et al. (1966).

Newton and Sampson (1951) tested the effectiveness of intravenous injections of various carbohydrates in alleviating hypoglycemic coma in pigs fasted soon after birth. Their results showed that glucose

was the most effective sugar in resuscitating pigs in hypoglycemic coma, whereas sucrose, fructose and lactose were ineffective in so doing. Other investigators have confirmed these observations and demonstrated the specificity of glucose in alleviating hypoglycemic coma (Mann and Magath, 1922; Herring et al., 1924; Drury and Salter, 1934 and Young et al., 1938). Why glucose per se is needed to resuscitate comatose pigs is still obscure. Drury and Salter (1934) postulated that the respiratory centers require an adequate glucose environment for normal functioning or that glucose undergoes metabolic reactions which supply energy in a specific and unique manner to the respiratory center, without which these tissues cannot function properly. Such a possibility seems likely in view of the experiments of Himwich and Nahum (1932), Maddock et al. (1939) and Mulder and Crandall (1942). Maddock et al. (1939) indicated that the intact molecule of glucose is essential for the normal functioning of the cortical cells, perhaps to form a special product of glucose metabolism which is essential for the synthesis of some chemical mediator.

The available evidence supports the conclusion that glucose is the most effective and probably the only sugar that is immediately capable of resuscitating animals in hypoglycemic coma. Other sugars that have a beneficial effect are probably first changed to glucose before alleviation of coma is possible (Newton and Sampson, 1951). If this is so, then alleviation of hypoglycemic coma by sugars other than glucose, may be used to determine whether such sugars are converted to glucose within the animal body.

On the Conversion of Fructose to Glucose by the Intestinal Wall

It has been recognized for some time that in certain species of animals a conversion of fructose to glucose occurs during the intestinal absorption of fructose. Bollmann and Mann (1931) demonstrated that fructose did not relieve hypoglycemia in the dog deprived of both liver and gastrointestinal tract, but did have such an effect, though not as great as that of glucose, when only the liver was exised. They concluded from these observations that the gastrointestinal tract of the dog was capable of converting fructose to glucose. They suggested that it was probably the mucosal cells of the intestine that were responsible for the conversion. Using perfusion techniques on isolated intestinal loops of the guinea pig, Fridhandler and Quastel (1955) found that with low concentrations of fructose, a considerable proportion of the fructose was transformed into glucose on its passage through the intestinal wall. With high concentrations, the fructose levels appearing in the serosal solution exceeded those of glucose. They suggested that the enzyme system responsible for the conversion of fructose to glucose becomes saturated, and that no further conversion above this maximum is possible. That an enzyme system is involved in the conversion is supported by the findings that anaerobic conditions or the presence of 2,4-dinitrophenol under aerobic conditions, suppresses glucose formation from fructose in the isolated intestine but has no influence on the rate of absorption of fructose. Wilson and Vincent (1955) reported that as much as 75 percent of the fructose absorbed by the isolated small intestine of the hamster was converted to glucose during absorption. In 1957,

Kiyasu and Chaikoff in a study with ^{14}C-fructose and jejunal loops of the rat and guinea pig demonstrated that a variable portion of the absorbed fructose was recovered in the portal plasma as lactate, fructose and glucose. From 25 to 75 percent of the ^{14}C present in the portal plasma at various intervals after the introduction of ^{14}C-fructose into the lumen of the rat loop was contained in fructose, 0 to 20 percent in glucose, 2 to 60 percent as lactate and 0 to 30 percent as unidentified compounds. In the guinea pig, 10 to 30 percent of the ^{14}C in portal plasma was present as fructose, 60 to 90 percent as glucose and about 0 to 10 percent as lactate. These results demonstrate quite clearly that there are wide individual and species differences in the ability to convert fructose to glucose in the intestinal wall. These authors also concluded that routes other than the portal blood are of negligible significance in the transport of absorbed fructose from the intestine. The experiments of Riklis and Quastel (1958b) also using ^{14}C-fructose and isolated guinea pig intestine demonstrated the conversion of fructose to glucose during its passage through the intestinal wall, but in this experiment the glucose and fructose that appeared in the serosal solution accounted for all the radioactivity found there. These authors also observed that the percentage conversion decreased with increased fructose concentration in the lumen. Perfusion experiments with rat and guinea pig intestine (Chain et al., 1960) showed that fructose was absorbed without epimerization to glucose in the rat, but that in the guinea pig, however, 36 percent of the carbohydrate appearing on the serosal side after perfusion was found to be glucose. The observation that intestine of the guinea pig can

convert fructose to glucose was verified by the experiments of Fisher and Parsons (1949), Hele (1953), Darlington and Quastel (1953) and Riklis and Quastel (1958a). That the rat does not convert fructose to glucose to any great extent during its absorption is also supported by the report of Boganove and Barker (1950). Ginsburg and Hers (1960) also demonstrated that inverted intestinal sacs of the guinea pig but not of the rat, converted fructose to glucose during its passage through the intestinal wall. Using ^{14}C-fructose they found that the radioactivity recovered inside the sacs was accounted for by glucose and fructose. Their work suggests that the mechanism involved in the conversion involves the formation of fructose-1-phosphate, triose phosphate, fructose diphosphate and hexose-6-phosphate. This hypothesis is supported by their demonstration of the presence of fructokinase and glucose-6-phosphatase in the guinea pig intestine. Fructokinase activity was also observed in the rat intestine, but glucose-6-phosphatase appeared to be absent. The experiments of Cori et al. (1951) and Hers and Kusaka (1953) showed that the conversion of fructose to glucose in the liver of the rat involved a similar pathway. The absence of glucose-6-phosphatase from the small intestine of the rat has also been verified by the experiments of Hers and deDuve (1950). The formation of fructose-1-phosphate in the small intestine of the rat has also been domonstrated by Kjerulf-Jensen (1942). According to his hypothesis the inability of the rat intestine to convert fructose to glucose is related to the absence of glucose-6-phosphatase. If this is true, then the fact that glucose-6-phosphatase could not be detected in human intestine might

indicate that fructose is not converted to glucose in the course of fructose absorption in man. This conclusion is supported by the experiments of Groen (1937).

Burget et al. (1932) reported that the intestine of the rat, rabbit and dog were unable to convert fructose to glucose. A similar series of experiments were conducted by Verzár (1936) with surviving intestine of the rabbit and he concluded that conversion of fructose to glucose does occur, but that the conversion is neither rapid nor extensive. He suggested that Burget et al. (1932) neglected to consider the possibility that only some of the absorbed fructose takes part in the reaction. This same criticism can be directed to the series of experiments of Cori in 1926, 1927, 1928. Cori observed that glucose and fructose each had their own characteristic utilization pathways in the animal body. Utilization was determined by the percentage of absorbed sugar which was oxidized, deposited as liver glycogen or deposited as body glycogen. These observations are not in harmony with the concept that fructose is converted into glucose by the liver and/or intestinal wall before it is utilized by the tissues. He suggested that if such a conversion were to occur to any large extent, then the same percentage of these sugars should be oxidized and converted into liver glycogen and into body glycogen. Cori conducted this series of experiments with rats, a species of animals in which it has been shown that the intestinal conversion of fructose to glucose does not occur to any appreciable extent.

The literature available suggests that the degree to which fructose is converted to glucose during its absorption varies with the

concentration of the fructose solution used, and between individual animals and species. There appears to be no direct evidence that the intestine of the pig can or cannot effect the conversion of fructose to glucose during fructose absorption. The results of the feeding trials previously reviewed, Becker et al. (1954a) and Johnson (1949), indicate only a limited conversion of fructose to glucose by either the intestine or the liver of newborn pigs.

Fructose and Glucose in the Blood of the Fetal and Newborn Pig

Though the presence of fructose in the fetal blood, amniotic and allantoic fluids was suggested by Paton et al. as early as 1907, it was not conclusively established that the Seliwanoff-positive material present in the fetal blood, was in fact fructose, until the experiments of Bacon and Bell (1948). These workers isolated D-fructose from the fetal blood of sheep and demonstrated that all, or practically all, the total reducing substances present could be accounted for by the presence of glucose and fructose. Goodwin (1956) suggested that the presence of fructose at high concentrations in the fetal blood of land mammals is a peculiarity of ungulates. He found fructose in addition to glucose to be present in high concentrations in the cord blood of the newborn pig, calf, kid and foal, whereas in the non-ungulate young, fructose was present in only trace amounts.

Since then, the presence of high concentrations of fructose in the fetal blood, amniotic and allantoic fluids of ungulates and its virtual absence from the maternal blood has been frequently observed. Barklay et al. (1949) measured the concentrations of fructose and non-fructose

reducing substances in the maternal and fetal fluids of the sheep at various stages of pregnancy. They found only traces of fructose in the maternal blood, whereas the fructose concentration in fetal oxygenated blood tended to exceed the values for non-fructose reducing substances at all stages of pregnancy. The amniotic and allantoic fluid levels for fructose and non-fructose were always higher than the blood sugar levels, but showed no correlation at any age to the blood sugar levels. These results were confirmed by Hitchcock (1949). He observed that there was a fall in fructose concentrations in the umbilical vessels during the second half of the gestation period and that the concentrations of fructose in the umbilical vein were on an average greater than those in the umbilical artery. The maternal blood glucose levels were greater than those of the fetus at all stages of pregnancy, but there was an apparent narrowing of this difference in glucose concentration during the second half of gestation. The infusion and perfusion experiments of Huggett et al. (1951) and Alexander et al. (1952, 1955a, 1955b) provided evidence that glucose was probably a precursor in the formation of fetal fructose and that the site of conversion of glucose to fructose was probably in the placenta. Alexander et al. (1952, 1955a, 1955b) also concluded that fructose was continually produced by the placenta at a constant rate, the rate of synthesis being independent of the glucose concentration in either the maternal or fetal bloods. Neil et al. (1961) and Walker et al. (1960) also used the infusion technique with twin goat fetuses, one fetus remained attached to the placenta, the other detached from its placenta and living an independent existence. Their

results confirm that in the goat the placenta is also the site of fructose synthesis from glucose.

The mechanism of fructose formation from glucose remains obscure. Neil et al. (1961) investigated the possibility that fructose was formed in sheep placenta by the action of a phosphatase on a fructose phosphoric acid ester. These authors however found that only fructose-1:6-diphosphate accumulated in the goat placenta to any great extent when large amounts of glucose were injected into the maternal circulation. They suggested that, whereas, the accumulation of fructose-1:6-diphosphate may well be a reflection of the mechanism for the formation of fetal-blood fructose, it may merely reflect enhanced glucose metabolism in the placenta.

A second hypothesis, that fructose may be formed from glucose via sorbitol arose from the experiments of Hers (1957, 1960a, 1960b). Hers demonstrated the presence of aldose reductase in the placenta of the ewe, ketose reductase in the liver of the sheep fetus and sorbitol in the fetal blood. These findings led Hers to postulate that the fructose in the fetal blood of the sheep is formed by reduction of glucose to sorbitol in the placenta, followed by the oxidation of sorbitol to fructose in the fetal liver. Andrews et al. (1959) reported that perfusion of the placenta showed that little sorbitol was formed compared to the amount of fructose produced, at least in the initial period of perfusion. They suggest that if fructose is formed from glucose via sorbitol that the entire reaction takes place in the placenta. Perfusion of goat placenta did not enhance the rate of increase of fructose

concentration in the perfusion fluid (Neil et al., 1961). Andrews et al. (1960) consistently demonstrated that the perfused fetal liver of the sheep converted sorbitol to fructose. Neil et al. (1961) confirmed the presence of sorbitol dehydrogenase in the placenta of both the sheep and the goat, but not in the pig.

Though aldose reductase has been found in the sheep placenta, the conversion of glucose to sorbitol in the placenta has also been shown to be low. Similarly the presence of ketose reductase in the placenta does not effect the conversion of sorbitol to fructose to any appreciable extent. These apparent contradictions may conceivably be attributed to failure of both the glucose and the sorbitol in the perfusion fluid to penetrate to the site of the placental enzymes (Neil et al., 1961). The demonstration that the placenta of both the goat and the sheep do produce fructose suggests that the fetal-blood fructose in these species may arise by a mechanism other than via sorbitol.

The role of fructose in the fetus is still unknown. Andrews et al. (1960) has shown that the perfused liver of the fetal and newborn lamb is unable to metabolize fructose before the fifth day after birth. There is general agreement that the first step in the metabolism of fructose is the phosphorylation of the sugar to fructose-1-phosphate by adenosine triphosphate in the presence of fructokinase (Cori et al., 1951; Hers, 1955; Salomon and Johnson, 1959; Ginsburg and Hers, 1960). Thus, the reports of Hers (1957) and Ballard and Oliver (1965) that they were unable to detect fructokinase activity in the fetal sheep liver explains the inability of the fetal liver to metabolize fructose. Ballard and Oliver (1965) also observed that fructose metabolism, as measured by the rates of incorporation of ^{14}C-fructose into glycogen and glucose,

was barely detectable in the liver of the fetal sheep but appeared soon after birth. Walker (1963) has also measured the activity of liver ketohexokinase at various ages in rats, guinea pigs and rabbits. He was unable to detect any activity in the livers from fetuses of these animals but found that the enzyme appeared at birth and increased to a maximum activity within a few days. Dickens and Greville (1932) reported that various embryonic tissues differed in their ability to metabolize fructose. They observed that in every species tested either the placenta or the fetal membranes, was able to convert fructose to lactic acid.

Alexander et al. (1955b) noted that though it is not clear what part fructose plays in the blood of the fetus, it cannot be regarded as inert. They suggested that the fetal fructose may play a part in the survival of the developing fetus under adverse circumstances, particularly since the rate of fructose production is continuous and unaffected by glucose concentration in either the fetal or maternal bloods. The impermeability of the placenta to fructose transport from the fetal to the maternal blood further ensures that fructose always exists in the fetal circulation at a reasonable concentration (Huggett, 1951; Karvonen 1954; Alexander et al., 1955b). Goodwin (1956) postulated that some species of mammals with a relatively low maternal blood glucose concentration cannot supply the fetuses with a sufficient concentration of blood sugar without the intervention of the fructose mechanism.

Shelley and Dawes (1962) reported that the fructose concentrations in the blood of the newborn lamb were still high at birth, but that the amount of this blood fructose that was metabolized was extremely small,

since the urinary excretion of fructose was more than sufficient to account for the apparent loss of fructose from the blood. They concluded from this experiment that fructose is of little importance as a source of energy to the newborn lamb. Goodwin (1956, 1957), Kidder et al. (1963b) and Curtis et al. (1966) reported fructose concentrations ranging from 16 to 69 mg. per 100 ml. in the blood of newborn pigs. Curtis et al. (1966) observed that the fructose concentration in the blood decreased significantly from 69 mg. per 100 ml. at birth to 28 mg. per 100 ml. at 8 hours of age. They concluded that this disappearance of fructose from the blood suggested a unique role of fructose as an energy substrate (Curtis et al., 1964). A similar disappearance of blood fructose was also noted by Goodwin (1957) and Kidder et al. (1963b) with the blood fructose concentrations being negligible in the two-day-old pig. Kidder et al. (1963b) administered fructose by continuous intravenous infusion to pigs of various ages from birth to 17 days of age. Their results suggest an extremely slow rate of metabolism and excretion of fructose by the newborn pig. The rate of metabolism, however, increased markedly with age.

Since most of the experiments conducted with the fetus have been with sheep, goats or monkeys and considering the paucity of information on the fate of fructose in the newborn pig, some additional experimentation in this subject should contribute to a more thorough understanding of the role of fructose in the energy utilization processes of fetal and newborn pigs.

EXPERIMENTAL PROCEDURE

General Procedure

The data from the experiments which comprise this dissertation are on file in the Animal Science Department of the Iowa Agricultural and Home Economics Experiment Station as Swine Nutrition Experiments 6506, 6513, 6717, 6719, 6720 and 6721. Any aspects of the experimental procedure that were common to two or more of the experiments will be described here to avoid unnecessary repetition in the discussion of each individual experiment.

All experimental animals were either purebred Landrace or Yorkshire or crossbreed pigs of Landrace, Yorkshire and Poland China breeding and were obtained from the swine nutrition breeding herd. Any pigs that were not on experiment before two days of age were ear marked and injected subcutaneously with 2 ml. of iron dextrin solution containing the equivalent of 100 mg. of elemental iron, in the first 24 hours after birth. The male pigs were castrated during the first week of life.

