

These data support the findings of earlier investigators(6) that the absorption rate increases with increasing luminal concentration of sugars, as contrasted to the view of others (7,8) that absorption rate is not related to concentration.

Summary. Studies were conducted to determine the alimentary absorption of L-arabinose and D-xylose in chicks during 30 minutes following dose by stomach-tube. The rates of absorption of L-arabinose and D-xylose were compared to that of D-glucose from 3-12 days of age. Absorption velocity of L-arabinose was lower than that of D-glucose and D-xylose. This age range did not influence the absorption rate of any of the test sugars. Increase in dose concentration correspondingly increased rate of absorption. The data suggest that at lower dose concentration D-glucose is absorbed more rapidly than D-xylose, but at higher concentration

absorption rates of the two sugars are reversed. It is confirmed that the chick's gut possesses selective absorption capacity as early as 3 days of age.

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Inorganic Ions in Cecal Content of Gnotobiotic Rats.* (31756)

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The enlargement of the cecum and its retention of a large quantity of fluid in germfree rodents have been noted since the advent of germfree rearing technique(1). However, little work has been done in clarifying the etiology of this anomaly. It was pointed out that a diet rich in starch gave rise to further enlargement of the cecum in germfree rats, and cesarian-born, hand-fed animals tended to develop still larger ceca(2). Skelly *et al* observed that contamination of germfree mice with intestinal flora obtained from conventional counterparts induced a rapid shrinkage of the cecal size and they noted that *Clostridium difficile* was most potent in this respect (3). Gustafsson and his associates observed a high concentration of mucus in the cecum of germfree rats and by bacterial contamination they were able to show an increase in

fecal excretion of mucus concomitant with the decrease of the cecal size(4). Recently Csaky proposed a hypothesis that a bioactive substance was involved in the cecal enlargement (5).

Inasmuch as inorganic ions, especially sodium and chloride, play a profound role in the intestinal absorption(6), it seems of importance to determine the concentrations of inorganic ions in the ileal and cecal contents together with other indices for the coligative property. References are made to the germfree and monocontaminated conditions.

Method. Animals. Experimental animals were germfree Lobund Wistar strain rats propagated in germfree condition for 20 generations at Lobund Laboratory. Male rats weighing between 300 g and 400 g were used. They were fed steam sterilized L-462 diet and water *ad libitum* in steel isolators with steam sterilized sawdust bedding. For the experiment pertaining to germfree condition,

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rats were sacrificed immediately after being brought out of the isolator. For the monocontamination experiment germfree rats were transferred to a plastic film isolator and maintained with the same diet before and during monocontamination.

Monocontamination. A strain of *Clostridium difficile* (ATCC #9689) was grown in fluid thioglycollate medium for 18 hours at 37°C. Ampules containing about 10 ml of bacterial suspension were introduced into the film isolator through the lock after peracetic acid sterilization of the ampule(7). The suspension was introduced into the mouth of the rat with a cotton swab. Also the suspension was added to drinking water. After 24 hours the rat was sacrificed. Before and after the 24-hour monocontamination period routine bacteriological examination was performed to ascertain the germfree and monocontaminated conditions(8).

Chemical analysis. Rats were lightly anesthetized with ether. A thin polyethylene tube was inserted into the common carotid artery and blood was slowly withdrawn into a syringe wet with heparin solution (Panheparin, Abbott, 5000 USP units/ml). The withdrawal of blood took place through the following experimental procedure and 5 to 8 ml of blood was collected. Plasma obtained from the blood was used only for osmolarity and conductivity determination.

The cecum was isolated *in toto* after ligation of ileum and colon immediately adjacent to the cecum. A clamp was applied to severed end of blood vessels in the mesentery to prevent blood-letting. The total weight of the cecum was obtained. The cecal content was squeezed out into a 50 ml centrifuge tube. The cecal wall was immediately weighed and the weight of the cecal content was calculated by subtraction.

The content of the terminal ileum was squeezed out into a pre-weighed centrifuge tube and the weight of the content was immediately determined. Five to 10 ml of distilled water was added and the ileal content was dissolved by vigorous shaking. The blood, cecal and ileal contents were centrifuged at 4°C at 2500 rpm (IEC Model PR-2)

for 20 minutes and the plasma and supernatants were separated.

The wet and dry weights of the cecal wall, cecal and ileal contents were determined and respective water contents were calculated. Drying was done in an oven maintained at 95°C overnight.

Following analyses were performed:

1) Osmolarity: Mechrolab vapor pressure osmometer model 301A was used with 0.1, 0.2, 0.3 and 0.4 M urea solutions as the standard.

2) Electric conductivity: After proper dilution the conductivity of the sample was determined with a conductometric cell with 1,000 cps using a 5 decade precision resistance box and an oscilloscope for the display of Lissajou figure. NaCl solutions at 0.4, 0.6 and 0.8 mM were used as the standard and the conductivity of the sample was expressed as the concentration of NaCl solution with the equal conductivity.

3) Na and K: Concentrations of Na and K were determined with Beckman DU-2 spectrophotometer with flame attachment.

4) Cl: Concentration of Cl was determined with Buchler Cotlove chloridometer.

5) Carbon dioxide: In a separate series of experiments the total carbon dioxide concentration in the cecal content was determined with Van Slyke manometer. Cecae of germfree and monocontaminated rats were isolated and the total cecal weight was obtained. The content was squeezed out into a 50 ml centrifuge tube and paraffin oil was superposed immediately. After centrifugation the supernatant was transferred to another tube without exposure to air and stored under paraffin oil. Thus loss of carbon dioxide to the air was prevented.