Analytical Methods

With the exceptions of experiments 6717 and 6721, all blood samples were taken by syringe from the anterior vena cava (Carle and Dewhirst, 1942). The dead space of the syringe contained a 10 percent sodium citrate solution as an anticoagulant (Hewitt, 1932). The blood samples were placed in centrifuge tubes which contained 0.05 ml. of the 10 percent sodium citrate solution, mixed and centrifuged immediately at 3500 R.P.M. for 20 minutes. One ml. of plasma was then transferred into a

a larger centrifuge tube, deproteinized by the addition of nine ml. of 95 percent ethyl alcohol (Chinard et al., 1956), and then centrifuged at 5000 R.P.M. for 20 minutes. The protein free plasma was then stored at -4°C. until analyzed.

Blood plasma fructose levels were determined by Cole's modification of Roe's (1934) resorcinol method, as described by Chinard et al. (1956). Equal parts of a solution containing 0.15 percent resorcinol in 95 percent ethyl alcohol and a 0.75 percent solution of ferric chloride in concentrated hydrochloric acid were mixed immediately before use. To one ml. of protein free plasma or clarified urine was added three ml. of the resorcinol-ferric chloride solution. After a thorough mixing of the contents, the tubes were tightly stoppered and placed in a water bath at 100°C. for 20 minutes. A blank, six fructose standards and two glucose standards were included with each series of determinations. On withdrawal from the water bath the tubes were cooled for three minutes in 25°C. water. Chromogenesis was then read in a Bausch and Lomb Spectrophotometer (Model 20) at 480 mμ.

Plasma glucose levels were determined directly by chromogenesis with benzidine. Considerable difficulty was encountered in trying to prepare a three percent solution of benzidine in glacial acetic acid (Chinard et al., 1956). A solution of 0.2 percent benzidine in glacial acetic acid containing 0.1 percent stannous chloride as described by Jones and Pridham (1954) was therefore used. The addition of stannous chloride produced a cloudy precipitate which was filtered off.

The benzidine solution was freshly prepared before each series of determinations. The final method used in glucose determinations was : to one ml. of protein-free filtrate was added five ml. of the benzidine reagent. After a thorough mixing, the screw topped culture tubes were tightly closed and heated in a water bath at 100°C. for a period of 30 minutes. The tubes were then cooled for three minutes in water at 25°C. after which chromogenesis was read in a Bausch and Lomb spectrophotometer (Model 20) at 425 mμ. Five glucose standards, two fructose standards and a blank were included with each series of determinations.

The removal of pigments and cellular debris from urine samples was carried out using acid-washed activated charcoal, as described by Roe (1934). Glucose and fructose determinations on the clarified urine-acetic acid solutions were then carried out by the procedures already described for blood.

In Experiment 6720, the blood samples of all piglets from sows number one and two and all blood samples collected in Experiment 6721 were analyzed for total reducing substances by the ferricyanide method and for those substances giving the Seliwanoff reaction by the resorcinol-ferric chloride method. Glucose content was estimated by subtracting fructose from total reducing sugars. These analyses were carried out simultaniously on a Technicon Autoanalyzer using a procedure described in Appendix II.

Fructokinase activity of intestinal, liver and placental samples was measured using a modification of the method described by Adelman

et al. (1966). For each assay, a 0.5 gm. sample of previously frozen tissue was homogenized with four volumes of cold 0.25 M mannitol in a Potter-Elvehjem tissue grinder for three minutes. The homogenate was centrifuged for one hour at 100,000 x g in a Beckman model L-2 ultracentrifuge at 0°C. All measurements were made with a Bausch and Lomb spectrophotometer (model 505) and recorder. Fructokinase activity was measured by coupling adenosine diphosphate formation with phosphoenolpyruvate, pyruvate kinase and lactate dehydrogenese, according to the following sequence of reactions:

Fructose+ATP	fructokinase →	Fructose-1-P+ADP
ADP+P-enolpyruvate	pyruvate kinase →	ATP+pyruvate
Pyruvate+NADH	lactate dehydrogenase →	Lactate+NAD+

A typical reaction mixture of 3 ml. contained 2 ml. of substrate (pH 7.0), 0.1 ml. lactate dehydrogenase, 0.1 ml. homogenate and 0.2 ml. ATP-$MgCl_2$ solution (pH 7.0). The substrate contained 100 μ moles of triethonolamine, 100 μ moles of KCl, 3 μ moles fructose, 50 μ moles NaF and 2 μ moles of phosphoenolpyruvate. The 0.1 ml. of lactate dehydrogenase contained 80 units of enzyme activity, and the 0.1 ml. of pyruvate kinase contained 0.25 mg. of pyruvate kinase preparation, sufficient to convert 0.6 μ moles of phosphoenolpyruvate to pyruvate per minute at pH 7.6 and 37°C. The ATP-$MgCl_2$ solution contained 12 μmole $MgCl_2$. The reaction was started by the addition of ATP-Mg, and absorbance change due to oxidation of NADH was measured at 340 mμ at 37°C. for 5 minutes.

In experiments 6720 and 6721, a modification of the paper chromatographic technique described by Adachi (1964) was used to establish the presence of fructose in the urine of the newborn pig. Clarified urine-acetic acid solutions whose fructose concentration had previously been determined were used. A standard fructose solution containing 1 g. per 100 ml. of deionized water was first prepared. Twenty ml. of this stock solution was then made up to 100 ml. with one percent acetic acid, giving a final concentration of 2 mg. of fructose per ml. of solution. Standard and test solutions were chromatographed using Eaton-Dikeman No. 613 filter paper and Brinkman Mn-300 cellulose sheets. The paper and thin layer chromatograms were developed in pre-equilibrated battery jars by the ascending technique with a solvent system containing n-propanol-ethyl acetate-water (7:1:2). The spray reagent for the paper chromatograms was prepared by dissolving 300 mg. of dimidon (5,5-dimethyl-1,3-cyclohexanedione) in 90 ml. of ethanol, followed by addition of 10 ml. of orthophosphoric acid. The spray used for the cellulose plates was the resorcinol-ferric chloride reagent described earlier for the fructose determination of blood and urine samples.

The developed paper and plates were dried in air and sprayed. The paper chromatograms were then allowed to nearly dry and heated to 110°C. for 5 minutes. The cellulose plates were allowed to dry in air after spraying and were then suspended in an oven at 100°C. for 2 minutes. The developed colors were then read in a Photovolt Densicord (Model 542).

All tables referred to in the dissertation are included in Appendix I and figures are included in the body of the thesis.

Experiment 6506 - Glucose, Lactose, Sucrose, and Fructose as the Carbohydrate Source in Synthetic Milk Diets of Baby Pigs of Various Ages

Objective

The purpose of this experiment was to determine the nutritive value of glucose, sucrose, fructose or lactose as the carbohydrate source in synthetic milk diets for pigs of two, four, six and seven days of age.

Experimental procedure

The composition of the basal diet is presented in Table 1. Glucose, sucrose, fructose or lactose was used as the carbohydrate source, (56.58 percent) and casein provided 24.6 percent protein. All tests were conducted in a temperature controlled unit equipped with germicidal (ultra violet) lamps. The unit was fumigated using formaldehyde prior to the start of each trial. The unit contained 36 individual wire-bottom metal pens. The concrete floor under the individual pens was heated by thermostatically controlled circulating water. The temperature of the room and floor was maintained at 28° C. for the first week of the trials and gradually lowered over the next week to 24° C. Each individual pen was equipped with a continuous-flow water fountain. All pens and troughs were cleaned and disinfected twice daily. Each trial was conducted for a 14-day period. The pigs were fed twice daily in metal troughs which were hinged to wooden platforms and positioned to allow the pigs sufficient access to the troughs but still prevent their lying in the troughs or spillage of the milk. The piglets were allowed no supplemental water during the first two days of each

trial. The pigs were fed as much as the previous feeding indicated that they would eat. Any milk remaining in the troughs at the next feeding was weighed back and recorded. The blended milk diets, mixed to contain 15 percent total solids, were prepared eight hours before feeding and stored in a cooler at 7° C. until fed.

In all tests the pigs were weighed on alternate days, and general observations were made at the time of feeding and weighing. Thirty-two two-day-old, twenty-eight four-day-old, eighteen six-day-old and twenty-eight seven-day-old pigs were used in the experiment. The average initial weights of these pigs were 1.90, 1.93, 2.4 and 2.64 kg., respectively. The two-, four- and seven-day-old pigs were allotted to a randomized complete block design of four experimental treatments. The six-day-old pigs were also allotted to a randomized complete block design but only three experimental treatments were used. The pigs were assigned at random from outcome groups of initial weight to the experimental treatments, with six to eight replications per treatment at each age.

Results and discussion

Total gains, feed required per unit gain and dry matter intake of all surviving pigs are presented in Table 4. The mortality of the two- and four-day-old pigs was so high that the data were not analyzed. The results of the trial with two-day-old pigs showed that the mortality was considerably higher on the sucrose and fructose diets than on the glucose and lactose diets. Pigs fed glucose or lactose also gained better and had a better feed efficiency. Two of the two-day-old pigs on both the sucrose and fructose diets survived for the fourteen days of the trial.

The pigs that survived on the fructose diets had better liveweight gains and feed conversion efficiencies than the surviving sucrose fed pigs.

The mortality of the four-day-old pigs was considerably less on sucrose and fructose than for the two-day-old piglets. Survival, liveweight gain and feed conversion efficiency were again better on the glucose and lactose diets than on the sucrose and fructose diets and better on the fructose diet than on the sucrose diet. Necropsy examination of the pigs that died during these trials showed the presence of a severe gastritis and enteritis in all cases. Bacteriological culture of tissues from these pigs did not reveal any significant microorganisms.

In contrast to the two-day-old and four-day-old pigs, there were no deaths on any of the treatments during the trials with six and seven-day-old pigs. Statistical analysis of the data from the trial with six-day-old pigs (Table 5) showed no significant difference between treatments in either weight gain or feed/gain. The weight gain and feed/gain data from the feeding trial with seven-day-old pigs was treated statistically according to the analysis of variance presented in Table 6. All statements concerning statistical significance are at a probability level of 5 percent or less. Pigs fed glucose and lactose diets gained significantly more than pigs fed the sucrose diet, but not the fructose diet. There were no significant differences in daily gain between the fructose, glucose or lactose diets, or between sucrose and fructose. There were no significant differences between any of the treatments in terms of feed efficiency.

The results of these experiments indicate that glucose and lactose are preferable to sucrose and fructose as the carbohydrate sources for pigs during their first week of life. Some piglets possess the ability to survive on diets containing as high as 56.6 percent sucrose or fructose as early as two days of age. All the pigs used in these trials had acquired the ability to utilize both sucrose and fructose at the age of six days. These results are in close agreement to those of Becker et al. (1954a, 1954b). It is possible that the observed improvement in survival with increase in age reflected the enzyme maturation needed to metabolize both sucrose and fructose.

Experiment 6513 - The Value of Certain Sugars in the Alleviation of Hypoglycemic Coma

Objective

This study was undertaken to test the value of glucose, sucrose, fructose and lactose in the alleviation of experimentally induced acute hypoglycemia.

Procedure

Twenty-four pigs were weaned at two days of age, caged and fasted until a state of hypoglycemic coma was reached. Room temperature was maintained at 27° C. throughout the trial and the pigs were allowed water ad libitum. Blood samples were taken from the anterior vena cava, for glucose analysis prior to fasting and again when the pigs reached coma. Some of the common features of progressive hypoglycemia were weakness, pallor, hypothermia, bradycardia, convulsions and finally

death. Piglets were considered to be in a state of coma when they exhibited strong and regular convulsive galloping movements of the forelegs. The pigs were then given an intraperitoneal injection of 20 ml. of a 10 percent solution of either glucose, sucrose, fructose or lactose. The pigs were then observed for any signs of alleviation of the hypoglycemic coma.

Results and discussion

A summary of changes in blood glucose levels and response of the comatose pigs to intraperitoneal injections of carbohydrate are given in Tables 7 and 8. The piglets reached coma at periods ranging from 22 to 148 hours, with the average time to coma being 90.7 hours. The average blood glucose level before fasting was 90.45 mg. per 100 ml. of plasma and at coma this average had fallen to 11.72 mg. per 100 ml. of plasma.

All six piglets given an intraperitoneal injection of 20 ml. of a 10 percent solution of glucose at coma were resuscitated and were usually on their feet within 10 minutes. The piglets given a similar injection of either sucrose or lactose did not recover. One piglet that had taken 148 hours to reach coma did recover when given an intraperitoneal injection of fructose.

These results show that of the four carbohydrates tested only glucose is capable of resuscitating pigs in hypoglycemic coma. Since one pig recovered from coma following fructose injection, it was thought that perhaps fructose would alleviate coma if coma were not reached until the pigs were six days of age.

To determine if this was so, nine piglets were weaned at four days of age and fasted under the same conditions as were the two-day-old pigs. The average time to coma for these piglets was 120 hours. Two piglets were given an intraperitioneal injection of glucose when they reached coma and seven were given fructose. In this experiment 20 ml. of a 10 percent solution of carbohydrate were also used. Both pigs given glucose recovered but all seven fructose treated pigs died.

In a third experiment, ten piglets were weaned at two days of age and fasted. The average time to coma for these 10 pigs was 60 hours. Eight of these piglets were given 20 ml. of a 10 percent solution of fructose-1-6-diphosphate when they reached coma and two received glucose injections. Both glucose pigs recovered but none of the piglets treated with phosphorylated fructose recovered.

The results obtained in this study are in agreement with those reported by Newton and Sampson (1951). Sucrose, lactose, fructose and fructose-1-6-diphosphate were not effective in resusciating hypoglycemic comatose pigs. Glucose was the only sugar that was capable of resuscitating pigs in hypoglycemic coma.

Experiment 6717 - Carbohydrate Studies Using Intestinal Loop Techniques

Objective

The objective of this experiment was to determine whether the injection of fructose directly into the small intestine would result in a rise in the blood glucose level of the portal blood and thus suggest an ability of the intestinal wall to convert fructose to glucose. The

intestinal loop technique of injecting a solution into a tied-off segment of intestine *in situ* has two disadvantages; one, that maintained anesthesia is necessary, and second, that with a readily absorbed substance, the change in concentration in the small amount of fluid introduced is so large that specification of a concentration at which absorption takes place is artificial. The first objection does not seriously negate the objectives of this experiment, since Wilson *et al.* (1960) observed that in general anesthetic agents do not seem to affect absorption. The question of the influence of concentration on absorption is, however, of major importance, in view of the observation of Verzár (1936, p. 123), that fructose absorption increased with increased concentration in the lumen. Verzár (1936, p. 144) observed that with low concentrations the fructose was absorbed in constant quantities because it was transformed to glucose, but with more fructose present than could be transformed a diffusion of unchanged fructose occurred. It was on this basis that a dilute solution of 10 mg. fructose per 100 ml. of solution was chosen for this experiment.

Experimental procedure

Thirty-six pigs were used in this experiment. Littermates, selected on the basis of weight, were removed from the sows in groups of four at the age of three, six and nine days after birth and allotted to four experimental treatments in a randomized complete block design with three replications per treatment at each age.

Pigs were starved for 16 hours prior to the intestinal loop operation. They were then anesthetized with ether and the abdominal

cavity opened. The small intestine was then ligated at both ends and a blood sample taken from the portal vein. All solutions that were to be injected were first heated to 37° C. and then injected into the small intestine within 2.54 cm. of the pylorus. One pig of each replication was given 20 ml. of a 10 percent solution of glucose, another was given 20 ml. of distilled water, and the remaining two were each given 20 ml. of a 10 percent solution of fructose. The incision was then clamped and the pigs kept under heating lamps. Blood samples were again taken from the portal vein at 0.5, 1 and 2 hours after injection. At exactly two hours after the injection the piglets were killed. The small intestine was removed and washed out with distilled water. One hundred ml. of the intestinal washings of each pig were then centrifuged at 5000 R.P.M. for 30 minutes to remove intestinal debris. The supernatant was stored as were blood samples at -4° C. until analyzed for glucose and fructose content.

Results and discussion

Summaries of the changes occurring in the blood plasma sugar levels of piglets as a result of injection of either glucose, fructose or water are presented in Table 9 and graphically in Figures 1 and 2. Statistical analysis of the combined trials is presented in Table 10 and analyses within age group are presented in Tables 11 and 12. As is obvious from the graphical presentations, glucose injections yielded a significantly greater increase in blood plasma glucose levels than either fructose or water injections. While the fructose treatment significantly increased blood plasma fructose, it did not significantly increase plasma glucose levels when compared to injection with water. Though not significant,

Figure 1. Experiment 6717 - Blood glucose response to intestinal injections of glucose, fructose or water

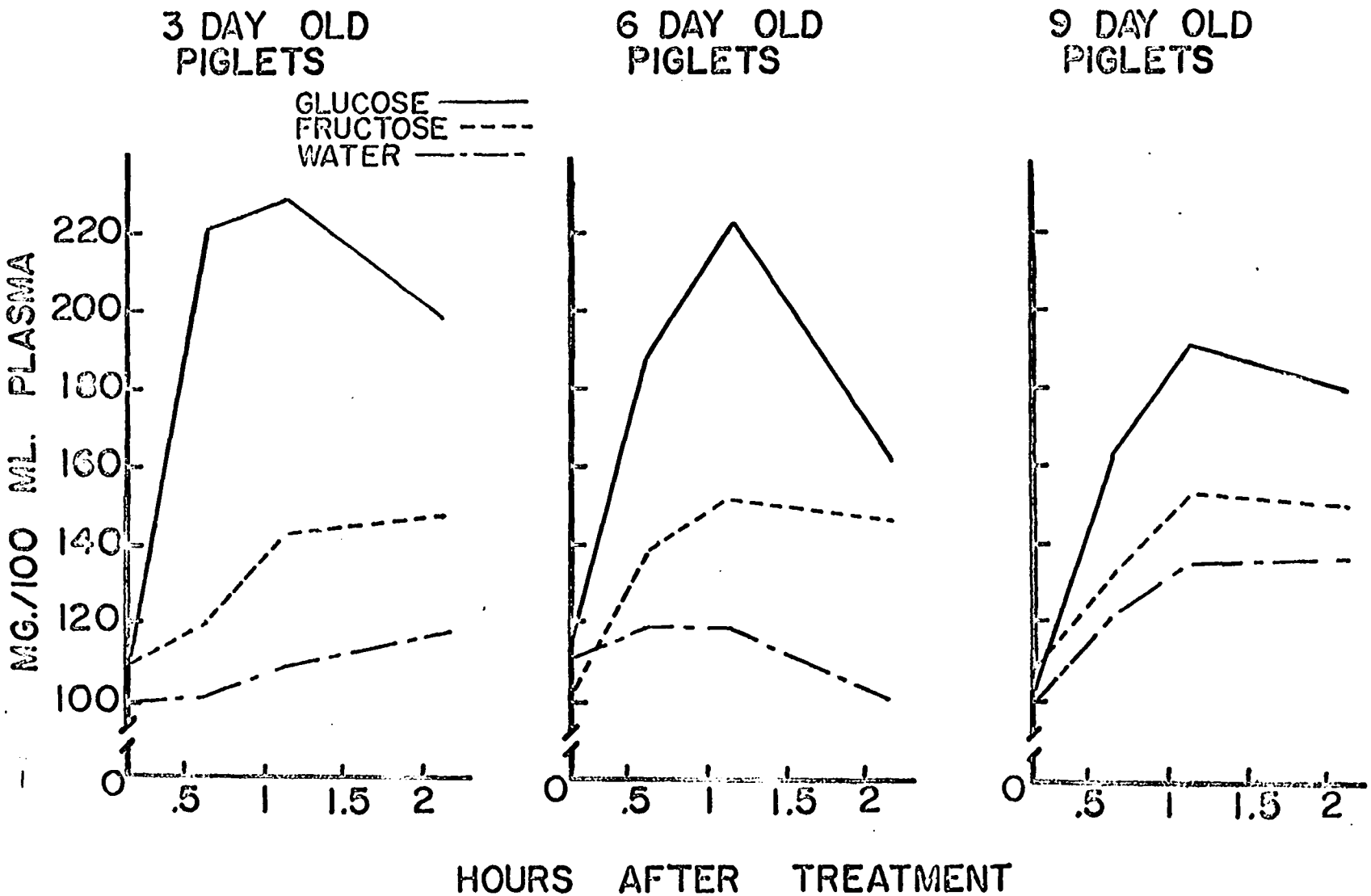
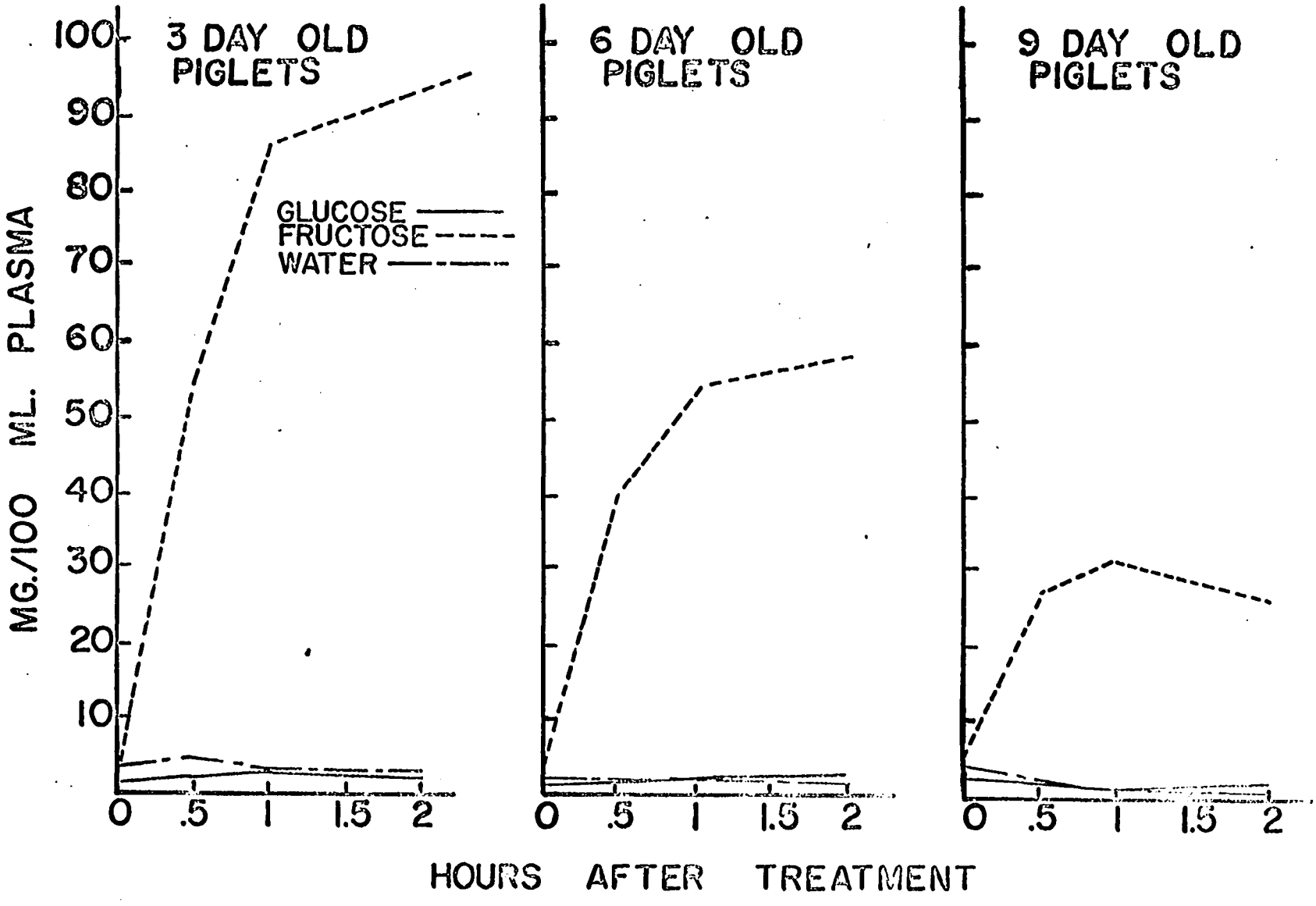

3 DAY OLD PIGLETS
6 DAY OLD PIGLETS
9 DAY OLD PIGLETS
GLUCOSE
FRUCTOSE
WATER
MG./100 ML. PLASMA
220
200
180
160
140
120
100
0
.5
1
1.5
2
HOURS AFTER TREATMENT

Figure 2. Experiment 6717 - Blood fructose response to intestinal injections of glucose, fructose or water


3 DAY OLD PIGLETS
6 DAY OLD PIGLETS
9 DAY OLD PIGLETS
GLUCOSE
FRUCTOSE
WATER
MG./100 ML. PLASMA
100
90
80
70
60
50
40
30
20
10
0
.5
1
1.5
2
HOURS AFTER TREATMENT

the rise in blood glucose following fructose injections was greater at all ages than the glucose rise observed following treatment with water. These observations suggest that fructose is absorbed predominantly as fructose, with perhaps a small amount of fructose being converted to glucose in the intestinal wall. There was no significant difference in response between fructose treated pigs within litters. One group of nine-day-old pigs showed a very small increase in blood fructose following treatment with fructose. Because this result was so contrary to that observed on the other two replications of this experiment it was decided to exclude this litter from the statistical analysis of the blood fructose response.

Age had a significant effect on the pig's response to fructose treatment but not to glucose. The observed rise in blood fructose levels following fructose injection decreased with increase in age. It is possible that as the weight of the pigs increased, the degree of dilution of the injected sugars in the stomach and small intestine increased with the weight of the pigs. This dilution effect would result in a decrease in the concentration of the injected sugar solutions in the intestine. Groen (1937) demonstrated that fructose absorption from the small intestine increased with an increase in the concentration of solution up to 10 percent, above which concentration the absorption of fructose remained constant.

Thus the apparent decrease in absorption of fructose with an increase in age may reflect differences in the concentration of the fructose solutions in the intestine. It is also possible that

fructose absorption decreases with increase in age due to permeability changes in the intestinal wall. The apparent decrease in blood fructose concentration following treatment with fructose could also be explained in part by the greater blood volume of the older and heavier pigs and by an increased ability to utilize fructose with increasing age.

A decreased fructose absorption with increase in age was also suggested by the observation that the average fructose recovery from the intestine of pigs treated with fructose accounted for 9.9, 13.9 and 17.9 percent of the injected dose in the three-day, six-day and nine-day-old pigs, respectively.

In the preparation of the intestinal contents for glucose and fructose determination a certain amount of contamination with blood and intestinal materials could not be avoided. Due to this contamination the presence of glucose was detected in small amounts even in the pigs treated with water. It was decided, therefore, to subtract the glucose values found in the water treated controls from the glucose values obtained in glucose and fructose treated pigs. On this basis no glucose was detected in the intestine of either the fructose or glucose treated pigs.

Experiment 6719 - Carbohydrate Studies Using Stomach Tubing Techniques

Objectives

Intestinal loop studies are subject to variations due to the effect of anesthesia and manipulation of the intestines during the operative procedure. Furthermore the period of study used in the intestinal loop

experiment was only of a two hour duration. Thus, it was the objective of this experiment to determine whether the administration of fructose by stomach tube to three-, six- or nine-day-old baby pigs would show similar changes in blood sugar levels as those observed in the intestinal loop experiment.

Experimental procedure.

Thirty-six pigs were used in this experiment. Littermates, selected on the basis of weight, were removed from the sows in groups of four at three, six and nine days after birth. The pigs were allotted to a randomized complete block design of four experimental treatments, with three replications per treatment at each age.

On removal from the sow, the piglets were caged and allowed only water for the sixteen hours prior to the administration of carbohydrate by stomach tubing. The temperature of the cages was maintained at 28°C. throughout the experiment. One of the four pigs from each replication received 20 ml. of a 10 percent solution of glucose, another received 20 ml. of distilled water and the remaining two were given 20 ml. of a 10 percent solution of fructose. Urine was collected from each pig during the starvation period and again following stomach tubing. Blood samples were taken from the anterior vena cava from each pig just prior to treatment and again at 0.5, 1.0, 2.0, 4.0 and 6.0 hours later. All blood and urine samples were analyzed for glucose and fructose content.

Results and discussion

Summaries of the changes occurring in blood sugar levels following the administration of glucose and fructose are presented in Table 13 and graphically in Figures 3 and 4. Statistical analyses of the data are presented in Tables 14 through 16. The changes in blood glucose following fructose administration were not significantly different from those obtained from treatment with water. Glucose treatment gave significantly greater increases in blood glucose at all ages than did fructose. The rise in blood fructose observed following fructose administration was significantly greater than that obtained from either glucose or water administration. These results indicate that all or practically all the fructose was absorbed as fructose, with little or no conversion of fructose to glucose in the intestinal wall. The rise in blood fructose following treatment with fructose was significantly decreased with increase in age. Whether this reflects a dilution change in the concentration of administered sugars, with a consequent reduction in absorption, as was explained previously in Experiment 6717, is unknown. It may possibly be the result of a decreased intestinal permeability to fructose absorption in the older pigs. There was no significant difference between fructose treated pigs within litters.

A comparison of the effects of glucose versus fructose plus water on the blood glucose levels was highly significant for the three-day-old pigs, significant for the six-day-old pigs but not significant for the nine-day-old pigs. This may be explained on the basis of an increased

Figure 3. Experiment 6719 - Changes in blood fructose concentration as a result of the administration of glucose, fructose or water by stomach tube

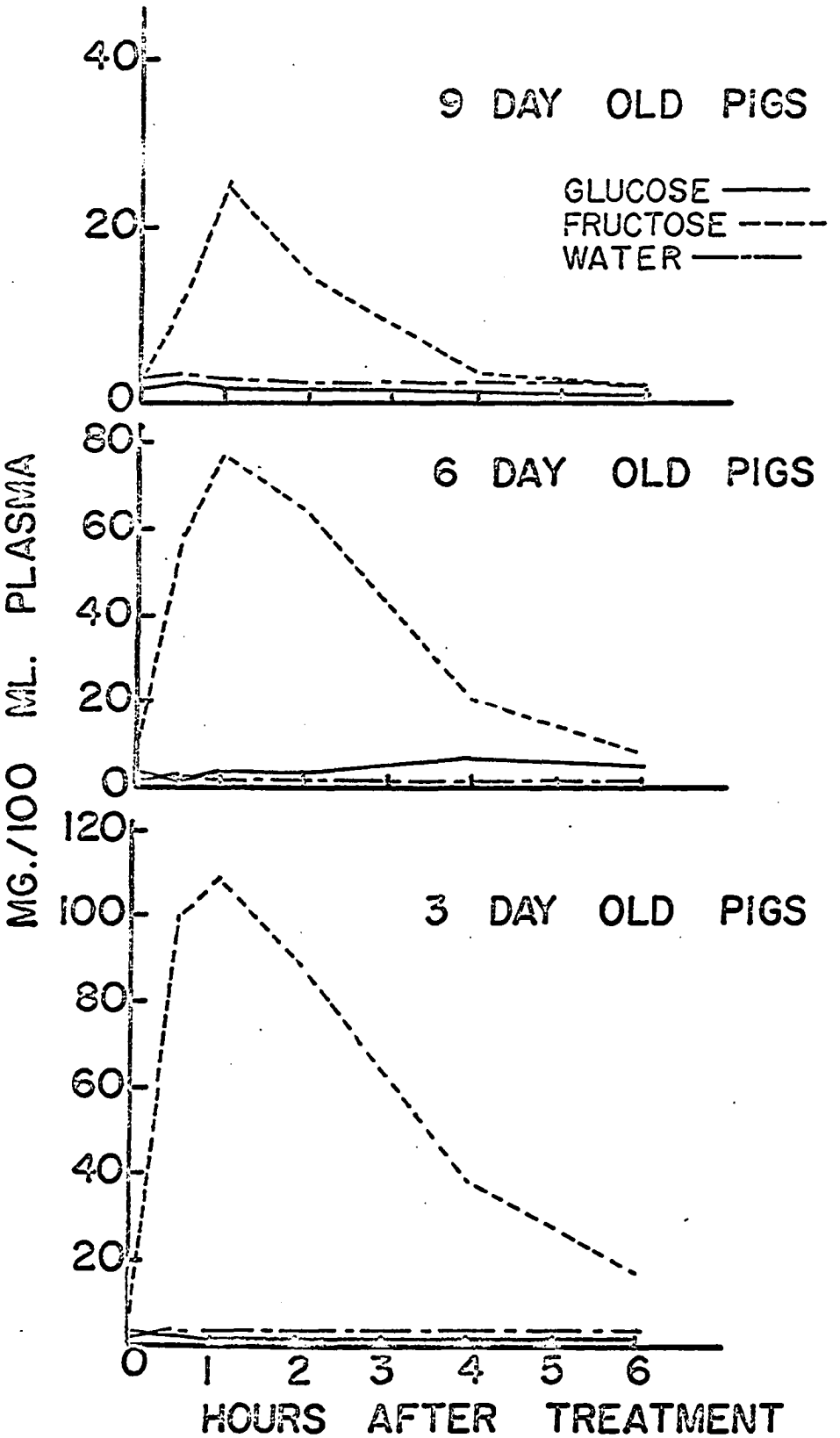
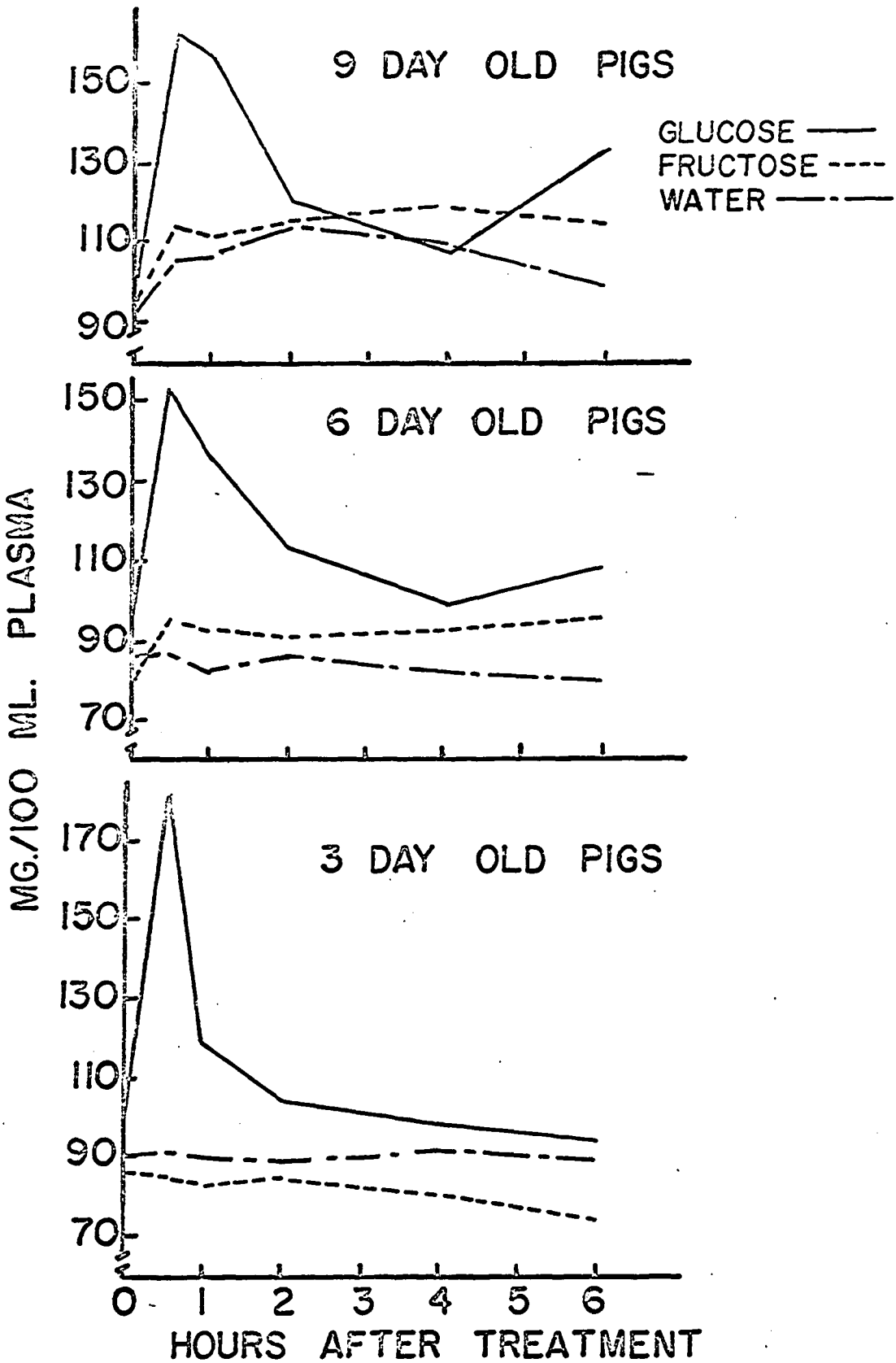

9 DAY OLD PIGS
GLUCOSE
FRUCTOSE
WATER
6 DAY OLD PIGS
3 DAY OLD PIGS
MG./100 ML. PLASMA
HOURS AFTER TREATMENT
0
20
40
60
80
100
120
1
2
3
4
5
6

Figure 4. Experiment 6719 - Changes in blood glucose concentration as a result of the administration of glucose, fructose or water by stomach tube


9 DAY OLD PIGS
GLUCOSE
FRUCTOSE
WATER
150
130
110
90
6 DAY OLD PIGS
150
130
110
90
70
MG./100 ML. PLASMA
3 DAY OLD PIGS
170
150
130
110
90
70
0 1 2 3 4 5 6
HOURS AFTER TREATMENT

glucose utilization with age but it may also indicate an increased conversion of fructose to glucose in the older pigs.

Fructose analysis of the urine (Table 17) showed that rate of fructose excretion before treatment was less than 1.0 mg. per hour for the pigs at the three ages tested. The average urine excretion during the six hours of the experiment following treatment was 20.7 ml. In each case the volume of urine collected during the experiment was diluted to 100 ml. with distilled water and the fructose determinations run on the diluted samples. The increases in the fructose content of the urine following the administration of glucose and water by stomach tube were so small that they fall within the limits of error of the chemical assay techniques. Urinary fructose excretion from the pigs treated with fructose for three-, six- and nine-day-old age groups accounted for 13.1, 11.0 and 4.7 percent, respectively, of the administered fructose. This decline in urinary fructose excretion with age also suggests an increased fructose utilization with age, but it may also be a result of reduced absorption resulting from increased dilution of a constant dosage level, as was explained previously. A reduced absorption would decrease the level to which the blood fructose was elevated above the renal threshold for fructose and thus reduce urinary fructose excretion.

Experiment 6720 - Blood Sugar Levels and Urinary Fructose Excretion of Baby Pigs

Objective

The objective of this experiment was to determine the blood levels of glucose and fructose of baby pigs from birth to 48 hours of age and to

measure glucose and fructose urinary excretion over the same period.

Procedure

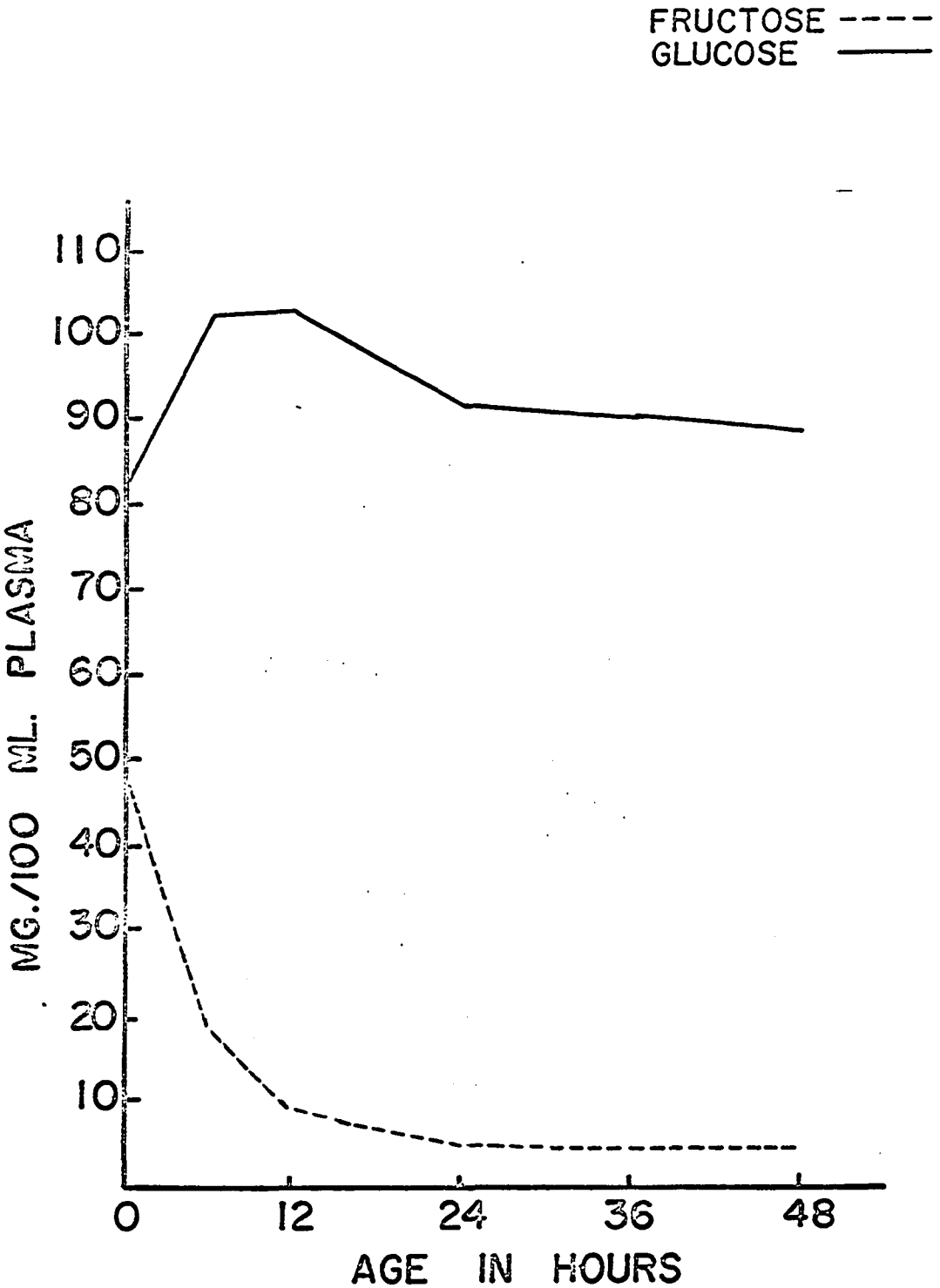
Five litters containing 48 piglets were used in this experiment. Piglets were collected at birth, weighed and blood samples obtained from the anterior vena cava. The piglets were then allowed to suckle for two hours after which they were placed in plastic lined cages of approximately 3½ x 31 x 31 cm. in size. The floors were of fine wire mesh and the cages were set on top of large glass funnels to allow for urine collection. The funnels were also covered by a fine wire mesh and contained glass wool to minimize urine contamination by feces. The urine was collected over four twelve-hour-periods, and then stored at -4°C. until analyzed. Most of the urine samples collected in the first twelve-hour-period required dilution with one percent acetic acid solution for analytical purposes.

The piglets were maintained at a temperature of 28°C. throughout the trial by use of heating lamps and were allowed to suckle every two hours for a period of approximately twenty minutes. Blood samples were taken from the anterior vena cava of each piglet at 6, 12, 24, 36 and 48 hours after birth. No deaths occurred during the experiment and piglets remained in excellent health.

Results and discussion

A graphical representation of changes in blood glucose and fructose levels from birth to 48 hours of age is presented in Figure 5. A statistical analysis of these changes (Table 18) showed no significant difference between litters, within litters or between methods of

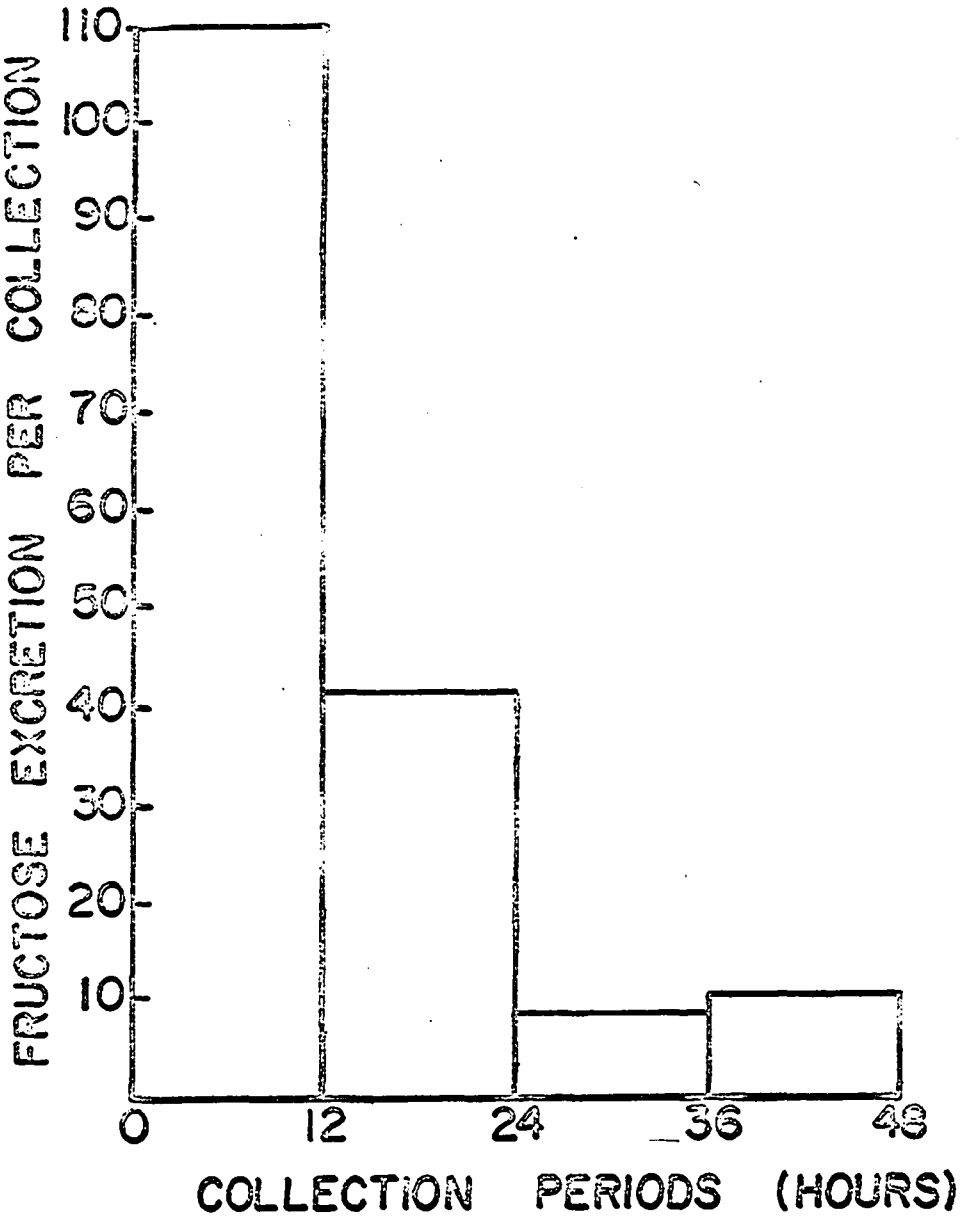
Figure 5. Experiment 6720 - Changes in blood glucose and fructose concentration from birth to 48 hours


FRUCTOSE ----
GLUCOSE ——
MG./100 ML. PLASMA
110
100
90
80
70
60
50
40
30
20
10
0
12
24
36
48
AGE IN HOURS

analysis. These analyses showed a significant linear increase in glucose levels of piglets blood from birth to 12 hours of age, (82.74 to 102.70 mg./100 ml. plasma) followed by a significant curvilinear decrease of glucose over the following 36 hours (102.70 to 88.7 mg./100 ml. plasma). The fructose levels of the piglets blood showed a highly significant curvilinear decrease over the 48 hours period (48.01 to 4.8 mg./100 ml. plasma). These values are in close agreement to those cited by Goodwin (1955).

Urine was excreted at a mean rate of 1.75 ml./pig/hour over the 48 hour period of the trial. Statistical analysis of fructose excretion data are presented in Table 19. These analyses show a significant curvilinear decrease in the amount of fructose excreted per 12-hour collection period. There was also a significant difference among the pigs in the amounts of fructose excreted during the trial. The mean fructose excretion over the 48 hours was 170.98 mg./pig, the majority of which was excreted in the first 12 hours (Figure 6). The apparent loss of fructose from the blood over the same time period was 42.50 mg. The total loss of fructose from the blood was calculated on the assumption that the blood plasma volume in ml. was equivalent to seven percent of body weight in grams (Talbot, 1964). The amount of fructose excreted in the urine was more than four times the apparent rate of fructose loss from the blood. Shelley and Dawes (1962) observed this same situation in lambs. They suggested that since urinary fructose must have come mainly from the blood, the difference was presumably due to fructose passing from the tissues and/or extracellular fluids into the blood, so leading to a low estimate of blood fructose loss.

Figure 6. Experiment 6720 - Urinary fructose excretion per 12-hour period from birth to 48 hours (Mg)


FRUCTOSE EXCRETION PER COLLECTION
110
100
90
80
70
60
50
40
30
20
10
0
12
24
36
48
COLLECTION PERIODS (HOURS)

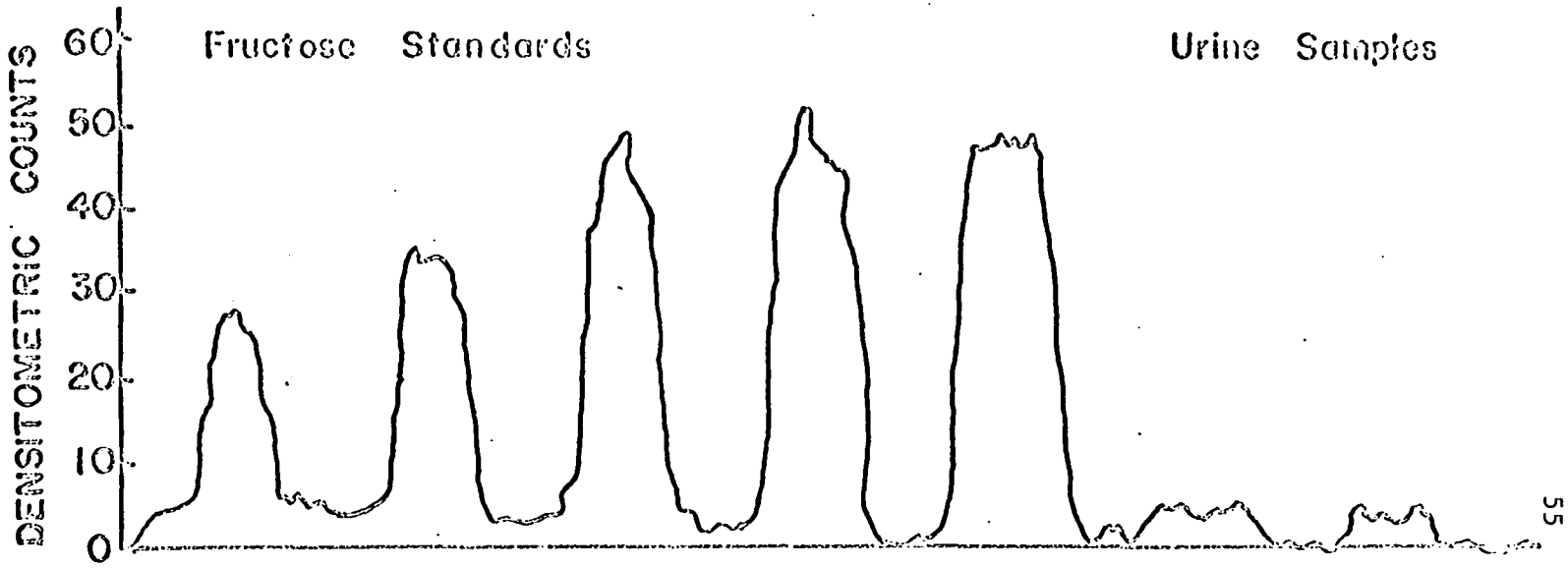
Bacon and Bell (1948) showed that the Seliwanoff-positive material in the blood of the fetal lamb was mainly D-fructose, but that the total reducing substances in fetal blood included not only fructose and glucose but also approximately 10 mg. of non-fermentable reducing materials per 100 ml. of blood. It is possible that the levels of non-fermentable reducing materials are higher in the urine of the newborn pig, and thus lead to the seemingly anomolous results in which fructose excretion exceeded the apparent loss of fructose from the blood.

To determine the levels of non-fermentable reducing substances present in the urine of the newborn pig the urine samples collected during the first 12 hours after birth were subjected to a yeast fermentation. This was carried out with a commercial bakers yeast. A sample of the pressed yeast was suspended in ten times its weight of tap water, left for an hour or more at room temperature and then centrifuged at 5000 R.P.M. for 10 minutes. The yeast was then resuspended in approximately ten times its weight of tap water. The urine-acetic acid samples were adjusted to pH 7.0 with 1 N sodium hydroxide. Equal volumes of yeast suspension and urine samples were then mixed and allowed to stand, with occasional mixing, for three hours at room temperature. The yeast was then centrifuged out at 5000 R.P.M. for 10 minutes. Estimations of fructose were carried out on the urine samples before and after fermentation. The results (Table 20) suggest the presence of an average of 8.3 mg. of non-fermentable reducing substances per 100 ml. of urine. Urea or uric acid at concentrations ranging from 10 to 100 mg. per 100 ml. of solution did not give any chromogenesis with the resoncinol-ferric chloride reagent.

Thin-layer and paper chromatography were used to determine if pig urine did contain fructose and to establish whether substances other than fructose gave a color with the resorcinol-ferric chloride reagent. Paper chromatograms were spotted with 4, 8, 12, 16 and 20µg. of fructose as standards and approximately 10 and 20µg. as urine-acetic acid samples of known fructose content. After the chromatograms were developed, they were sprayed with an ethanol solution of dimedon and orthophosphoric acid, dried and heated to 110°C. for five minutes. When viewed under white light spots containing fructose showed a grayish-yellow color which was stable for several days. A typical densitometric reading of these chromatograms is given in Figure 7. Quantitative densitometry of the sheets was obtained by the use of an automatic integrator. The results of integration showed that the fructose content of the urine samples was only 20 to 25 percent of the levels obtained by chromogenesis with resorcinol ferric chloride. Since dimedon has been shown by Adachi (1964) to have a sensitivity for ketoses of approximately 1µg., these results suggest that the resorcinol-ferric chloride reagent over estimates the fructose content of urine samples, possibly because of chromogenesis with some substance other than fructose.

Thin-layer cellulose chromatoplates were spotted with fructose standard and urine-acetic acid solutions. The developed plates were dried in air and sprayed with resorcinol-ferric chloride reagent. The plates were again allowed to dry and then suspended in an oven at 100°C. for two minutes. When viewed in white light the fructose standard showed an orange-yellow spot with an R_f value of 0.48, followed by a dark brown spot with an R_f value of 0.54. These chromatoplates gave

Figure 7. Experiment 6720 - Densitometric measurement of a paper chromatogram



Estimated Fructose Content µg	4	8	12	16	20	20	10
Integration Markings	29	40	58	64	79	19	15
Integration Markings Corrected for Baseline Shift	19	35	53	64	79	19	15

curves similar to the one presented in Figure 8 when read on the densitometer. The nature of the interfering substance was not determined. These results clearly demonstrate that resorcinol-ferric chloride reagent gives a color reaction with some substance in the urine which is not fructose. The results also demonstrate that the resorcinol-ferric chloride reagent does over-estimate the fructose content of pig urine, and thus explain the observation that fructose excretion during the first 48 hours after birth exceeded the fructose loss from the blood by approximately four times.

Experiment 6721 - Blood Sugar Levels of Fetal and Maternal Blood

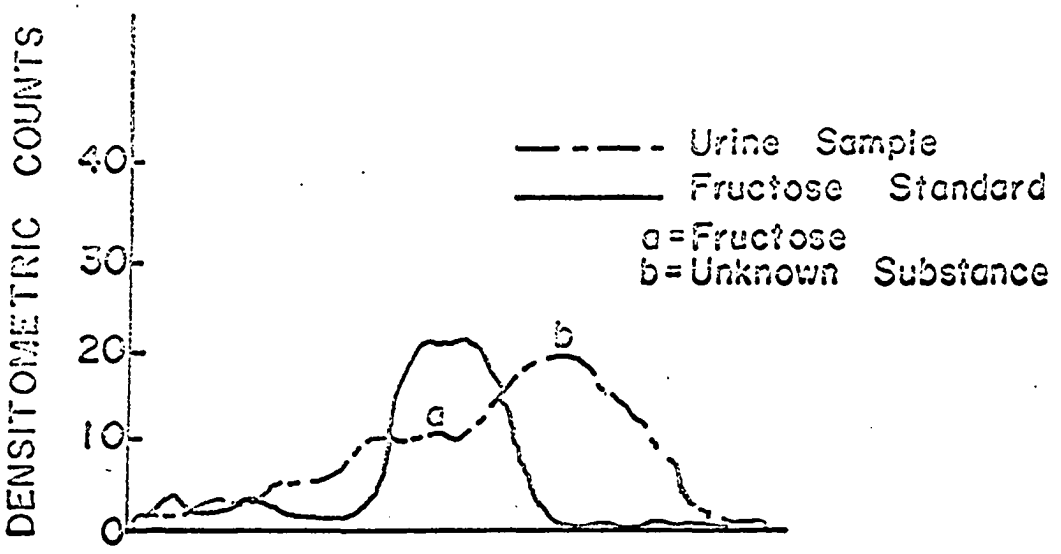
Objective

The objective of this experiment was to determine the glucose and fructose levels in the fetal and maternal bloods of pigs throughout the second half of pregnancy.

Procedure

Six sows, of varying stages of pregnancy ranging from 80 to 110 days were used in this experiment. The sows were fasted for 12 hours before the operation. The sows were washed and brought to a holding-pen near the operating room several hours before the operation. Shortly before the operation the sows were given one gram of a 10 percent solution of surital (sodium thiamylal) through an ear vein. The sows were then tied in a frame with their abdomens facing upward and carried to the operating table. Anesthesia was maintained throughout the operation using fluothane (halothane) in a closed-circuit anesthetic machine.

Figure 8. Experiment 6720 - Densitometric measurement of thin-layer cellulose chromatoplate


DENSITOMETRIC COUNTS
40
30
20
10
0
Urine Sample
Fructose Standard
a = Fructose
b = Unknown Substance
a
b

Collection of blood

The abdominal cavity was opened and samples from the uterine vein and artery were obtained. The uterus was then opened, and the two piglets nearest the cervix in one horn of the uterus were exposed. Blood samples were then obtained from the umbilical vein and artery of these piglets. Samples of allantoic and amniotic fluids were also obtained from these piglets where possible. All samples were obtained by means of a hypodermic syringe and stored in tubes containing 50 mg. of sodium fluoride and 0.05 ml. of a 10 percent sodium citrate solution. Two piglets were then removed from the sow and quickly frozen in dry-ice and acetone. A sample of the placenta was also obtained and frozen. Glucose and fructose determinations of the blood, amniotic and allantoic fluids were carried out using the procedure described in Appendix II. Fructokinase activity of the fetal livers, intestines and placenta were measured at the various stages of pregnancy using a modification of the procedure describe by Adelman et al. (1966). For comparitive purposes the fructokinase activity of adult rat liver was also determined.

Results and discussion

Table 21 gives the values for total reducing substances (T.R.S.), fructose and glucose found in the uterine and umbilical vessels and in the amniotic and allantoic fluids. The glucose values were obtained by subtracting the fructose from the total amount of reducing substance present. Bacon and Bell (1948) demonstrated that the total reducing substances in the fetal blood of sheep include not only fructose and glucose but also about 10 mg. per 100 ml. of non-fermentable reducing

materials. The levels of non-fermentable reducing substances in the fetal blood or in the amniotic and allantoic fluids of the pig at different ages of fetal life have not been determined. The glucose values reported in this series of determinations are therefore more correctly the non-fructose reducing substances of which perhaps 10 mg. per 100 ml. are non-fermentable and the remainder glucose.

The fructose contents of both the umbilical arteries and veins tended to decrease with increase in fetal age. If the placenta is the site of formation of fructose, a higher concentration of this sugar might be expected in the blood of the umbilical vein leaving the placenta than that in the umbilical artery entering the placenta. The fructose content of the umbilical artery was higher than that of the vein by an average amount of 1.4 mg. per 100 ml. of blood (Table 22). Half of the fetuses showed greater or equal amounts of fructose in the umbilical vein as that observed in the umbilical artery. These results therefore do not allow the conclusion that the placenta is the site of formation of fructose for the fetal pig. With the exception of one fetus, the glucose content of the umbilical vein was higher than that of the umbilical artery, the average difference being 5.8 mg. per 100 ml. The average glucose levels of the uterine artery and vein were 58.6 and 54.7 mg. per 100 ml. respectively, a mean loss of glucose from the maternal blood of 3.9 mg. per 100 ml. The maternal glucose loss and the umbilical vein gain in glucose content indicate the passage of glucose from dam to fetus. The concentration of fructose in the maternal blood was low at all stages of pregnancy tested, being an average 1.93 and 1.63 mg. per

100 ml. in the uterine artery and vein respectively. These fructose levels lie within the limits of experimental error and therefore the apparent loss of fructose from the maternal blood of 0.3 mg. per 100 ml. cannot be considered to be meaningful. The fluctuations in the glucose levels of the uterine vessels at the different stages of pregnancy are probably due more to individual differences than to differences in response to handling prior to the operation, since the observed sugar levels are within the normal limits observed for sows (Bunding et al., 1956).

The values for fructose and for non-fructose reducing substances of the amniotic and allantoic fluids are also included in Table 21. Very high levels of both glucose and fructose were observed sporadically, with no apparent relationship to either fetal blood sugar levels or to fetal age. Barklay et al. (1949) observed similar variations in the amniotic and allantoic fluids of the fetal lamb. The average glucose and fructose levels of the amniotic fluids were 35.9 and 113.2 mg. per 100 ml. respectively, whereas the average glucose and fructose values for the allantoic fluids were 175.5 and 120.3 mg. per 100 ml. respectively. The amniotic sugar levels were on an average lower than those found in the allantoic fluids and were more similar in sugar content to the fetal blood levels than were the allantoic levels.

The fructokinase activities of the fetal intestine, liver, and placental samples are presented in Table 23. In all cases the fructokinase activity of the liver was considerably higher than that observed in either the placenta or in the fetal intestine. The

fructokinase activity of the adult rat liver and of the liver and intestine of the three-day, six-day and nine-day old pig were also determined and are included in Table 23. Though the fructokinase concentrations of the neonatal pig were higher than those observed in the fetus, the levels of enzyme activity were still considerably lower than those obtained in the adult rat liver. The enzyme activity of the liver of the newborn pig was higher in all cases then the activity observed in the intestinal wall. Since it has been shown that the conversion of fructose to glucose in the liver of the rat involves the intermediary formation of fructose-1-phosphate, (Kjerulf-Jensen, 1942; Cori et al., 1951; Hers and Kusaka, 1953), then it is apparent that with the pig, the ability of the placenta, liver and intestine to convert fructose to glucose is considerably lower than that of the adult rat liver.

These results are difficult to interpret in view of the results of Experiment 6506 in which it was shown that pigs fed synthetic milk diets containing 56.58 percent fructose acquired the ability to utilize fructose by six days of age. It is possible that the improved utilization resulted from the development of some other pathways of utilization other than through the intermediate substance fructose-1-phosphate. The present results do not give any information on the mechanisms involved in the improved fructose utilization. Alternatively, fructokinase activity may increase only in the presence of its substrate fructose. The fructokinase activity of the tissues of the pigs fed fructose diets was not determined.

GENERAL DISCUSSION

The results of experiment 6506 comparing 56.58 percent glucose, lactose, sucrose or fructose as the carbohydrate source in synthetic milk diets for baby pigs during their first week of life, demonstrated that glucose and lactose yielded better live weight gain and feed/gain ratio than did either sucrose or fructose. The percentage mortality of two-day and four-day-old pigs fed the glucose or lactose diets was considerably lower than that observed with the sucrose or fructose diets. Seventy-five percent of the two-day-old pigs fed sucrose or fructose diets did not survive the fourteen day trial. When these diets were fed to four-day-old pigs the mortality was reduced to 57 percent on the sucrose diet and 29 percent on the fructose diet, and no deaths occurred when these diets were fed to six-day and seven-day-old pigs. The observed improved utilization of sucrose and fructose with increase in age and the marked individual and strain differences in the age at which pigs can utilize sucrose or fructose are in agreement with the studies reported by Johnson (1949) and Becker *et al.* (1954a, 1954b). The observed differences in the utilization of sucrose may be due to differences in the degree of infection, disease resistance and/or levels of sucrase activity in the young pig. Walker (1959) and Dahlqvist (1961) observed that the sucrase activity of the intestinal mucosa was very low or absent at birth and developed gradually as the pigs matured. Walker (1959) also reported very marked differences in the levels of sucrase activity observed in individual pigs of the same age.

Becker et al. (1954a) reported that 60 percent of two-day-old pigs fed a diet containing 56.58 percent sucrose developed the ability to utilize sucrose by seven days of age. The results of experiment 6506 showed that all pigs had developed the ability to grow and survive on sucrose diets when they were six days of age.

Since lactose is the carbohydrate of sow milk and the lactase activity of the pig is high at birth (Dahlqvist, 1961) it is not surprising that dietary lactose produced satisfactory gain, feed/gain and survival at all ages tested. One would also expect that the newborn pig would be able to utilize glucose since it is readily absorbed from the intestine, is the principle carbohydrate of the blood and is readily oxidized within the body. The results of experiment 6506 and those of Johnson (1949) and Becker et al. (1954a, 1954b) all showed very satisfactory weight gains and survival when newborn pigs were fed diets containing high levels of glucose.

The improved utilization of fructose with age is more difficult to explain. There is still controversy regarding the mechanism of fructose absorption. This controversy has arisen mainly due to species differences in the nature of fructose metabolism in the intestinal mucosa and in the liver. The extent to which fructose is converted to glucose in the intestinal wall varies greatly between different species of animals and even within a species. At least three pathways for the conversion of fructose to glucose have been proposed (Crane, 1960). One involves the conversion of fructose to fructose-6-phosphate

through the action of a hexokinase. Another, catalyzed by fructokinase involves the formation of fructose-1-phosphate, while the third sequence of reactions is based on the reversible conversion of glucose to fructose through the intermediate substance sorbitol and the action of aldose and ketose reductases. The "sorbitol cycle" as proposed by Hers (1960b) has been shown to exist in the seminal vesicles of some species but attempts to find evidence of its presence in the intestinal wall have yielded negative results.

Crane (1960) suggests that fructose can form either fructose-6-phosphate or fructose-1-phosphate, depending on the source of the fructose and on the relative rates of the hexokinase and fructokinase reactions. Fructose which arises from invertase action will probably undergo the fructokinase reaction preferentially. The reason for this is that the glucose liberated from sucrose will compete with fructose for hexokinase but not fructokinase. The difference in the extent of conversion of fructose to glucose between and within certain species of animals may be explained by the absence or the levels of activity of either or both hexokinase and fructokinase (Hers and deDuve, 1950). The presence of the hexokinase pathway in the rat intestine was substantiated by the report of Sols (1956) but the evidence suggesting that fructose metabolism occurs via the fructokinase pathway is more convincing (Ballard and Oliver, 1965 and Adelman et al., 1966).

The observation in experiment 6506 that fructose does not support life in the early stages of piglet life suggests that during this period no substantial conversion of fructose to glucose occurs in the

intestinal wall of the young pig.

The results of experiment 6513 support the conclusion that fructose is not utilized by the piglet during the first four or five days of life. In this experiment fructose administered intraperitoneally failed to resuscitate comatose pigs starved from two and four days of age. This observation suggests that very little, if any, of the fructose administered was converted to glucose in the liver of the comatose pigs. Glucose was the only sugar tested that alleviated the symptoms of induced hypoglycemia. Similar observations have been reported by Newton and Sampson (1951). Sucrose, lactose and fructose-1-6-diphosphate were also ineffective in resuscitating the comatose pigs. These results support the observation that practically no hydrolysis or metabolism of disaccharides occurs in the blood (Wilson, 1962, p. 70). The failure of fructose-1-6-diphosphate to alleviate the hypoglycemic coma may possibly have been due, either, to its dephosphorylation during the process of cellular absorption or its inability to substitute for glucose in some essential body function which requires the intact glucose molecule.

Experiments 6717 and 6719 and part of the data from experiment 6721 were designed to further test the new born pigs ability to convert fructose to glucose in the liver or intestinal wall. In experiment 6717 the injection of 20 ml. of a dilute solution (10 mg./100 ml.) of fructose into a tied-off segment of the intestine of three-day, six-day and nine-day-old pigs did increase the fructose content of the portal blood but it did not significantly increase the glucose levels of portal blood

more than did injections with water. Though not significant, the rise in portal blood glucose following fructose injections was greater at all ages than the glucose rise observed following treatment with water. These observations suggest that fructose, is absorbed predominantly as fructose, with perhaps a small amount of fructose being converted to glucose in the intestinal wall. The levels to which the portal blood fructose rose following intestinal injection of fructose decreased significantly as the age of the pigs increased. The quantity of fructose recovered from the intestine two hours after the fructose injection increased with an increase in age. Thus, it appears from these results that fructose absorption from the intestine of young pigs decreased as the pigs got older. Whether these results reflect a decrease in fructose absorption due to a decreased permeability to fructose or whether the decreased absorption was due to greater dilution of the injected fructose in the intestine of the older pigs is unknown. This dilution effect would result in a decrease in the concentration of the injected sugar solutions in the intestine. Since Groen (1937) has shown that fructose absorption from the small intestine increases with an increase in concentration of administered solution up to 10 percent, then one would expect that absorption would be lower in the older pigs in which dilution would be greatest.

The changes in blood sugar levels following the administration of 20 ml. of a 10 percent solution of glucose or fructose via stomach tube were very similar to those observed in experiment 6717 using the intestinal loop technique. Fructose administration resulted in a steep rise in

blood fructose levels but caused only a slight increase in blood glucose. These results again suggest that very little conversion of fructose to glucose occurs in the intestinal wall or liver of the young pig. The rise in blood fructose following administration of fructose decreased significantly with increasing age. This may have been due to a decreased absorption of fructose in the older pigs. Whether the decreased absorption resulted from a decreased pereability to fructose or whether it reflects a possible increase in the degree of dilution of the administered sugar is unknown. Any decrease in the concentration of the administered solutions resulting from dilution in the stomach or intestine could have resulted in differences in the absorption rate of fructose between the different age and weight groups. Differences in blood volume between the different weight pigs would also contribute to the observed decrease in blood fructose rise following treatment with fructose. It is not possible therefore, to conclude from the blood sugar curves that fructose utilization improved with age. In experiment 6719 the urinary fructose excretion of the pigs treated with fructose decreased as the pigs increased in age. This may have been due to either decrease in fructose absorption or an increase in fructose utilization, or both. Glucose was rapidly absorbed from the intestine at all ages tested and no glucose was detected in the urine excreted during the six hours of the experiment.

The fructokinase activity of the liver and intestinal wall of three-day, six-day and nine-day-old pigs were determined in experiment 6721 and were found to be approximately one-third the values reported

in the liver of the adult rat. There appeared to be no difference in the fructokinase activity between the pigs of the three age groups. This observation supports the conclusion that very little conversion of fructose to glucose occurs in the liver or intestinal wall of the young pig, at least via the intermediate fructose-1-phosphate. These results appear somewhat in conflict with the results of the feeding trials reported earlier, in which it was demonstrated that piglets acquired the ability to utilize fructose by six days of age. It is possible that the observed improvement in fructose utilization resulted from the development of some other enzyme system which allowed the utilization of fructose by some other metabolic pathway. Crane (1960) suggests that fructose can be phophorylated to fructose-6-phosphate by a non-specific hexokinase. The presence of the hexokinase pathway in the intestine was substantiated by the studies of Sols (1956) who found fructose to be phosphorylated by the hexokinase of rat intestinal mucosa. Alternatively, the low fructokinase of the pig liver and intestine may reflect the absence of fructose, its substrate. It is possible that the feeding of fructose induced the development of an active fructokinase enzyme system, and that the improved utilization of fructose with increased age reflected the increased fructokinase activity.

In experiment 6720 the blood glucose levels of the pig showed a significant linear increase from birth to 12 hours of age, followed by a significant curvilinear decrease over the following 36 hours. The fructose levels of the piglets blood showed a highly significant curvilinear decrease over the 48 hour period. Urine collections over the

period from birth to 48 hours of age, showed that the fructose levels of the pigs urine were high at birth, but decreased significantly over the 48 hour period. The fructose excreted in the urine during the collection period was more than four times the apparent loss of fructose from the blood. Shelley and Dawes (1962) observed a similar situation in young lambs. They suggested that blood loss estimates were low because of the constant diffusion of fructose from the tissues and/or extracellular fluids. Bacon and Bell (1948) reported the presence of 10 mg. of non-fermentable reducing materials per 100 ml. of blood in the fetal lamb. These results illustrate that the Seliwanoff reaction is not specific for fructose and may therefore give an elevated value for the fructose levels present. The non-fermentable reducing substances in the urine excreted by the baby pig during the first 12 hours after birth were shown by a yeast fermentation technique to average 8.3 mg. per 100 ml. of urine. Chromatographic studies of the piglet urine showed that the resorcinol-ferric chloride reagent of the Seliwanoff reaction does over-estimate the fructose content of urine samples. Paper and thin-layer chromatograms demonstrated that the resorcinol-ferric chloride reagent gives a color reaction with substances in urine which are not fructose. Densitometric readings of the developed chromatoplates indicated that the urine samples contained only 20 to 25 percent of the fructose levels indicated using the Seliwanoff reaction. The nature of the interfering substance was not determined, but urea, uric acid and lactic acid were shown not to give a color reaction with resorcinol-ferric chloride. Glucuronic acid was not tested for chromogenesis

with resorcinol-ferric chloride and may possibly be the interfering substance.

The results of experiment 6721 clearly demonstrate that fructose is the principal sugar of the fetal blood throughout the second half of pregnancy. The maternal blood contained only traces of fructose, but the levels of glucose present were higher than those in the fetal blood. The fructose content of both the umbilical artery and vein blood tended to decrease toward the end of pregnancy. In 50 percent of the fetuses tested, the fructose level of the umbilical artery was equal or greater than that observed in the umbilical vein. These results therefore do not allow the conclusion that the placenta is the site of formation of fructose for the fetal pig. In the majority of cases the glucose concentration of the umbilical vein was higher than that of the umbilical artery, while the uterine artery showed higher glucose levels than the uterine vein. The maternal glucose loss and the umbilical vein gain in glucose content indicated the passage of glucose from the dam to the fetus. The within litter differences in the observed blood sugar levels were as large or larger than those observed between litters of different ages. The levels of fructose and glucose in the amniotic and allantoic fluids varied greatly from fetus to fetus and bore no apparent relationship to either fetal blood sugar levels or to fetal age. The amniotic sugar levels were on an average lower than those of the allantoic fluids and were more similar to the fetal blood sugar values than were the allantoic fluid values.

The fructokinase activity of the fetal intestine, liver and placenta was lower than the values obtained in the adult rat liver or in the tis-

sues of the neonatal pig. The enzyme levels were higher in the liver than in the intestinal wall or in the placenta, the latter tissues having values which were approximately equal. There was very little change in the fructokinase activity of any of the tissues throughout the period of pregnancy tested. These enzyme levels suggest that fructose metabolism via the intermediate fructose-1-phosphate is only of minor importance in the fetal pig. Though low fructokinase activity does not exclude the possibility of fructose metabolism by some other pathway, it does suggest the possibility that the fetal pig is similar to the fetal sheep in that it does not metabolize fructose (Andrews et al., 1960), in spite of the high levels of fructose present in the fetal blood.

SUMMARY AND CONCLUSIONS

A Comparison of Carbohydrates in Synthetic Milk Diets for Young Pigs

A total of 106 pigs were used in four experiments to determine the nutritive value of lactose, glucose, sucrose or fructose as the carbohydrate source in synthetic milk diets for two-, four-, six- and seven-day-old pigs. Weight gain, feed/gain ratio and survival of two-day-old pigs was better on the lactose and glucose diets than on diets containing either sucrose or fructose. The pigs that survived on the fructose diets gained more on less feed than the surviving sucrose fed pigs. Mortality was considerably lower when sucrose and fructose diets were fed to four-day-old pigs than to two-day-old pigs. There were no deaths on any of the treatments during the tirals with six- and seven-day-old pigs. There was no significant difference between treatments in either weight gain or feed/gain ratio of the six-day-old pigs. In the trials with seven-day-old pigs, glucose and lactose fed pigs gained significantly faster than pigs fed the sucrose diet, but not the fructose diet. There was no significant difference in daily gain between fructose, glucose or lactose fed pigs or between pigs fed sucrose or fructose. There were no significant differences between any of the treatments in feed/gain ratio. These results suggest that glucose and lactose yield better performance than sucrose or fructose when included in the diets of young pigs up to four days of age. Sucrose and fructose utilization improved considerably during the first six days of the pigs life.

Resuscitation of Experimentally Induced Hypoglycemia

Fasted four-day-old pigs were more resistant to the development of hypoglycemic coma than were two-day-old pigs. The blood glucose levels of the two-day-old pigs fell to an average value of 11.7 mg. per 100 ml. of plasma at the time of coma. Intraperitioneal injections of 20 ml. of a 10 percent solution of either sucrose, lactose, fructose or fructose-1-6-diphosphate were ineffective in resuscitating pigs in hypoglycemic coma. Glucose effected an immediate recovery of all pigs in hypoglycemic coma.

Absorption of Sugar From Intestinal Loops of Young Pigs

Twenty ml. of a 10 percent solution of either glucose or fructose or water were injected into the intestinal loops of 36 pigs of three, six or nine days of age. The increase in blood glucose following the intestinal injection of fructose was significantly lower than that obtained from glucose injection, and not significantly greater than that resulting from injection with water. Fructose injections into the intestinal loops significantly increased blood plasma fructose. These observations suggest that fructose is absorbed predominantly as fructose and that very little conversion of fructose to glucose occurs in the intestinal wall of the three-, six- or nine-day-old pig. There was a significant decrease in the blood fructose response to fructose injections as the pigs increased in age. Whether this decrease in absorption of fructose with increase in age was due to changes in the concentration of the administered fructose solutions resulting from variations in the degree

of dilution in the intestine of the different age and weight groups or whether it reflects a permeability change in the intestinal wall, was not determined. A decreased fructose absorption with increase in age was also suggested by the observation that the average fructose recovery from the intestine of pigs treated with fructose accounted for 9.9, 13.9 and 17.9 percent of the injected dose in the three-, six- and nine-day-old pigs, respectively. No glucose was detected in the intestine of either the glucose, water or fructose treated pigs.

Absorption and Excretion of Sugars Administered by Stomach Tube

In this experiment 20 ml. of a 10 percent solution of glucose or fructose or water was administered by stomach tube to 36 three-, six- or nine-day-old pigs. Urine was collected from each pig for a period of six hours after treatment. The results obtained agreed very closely to those obtained using the intestinal loop technique. The changes in blood glucose following fructose administration were not significantly different from those obtained from treatment with water. Glucose treatment yielded significantly greater increases in blood glucose at all ages than did fructose. The rise in blood fructose observed following fructose administration was significantly greater than that obtained from either glucose or water administration. These results indicate that all or practically all the fructose was absorbed as fructose, with little or no conversion of fructose to glucose in the intestinal wall. The rise in blood fructose following treatment with fructose was significantly decreased with an increase in age. Whether this reflects a dilution

change in the concentration of administered sugars, with a consequent reduction in absorption is unknown. It may be the result of a decreased intestinal permeability to fructose absorption in the older pigs. Urinary fructose excretion from the pigs treated with fructose for the three-, six- and nine-day-old age groups accounted for 13.1, 11.0 and 4.7 percent, respectively. This decline in urinary fructose excretion with age may be a result of reduced absorption or it may indicate an improved utilization with age.

The Fate of Blood Fructose and Glucose in the Newborn Pig

In this experiment five litters containing 48 piglets were used to determine the changes in blood sugars of the baby pigs from birth to 48 hours of age and to measure glucose and fructose excretions over the same period. There were no significant differences in blood glucose and fructose levels between litters, within litters or between methods of analysis. There was a significant linear increase in glucose levels of piglets blood from birth to 12 hours of age, followed by a significant curvilinear decrease over the following 36 hours. The fructose levels of the piglets blood showed a highly significant curvilinear decrease over the 48 hour period. The amount of fructose excreted per 12-hour period also showed a significant curvilinear decrease. Most of the fructose was excreted in the first 12 hours after birth. There was a significant difference among the pigs in the amounts of fructose excreted during the trial.

The levels of non-fermentable reducing material in the urine of the newborn pig was shown to be 8.3 mg per 100 ml. of urine. Chromatographic analyses of the urine showed that only 20 to 25 percent of the material giving a positive Seliwanoff reaction was in fact fructose. The nature of the interfering substance was not determined. Urea or uric acid did not give a color reaction with resorcinol-ferric chloride.

Fructose and Glucose in the Blood, Amniotic and Allantoic Fluids of the Fetal Pig

Fructose was shown to be the principal sugar of the fetal pigs blood. The glucose levels of the fetal blood were lower than those of the maternal blood. Only traces of fructose could be detected in the maternal blood. There appeared to be no consistant difference between the levels of fructose in the umbilical artery and vein. The glucose levels of the umbilical vein was greater than that of the umbilical artery, while the glucose concentration of the uterine artery was greater than that of the uterine vein. The loss of glucose from the maternal blood and the glucose gain by the fetal blood indicates the passage of glucose from dam to fetus. The amniotic sugar levels were on an average lower than those found in the allantoic fluids and were more similar in sugar concentration to the fetal blood levels than were the allantoic levels. There was considerable variation both between litters and within litters in the sugar levels of the amniotic and allantoic fluids. Very high glucose and fructose levels occurred sporadically with no apparent relationship to either fetal blood sugar levels or to fetal age.

The fructokinase activity of the fetal liver, intestine and placenta remained constant throughout pregnancy. The fetal enzyme concentrations were lower than those observed in the liver or intestine of the neonatal pig or in the liver of the adult rat. The fructokinase activities of the liver and intestine of the neonatal pig showed very little change from three to nine days of age and were considerably lower than the activities observed in the adult rat liver.

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APPENDIX I

Table 1. Experiment 6506 - Composition of experimental diets

Ingredient	%
Carbohydrate	56.58
Casein	30.00
Lard oil and Lecithin	6.00
Potassium phosphate (dibasic)	1.55
Sodium bicarbonate	1.42
Mineral mixture[a]	4.45
Vitamin mixture[b]	+
Antibiotic mixture[b]	+
TOTAL	100.00

[a]Composition given in Table 2.
[b]Composition given in Table 3.

Table 2. Experiment 6506 - Minerals added per kg. of diet

Ingredient	
Potassium phosphate (dibasic), g.	15.51
Sodium bicarbonate, g.	14.21
Calcium phosphate (dibasic), g.	20.28
Calcium carbonate, g.	13.72
Sodium chloride, g.	7.12
Magnesium sulfate, g.	2.12
Ferric citrate, g.	1.16
Manganese sulfate, mg.	60.00
Copper sulfate, mg.	11.00
Zinc chloride, mg.	8.80
Cobalt chloride, mg.	4.40

Table 3. Experiment 6506 - Vitamins and antibiotic additions per kilogram of complete diet

Constituent	Added
Vitamins: units/kg.	
Vitamin A, I.U.	5500.0
Vitamin D_2, I.U.	1093.0
Vitamin B_{12}, mcg.	44.0
Vitamin E, mg.	110.0
Vitamin C, mg.	33.0
Vitamin K, mg.	4.4
Thiamine, mg.	5.0
Riboflavin, mg.	11.0
Ca. Pantothenate mg.	22.0
Choline Chloride, mg.	1100.0
Biotin, mcg.	198.0
Folic Acid, mcg.	990.0
Inositol, mg.	198.0
Pyridoxine, mg.	1.1
Niacin, mg.	66.0
Ethyoxyquin, mg.	150.0
Penicillin, mg.	55.0
Sulfamethazine, mg.	110.0
Chloretetracycline, mg.	110.0

Table 4. Experiment 6506 - Performance data of two-, four-, six- and seven-day-old pigs fed synthetic milk diets

Treatment	Glucose				Sucrose			
Age in Days	2	4	6	7	2	4	6	7
No. pigs started	8	7	6	7	8	7	6	7
No. deaths	3	1	0	0	6	4	0	0
Av. time until death (days)	5.3	4.0	---	---	7.8	4.7	---	---
Av. gain per pig (grams)	1316.8	854.8	854.8	1380	635.5	514.7	703.1	1130
Solids intake per pig (grams)	1871.7	1347.2	1275.5	1970	1435.3	313.3	1072.2	1760
Feed/gain	1.46	1.67	1.57	1.48	2.79	10.20	1.75	1.59
	Fructose				Lactose			
No. pigs started	8	7	6	7	8	7	---	7
No. deaths	6	2	0	0	1	1	---	0
Av. time until death (days)	7.8	4.7	---	---	9.0	6.0	---	---
Av. gain per pig (grams)	863.0	671.6	452.1	1200	985.9	809.5	---	1380
Solids intake per pig (grams)	1832.5	1269.0	966.42	1570	1783.1	1312.2	---	1820
Feed/gain	1.94	2.02	2.59	1.62	2.53	1.77	---	1.34

Table 5. Experiment 6506 - Analysis of variance for average total gain and feed required per unit gain of six-day old pigs[a]

Source	d.f.	Mean squares	
		Total gain	Feed/gain
Total	17	113417.30	0.7424
Replication (Rep.)	5	71236.89	0.3358
Treatment (Trt.)	2	245625.56	1.7577
Rep. x Trt.	10	108065.86	0.7427

[a]Coefficient of variation for feed/gain and total gain 43.76 49.04 percent, respectively.

Table 6. Experiment 6506 - Analysis of variance for average total gain and feed required per unit gain of seven-day-old pigs[a]

Source	d.f.	Mean squares	
		Total gain	Feed/gain
Total	26	83500.	0.1704
Replication (Rep.)	6	190100.	0.3401
Treatment (Trt.)[b]	3	112900.*	0.1150
Rep. x Trt.	17	40700.	0.1203

*Indicates significant difference at P=0.05 or less.

[a]Coefficient of variation for total gain and feed/gain were 15.88 and 22.97 percent, respectively.

[b]Duncans Multiple Range test was used to test the difference between treatment means. Pigs fed the glucose or lactose diets gained significantly more than did pigs fed the sucrose diets.

Table 7. Experiment 6513 - Blood glucose changes and hours to coma in fasted pigs[a]

No. Pigs	No. hours to coma		Blood glucose (mg./100 ml.)			
	Range	Average	at start		at coma	
			Range	Average	Range	Average
24	22-148	90.70 ±8.20	56-119	90.45 ±4.00	10-20	11.72 ±0.56

[a]Each value represents the mean ± the standard deviation of the mean on 24 pigs.

Table 8. Experiment 6513 - Response of comotose pigs to intraperitoneal injections of carbohydrates

Treatment	No. of pigs treated	No. of pigs resuscitated
Glucose	6	6
Sucrose	6	0
Fructose	6	1
Lactose	6	0

Table 9. Experiment 6717 - Summary of glucose and fructose changes in blood plasma following sugar injection into the intestinal loops

Age (days)	Treatment bleeding time (hours)	Water	Glucose	Fructose	Fructose	Water	Glucose	Fructose	Fructose
		Glucose (mg/100 ml)				Fructose (mg/100 ml)			
3	0	99.3	104.7	110.4	108.6	3.9	1.9	1.5	1.9
	0.5	100.3	220.4	120.1	119.6	4.9	2.4	50.8	55.9
	1	109.0	229.0	140.0	145.4	3.5	3.1	80.1	92.2
	2	116.7	198.0	154.6	139.2	3.4	2.9	87.9	98.9
6	0	110.4	112.4	96.0	105.6	1.5	1.4	1.8	1.5
	0.5	119.8	189.0	140.3	138.9	1.8	1.7	46.2	33.5
	1	118.7	222.6	156.3	147.9	2.5	2.3	60.0	48.4
	2	100.8	160.8	144.6	149.5	1.9	2.4	56.7	60.1
9	0	108.9	110.2	98.7	117.4	2.7	4.6	3.4	3.0
	0.5	133.3	164.7	130.1	137.1	2.1	2.7	22.5	16.6
	1	147.0	202.2	143.6	166.4	1.4	1.4	25.2	22.3
	2	148.0	191.7	147.6	156.2	1.2	2.8	20.1	21.5

Table 10. Experiment 6717 - Intestinal loop studies. Analysis of variance of glucose and fructose changes in blood plasma[a]

Source	d.f.	Mean squares	
		Glucose	Fructose
Total	143	2158.05	877.30
Bleeding time (B.T.)	3	19755.63**	5770.30**
Among pigs	35	5186.38**	2165.15*
Litters	8	11758.05**	1863.57
Age	2	488.18	5474.16**
Litters/Age	6	15514.68	660.04
Treatment (Trt.)	2	32951.04**	22693.35**
Water versus glucose + fructose	1	24435.19**	15034.38**
Glucose versus fructose	1	41466.90**	30352.59**
Among fructose pigs/litters	9	445.46	231.64
Litters x Treatment	16	1096.70	837.49
B.T. x Pigs	105	645.82	308.22
B.T. x Litters	24	383.45	291.71*
B.T. x Trt.	6	3984.57**	2945.83**
B.T. x Litters x Trt.	48	479.30	135.30
B.T. x Fructose/Litters	27	433.20	44.15

[a]Glucose and fructose levels expressed as mg./100 ml.

*Indicates significant difference (P = 0.05 or less).

**Indicates significant difference (P = 0.01 or less).

Table 11. Experiment 6717 - Intestinal loop studies. Analysis of variance within age of glucose changes in blood plasma[a]

Age		Mean squares 3 days	6 days	9 days
Source	d.f.			
Total	47	2097.74	1398.15	3049.33
Among pigs	11	4593.87	2575.52	9243.97
Treatment (Trt.)	2	21825.44**	10675.30**	4357.01
Glucose versus fructose + water	1	39276.63**	17318.56**	8949.00*
Fructose versus water	1	4374.24	4032.02	64.98
Litters	2	115.79	1917.40	44510.85
Litters x Trt.	4	1486.10	404.89	542.58
Among fructose pigs/litters	3	235.22	508.59	592.56
Bleeding time (B.T.)	3	6254.06**	6590.35**	7860.37**
B.T. x Pigs	33	887.86	533.67	547.08
B.T. x Trt.	6	2826.39*	1725.62**	506.62
B.T. x Litters	6	80.07	694.35*	273.12
B.T. x Litters x Trt.	12	903.48	217.72	264.77
B.T. x (Among fructose pigs/litters)	9	113.19	53.18	113.11

[a]Glucose levels expressed as mg./100 ml.

*Indicates significant difference (P = 0.05 or less).

**Indicates significant difference (P = 0.01 or less).

Table 12. Experiment 6717 - Intestinal loop studies. Analysis of variance within age of fructose changes in blood plasma[a]

Age		Mean squares 3 days	6 days		Mean squares 9 days[b]
Source	d.f.			d.f.	
Total	47	1647.25	650.27	31	186.41
Among pigs	11	3948.44	1547.19	9	413.86
Treatment (Trt.)	2	18431.51**	8050.18**	2	1509.28*
Glucose versus fructose + water	1	12889.81**	5369.22**	1	906.51
Fructose versus water	1	23973.20**	10731.13**	1	2112.05*
Litters	2	1413.62	68.78	1	453.01
Litters x Trt.	4	601.74	39.36	2	76.64
Among fructose pigs/litters	3	445.20	207.91	4	24.96
Bleeding time (B.T.)	3	5264.94**	2060.89**	3	237.68**
B.T. x Pigs	33	551.31	223.06	19	70.57
B.T. x Trt.	6	2602.90**	972.62**	6	190.40**
B.T. x Litters	6	1688.46**	86.99	3	15.72
B.T. x Litters x Trt.	12	81.66	40.56	6	12.16
B.T. x (Amg. fructose pigs/litters)	9	646.94	57.42	4	19.56

[a]Fructose levels expressed as mg./100 ml.
[b]Analysis based on two litters only.
*Indicates significant difference (P = 0.05 or less).
**Indicates significant difference (P = 0.01 or less).

Table 13. Experiment 6719 - Glucose and fructose changes in blood plasma following the administration of carbohydrates by stomach tube

Age (days)	Treatment bleeding time (hours)	Water	Glucose	Fructose	Fructose	Water	Glucose	Fructose	Fructose
		(Glucose mg/100 ml)				(Fructose mg/100 ml)			
3	0	91.4	94.9	85.2	89.0	2.1	2.7	4.4	2.4
	0.5	92.0	181.7	84.0	89.7	3.2	1.5	110.7	87.5
	1	90.1	119.7	81.1	85.9	3.1	1.6	123.0	96.2
	2	89.7	104.3	82.7	88.4	3.2	1.8	94.5	81.8
	4	92.2	98.9	81.0	79.6	3.1	1.8	40.8	37.3
	6	90.1	95.6	79.0	71.9	2.7	2.0	17.7	16.3
6	0	86.2	87.8	74.7	83.9	2.5	3.4	7.9	4.3
	0.5	87.4	152.1	89.2	101.9	2.2	1.7	67.6	47.9
	1	83.2	137.4	84.3	102.8	1.4	3.2	77.0	79.6
	2	86.1	113.9	85.4	96.7	1.7	3.2	55.9	74.4
	4	82.7	99.6	93.0	96.9	1.8	6.8	18.2	23.4
	6	81.6	108.8	99.2	93.6	2.0	4.8	7.6	8.5
9	0	93.1	96.9	96.8	90.1	2.7	1.6	2.2	1.4
	0.5	106.2	163.2	113.1	114.8	3.0	2.2	20.1	25.7
	1	106.4	156.4	110.4	113.2	2.7	1.6	24.2	27.0
	2	114.1	121.1	103.2	126.5	2.3	1.7	13.5	15.6
	4	110.3	108.0	103.1	136.1	2.5	1.3	3.3	4.1
	6	100.0	135.0	106.3	124.6	2.3	1.3	1.7	2.3

Table 14. Experiment 6719 - Stomach tubing studies. Analysis of variance of glucose and fructose changes in blood plasma[a]

		Mean squares	
		Glucose	Fructose
Source	d.f.		
Total	215	654.84	1011.96
Bleeding time (B.T.)	5	2601.57*	7449.45**
Among pigs	35	2215.11*	3253.01*
Litters	8	3466.65*	3068.42
Age	2	9643.58**	10433.26**
Litters/Age	6	1407.67	613.48
Treatment (Trt.)	2	13842.80**	31937.00**
Water versus glucose + fructose	1	4390.36*	21201.98**
Glucose versus fructose	1	23295.24**	42672.09**
Among fructose pigs/litters	9	786.78	266.61
Litters x Trt.	16	939.32**	1439.64**
B.T. x pigs	175	287.16	379.83
B.T. x Litters	40	245.55	284.89**
B.T. x Trt.	10	2089.50**	3816.51**
B.T. x Litters x Trt.	80	183.39	145.64
B.T. x (Fructose/Litters)	45	108.12	116.83

[a]Glucose and fructose levels expressed as mg./100 ml.

*Indicates significant difference (P = 0.05 or less).

**Indicates significant difference (P = 0.01 or less).

Table 15. Experiment 6719 - Stomach Tubing Studies. Analysis of variance within age of glucose changes in blood plasma[a]

Age		Mean squares 3 days	6 days	9 days
Source	d.f.			
Total	71	551.81	532.56	626.94
Among pigs	11	1742.17	1938.53	1633.01
Treatment (Trt.)	2	6499.38*	5354.62*	3155.94
Glucose versus fructose + water	1	12271.81**	10116.09*	5797.05
Fructose versus water	1	726.96	593.14	514.82
Litters	2	15.62	3666.34*	541.06
Litters x Trt.	4	514.09	498.28	2161.34
Among fructose pigs/litters	3	1293.17	429.58	2741.54
Bleeding time (B.T.)	5	1133.53**	838.40**	1355.00**
B.T. x Pigs	55	264.45	233.56	359.74
B.T. x Trt.	10	1185.00**	571.79**	708.00
B.T. x Litters	10	76.89	199.40	343.25
B.T. x Litters x Trt.	20	81.56	144.99	319.36
B.T. x (Among fructose/litters	15	19.66	112.28	192.41

[a]Glucose values expressed as mg./100 ml.
*Indicates significant difference (P = 0.05 or less).
**Indicates significant difference (P = 0.01 or less).

Table 16. Experiment 6719 - Stomach Tubing Studies. Analysis of variance within age of fructose changes in blood plasma[a]

Age		Mean squares 3 days	6 days	9 days
Source	d.f.			
Total	71	1807.77	871.49	91.24
Among pigs	11	5765.28	2478.59	209.65
Treatment (Trt.)	2	29247.06**	11990.56**	851.65*
Glucose versus fructose + water	1	20296.28**	7161.07**	685.16
Fructose versus water	1	38197.84**	16820.04**	1018.13*
Litters	2	925.10	881.90	33.44
Litters x Trt.	4	380.46	280.33	21.63
Among fructose pigs/litters	3	517.31	132.76	149.81
Bleeding time (B.T.)	5	6140.87**	2858.58**	362.75
B.T. x Pigs	55	622.35	369.42	42.88
B.T. x Trt.	10	3092.59**	1544.23**	168.75**
B.T. x Litters	10	57.22	110.08	15.87
B.T. x Litters x Trt.	20	24.79	55.22	8.05
B.T. x (Among fructose/litters)	15	149.02	178.05	23.42

[a]Fructose levels expressed as mg/100 ml.

*Indicates significant difference (P = 0.05 or less).

**Indicates significant difference (P = 0.01 or less).

Table 17. Experiment 6719 - Stomach tubing trials. Urinary excretion of fructose prior to and following the administration of carbohydrates by stomach tube

Age	Treatment	Urinary Fructose excretion (mg./hr.) Before treatment	After treatment
3 days	Glucose	0.57	2.11
	Fructose	0.38	43.63
	Water	0.35	0.67
6 days	Glucose	0.27	0.69
	Fructose	4.44	36.76
	Water	0.57	0.59
9 days	Glucose	0.46	1.56
	Fructose	0.72	15.78
	Water	0.46	1.67

Table 18. Experiment 6720 - Analysis of variance of fructose and glucose in the blood of the new-born pig[a]

Source	d.f.	Mean squares Fructose	Glucose
Totals	287	302.24	340.64
Among pigs	47	101.24	510.24
Litter	4	403.90	576.28
Method 1 versus 2	1	468.23	1156.15
Within methods	3	382.46	382.98
Among pigs/litters	43	73.08	504.09
Bleeding time (B.T.)			
0-6-12 hours	2	19382.62**	6223.85**
Linear	1	36138.32**	9564.03*
Quadratic	1	2626.91**	2874.08
Bleeding time			
0-12-24-36-48 hours	4	17141.52**	2510.82
Linear	1	39830.74**	4.48
Quadratic	1	22637.41**	4130.29
Remainder	2	6097.95**	5908.51
Bleeding time x pigs	235	53.51	251.87
B.T. x litter	20	287.24	912.29
B.T. x pigs/litter	215	31.76	190.44

*Indicates significant difference (P = 0.05 or less).
**Indicates significant difference (P = 0.01 or less).
[a]Glucose and fructose levels expressed as mg./100 ml. plasma.

Table 19. Experiment 6720 - Analysis of variance of urinary fructose excretion of piglets from birth to 48 hours of age[a]

Source	d.f.	Mean squares Fructose
Total	191	3021.17
Among pigs	47	1733.89*
Litters	4	10981.68
Among pigs/litters	43	873.63
Collections	3	105715.25**
Linear	1	259584.98**
Quadratic	1	57560.60*
Cubic	1	0.17
Collections x pigs	141	1265.29
Collections x litters	12	6325.26
Collections x pigs/litter	129	794.59

*Indicates significant difference (P = 0.05 or less).
**Indicates significant difference (P = 0.01 or less).
[a]Fructose levels expressed as mg./100 ml. urine.

Table 20. Experiment 6720 - Effect of yeast fermentation on the fructose levels of urine samples of pigs from birth to 12 hours

Sample	Fructose mg./100 ml. Unfermented	Yeast fermented
1	36.1	8.1
2	37.2	7.6
3	26.2	8.6
4	19.4	6.7
5	15.8	7.6
6	36.9	11.1
7	80.2	8.6
Average	35.97	8.33

Table 21. Experiment 6721 - Summary of sugar levels in maternal and fetal bloods (mg./100 ml.)

Sow no.	2510		4252		5192		1313		6161		2402	
Fetal age (days)	82		95		96		110		111		112	
Fetal weights (g.)	I 402	II 455	I 485	II 800	I 1050	II 712	I 1222	II 556	I 908	II 934	I 1063	II 1478
Uterine artery												
Total reducing substances	73.0		59.0		63.0		48.0		70.0		50.0	
Fructose	1.8		3.3		3.0		1.0		2.0		0.5	
Glucose	71.2		55.7		60.0		47.0		68.0		49.5	
Uterine vein												
Total reducing substances	70.0		50.0		56.0		46.0		65.0		50.0	
Fructose	2.0		1.3		2.0		1.0		2.0		0.5	
Glucose	68.0		48.7		54.0		45.0		63.0		49.5	
Umbilical artery												
Total reducing substances	240	234	142	160	134	126	162	108	116	124	106	110
Fructose	186	188	101	120	95	92	120	90	76	81	72	80
Glucose	54	46	41	40	39	34	42	18	40	43	34	30
Umbilical vein												
Total reducing substances	225			161	138	135	180	114	120	124	118	115
Fructose	176			115	95	92	123	87	73	78	77	82
Glucose	49			46	43	43	57	27	47	46	41	33
Amniotic fluid												
Total reducing substances		240	146			120	174	120	108	128	138	158
Fructose		201	105			93	140	104	76	88	96	116
Glucose		49	41			27	34	16	32	40	42	42
Allantoic fluid												
Total reducing substances	510		122	224	192	186	666		326		140	
Fructose	417		97	202	105	111	278		93		101	
Glucose	93		25	22	87	75	388		233		39	

Table 22. Experiment 6721 - Fructose and glucose contents of umbilical arteries (U.A.) and umbilical veins (U.V.)

Fructose mg./100 ml.			Glucose mg./100 ml.		
U.V.	U.A.	V-A	U.V.	U.A.	V-A
176	186	-10	49	54	-5
115	120	- 5	46	40	6
95	95	0	43	39	4
92	92	0	43	34	9
77	72	5	41	34	7
82	80	2	33	30	3
123	120	3	57	42	15
87	90	-3	27	18	9
73	76	-3	47	40	7
78	81	-3	46	43	3
Average					
99.8	101.2		43.2	37.4	

Table 23. Experiment 6721 - Fructokinase activity of various tissues of the fetal and neonatal pig

Tissue	Units per gram of tissue[a]									Adult rat
	Fetal age (days)						Neonatal age (days)			
	82	95	96	110	111	112	3	6	9	
Liver	1.50	1.42	1.40	1.11	1.13	1.15	2.37	1.98	2.81	6
Placenta	0.22	0.37	0.37	0.37	0.25	0.22				
Intestine	0.58	0.57	1.14	1.14	0.67	0.93	1.64	1.64	1.33	

[a]One unit of fructokinase activity corresponds to the oxidation of 1 μ mole of NADH per minute. Each value represents the mean of two animals.

APPENDIX II

Simultaneous Determination of Glucose and Fructose in Serum, Plasma or Urine Using a Technicon Autoanalyzer[a]

Principle: The sample is dialized against water and the recipient stream split to allow determination of both sugars. "Glucose" or total reducing substances is determined by ferricyanide reaction while fructose is measured by the ferric chloride-resorcinol reaction. True glucose content is obtained by subtracting fructose from the total reducing sugars.

Reagents:

Saline: Dissolve 9 grams of sodium chloride (NaCl) in water and dilute to 1,000 ml. Add 0.5 ml. of Brij-35.

Water + Brij-35: Add 0.5 ml. of Brij-35 to 1,000 ml. of deionized water.

Alkaline potassium ferricyanide: Dissolve 9.0 grams of sodium chloride (NaCl) in approximately 500 ml. of deionized water. Add 0.25 grams of finely powdered potassium ferricyanide ($K_3Fe(CN)_6$) and shake until dissolved. Dissolve 20 grams of sodium carbonate in a portion of deionized water and add it to the flask. Dilute to 1,000 ml. with deionized water. Add 0.5 ml. of Brig-35 and mix.

Ferric chloride resorcinol: Dissolve 1.5 grams of resorcinol in 95 percent ethyl alcohol and dilute to 1,000 ml. with ethyl alcohol. Dissolve 7.5 grams of ferric chloride ($FeCl_3 \cdot 6H_2O$) in concentrated hydrochloric acid and dilute to 1,000 ml. with hydrochloric acid. Mix equal parts of these two solutions and use immediately.

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[a] This procedure was devised by Dr. R. C. Ewan of the Department of Animal Science at Iowa State University.

Glucose stock standard: Dissolve 1.0 gram of D-Glucose in deinoized water saturated with benzoic acid and dilute to 100 ml. with saturated benzoic acid.

Fructose stock standard: Dissolve 1.0 gram of fructose in deionized water saturated with benzoic acid and dilute to 100 ml. with saturated benzoic acid.

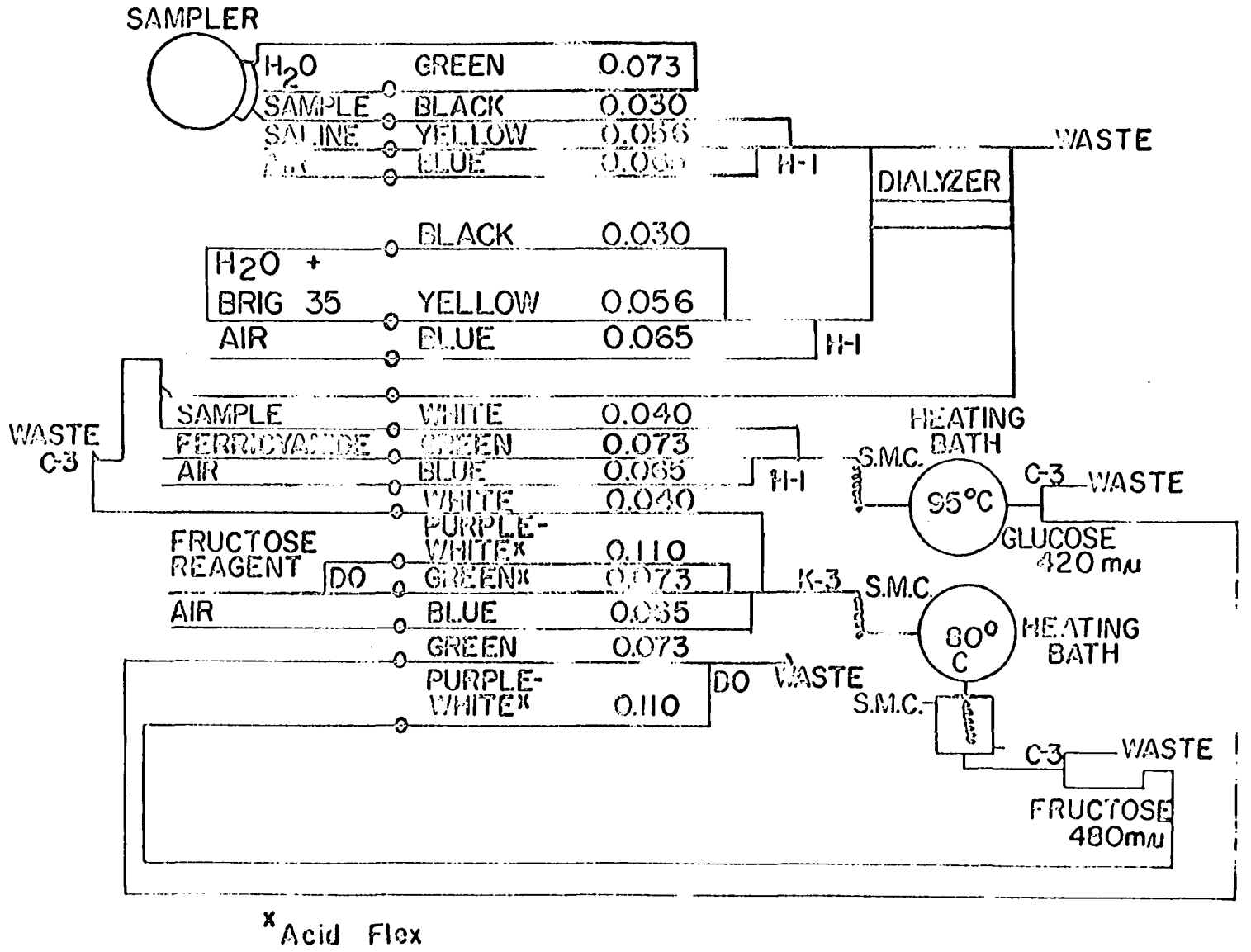
Working standards: Dilute the following to 100 ml. with deionized water saturated with benzoic acid.

Std.	Ml. of Glucose Stock	Ml. of Fructose Stock	Mg. Glucose/ 100 ml.	Mg. Fructose/ 100 ml.	Total/100 ml.
1	4	2	40	20	60
2	6	4	60	40	100
3	8	6	80	60	140
4	10	8	100	80	180
5	12	10	120	100	220
6	14	12	140	120	260

Procedure:

1. Fill the sample cups with at least 0.5 ml. of serum or plasma. Run a sequence of standards followed by the samples.
2. The determination is run at 40 per hour with a sample wash ratio of 1/2.
3. Stepwise procedure for running the analysis.
    a. Start the proportioning pumps with all reagent lines in deionized water.
    b. Turn on both colorimeters and the instrument switch of the recorder.
    c. After 20 minutes, turn on the recorder chart drive and adjust the glucose base line to 98 percent transmittance.
    d. Transfer the reagent lines to the reagent bottles.

Figure 9. Flow diagram for automated glucose and fructose determination.


SAMPLER
H_2O
GREEN 0.073
SAMPLE
BLACK 0.030
SALINE
YELLOW 0.056
BLUE 0.065
H-1
DIALYZER
WASTE
BLACK 0.030
H_2O +
BRIG 35
YELLOW 0.056
AIR
BLUE 0.065
H-1
WASTE C-3
SAMPLE
WHITE 0.040
FERRICYANIDE
GREEN 0.073
AIR
BLUE 0.065
WHITE 0.040
H-1
HEATING BATH
S.M.C.
95°C
C-3 WASTE
GLUCOSE 420 mμ
FRUCTOSE REAGENT
DO
PURPLE-WHITE* 0.110
GREEN* 0.073
AIR
BLUE 0.065
GREEN 0.073
PURPLE-WHITE* 0.110
K-3
S.M.C.
80° C
HEATING BATH
DO
WASTE
S.M.C.
C-3 WASTE
FRUCTOSE 480mμ
*Acid Flex

e. After the reagents reach the colorimeters, adjust the fructose base line to 100 percent transmittance.

f. The glucose pen should read between 0.8 and 0.9 O.D units. If it does not adjust the base line by altering the composition of the alkaline ferricyanide reagent as follows:

   1. To raise the base line add 5 percent potassium ferricyanide in saline. (0.5 ml. is equivalent to approximately 0.06 O.D. units)
   2. To lower the base line dilute the alkaline ferricyanide with 2 percent sodium carbonate in saline.

g. Start the sampler

h. After the samples have been recorded, transfer the reagent line to deionized water.

i. Turn off the coloimeters, both recorder switches and the sampler.

j. After deionized water has been pumped through the system for 15 minutes, the proportioning pumps are stopped and the pump tubes relaxed.