The concentrations in the plasma and cecal content were directly measured. With ileal contents the concentration in the water phase in the ileal content was calculated from the concentration in the supernatant, total weight, water content and amount of distilled water added. In this calculation an assumption is made that ions are present in water phase of the ileal content and not sequestered in solid phase.

Results. Table I shows the cecal weight,

TABLE I. Cecal Weight, Weight of Cecal Content and Cecal Wall, and Percentage of Water in Germfree and *C. difficile* Monocontaminated Rats. (Mean \pm S.E.)

Status	No. of rats	Cecal wt (wet), g	Cecal wt as % of body wt	Wt of cecal content, g	% of water in cecal content	Wt of cecal wall (wet), g	% of water in cecal wall
Germfree	12	30.3 \pm 1.1	8.53 \pm .24	26.9 \pm .9	85.5 \pm .2	3.32 \pm .16	72.0 \pm .7
<i>C. difficile</i> contaminated	12	13.5 \pm 1.3	3.97 \pm .39	10.4 \pm 1.3	85.7 \pm .7	3.14 \pm .09	74.7 \pm .9

weight of cecal contents, weight of the cecal wall and water percentage in the cecal content, and the cecal wall in germfree and *C. difficile* monocontaminated rats. It is clearly seen that the enlarged cecum of the germfree rat shrinks very rapidly following *C. difficile* contamination, and this change is accounted for by the decrease in cecal content and not by any change in the cecal wall. The water content in the cecal contents or in the cecal wall is not affected 24 hours after contamination.

Table II shows the osmolarity, electrical conductivity, concentrations of Na, K and Cl in the cecal content. The osmolarity and conductivity of blood plasma determined with the same animals are also shown. It can be pointed out first that plasma does not show any difference in the osmolarity and conductivity between the germfree and contaminated condition. This must indicate that whatever changes take place in the cecum the effect on plasma is well compensated by other adaptive mechanisms. In the germfree cecum the osmolarity is slightly hypertonic with respect to plasma indicating that there is no osmotic equilibrium across the mucosal membrane despite the large quantity of fluid, and that there must be some process counteracting the osmosis of water from the plasma to the lumen. Of further interest is the low electrical conductivity. The ion analysis shows low concentration of Na and extremely low concentration of Cl, although the K concentration is relatively high. The scarcity of inorganic ions accounts for low conductivity and in the face of hypertonicity it must be concluded that the contents of the germfree cecum contain relative abundance of non-electrolytes or ions with low mobility.

With the introduction of *C. difficile* the conductivity and ion concentrations increase

though the osmolarity remains unchanged. This observation immediately excludes a possibility that the reduction of cecal size is brought about by the increase in the muscle tone along the cecal wall which expels the contents into the colon. If this explanation were correct, there should be no change in the concentrations of solutes. Instead it must be assumed that the concentration changes are instrumental in inducing the volume change. The unchanged osmotic pressure is of importance indicating selective absorption of solutes and water in iso-osmotic fashion.

Table III shows the total carbon dioxide concentration in the cecal content under germfree and after 24 hours of contamination as determined in a separate series of experiments. The reduction of the cecal size expressed as percent of body weight is similar to that reported in Table I. The CO₂ concentration in the germfree cecal content is low and comparable to that of Cl. Since the pH of the germfree cecal content is reported to be slightly alkaline(9), the carbonic acid concentration must be very low and the bicarbonate concentration must be very close to the total CO₂ concentration. There is no report on the pH of *C. difficile* monocontaminated cecal contents. But the increase in total carbon dioxide concentration is so large that even if there is uncertainty in CO₂, the bicarbonate ion concentration must have increased similarly to the Cl concentration increase.

Table IV shows the total amount of solutes in the cecal content. In calculating the total amount, it is assumed that the solute is present in the water phase at the equal concentration as that determined in the supernatant of the cecal content. The amount of water is calculated from the water percentage and the weight of the content. Because the con-

centration in water and that in the supernatant are not strictly identical, there is a slight error in calculation. However, the change observed is far greater than the error and it is clearly seen that the amounts of Na and K decrease despite the increased concentration, while that of Cl increases.

Table V shows results obtained with the terminal ileum. Since the analysis involves

TABLE II. Concentrations in Cecal Content in Germfree and *C. difficile* Monocontaminated Rats. (Mean \pm S.E.) Osmolarity and conductivity of plasma are also shown.

Status	No. of rats	Osmolarity, mOsmol/l	Conductivity, mM NaCl	Na, mM	K, mM	Cl, mM	Plasma osmolarity, mOsmol/l	Plasma conductivity, mM NaCl
Germfree	12	318 \pm 3.6	84.6 \pm 1.7	49.0 \pm 2.0	11.59 \pm .45	1.66 \pm .29	292 \pm 1.4	144.2 \pm 2.2
<i>C. difficile</i> contaminated	12	321 \pm 4.3	114.4 \pm 2.5	87.8 \pm 6.2	13.50 \pm .43	13.78 \pm 2.52	292 \pm 1.3	141.4 \pm 1.3

TABLE III. Carbon Dioxide Concentration in Cecal Content. (Mean \pm S.E.)

Status	No. of rats	Cecal wt as % of body wt	CO ₂ concentration, mM
Germfree	8	8.57 \pm .27	4.17 \pm .13
<i>C. difficile</i> contaminated	12	4.93 \pm .62	18.98 \pm 3.01

dilution with distilled water and calculation under an assumption, the accuracy of determination is lower than that with the cecal content. However, the distinction from the cecal content is quite obvious: lower water percentage, much higher osmolarity, conductivity and Na concentration. In germfree animal the difference in water percentage and osmolarity between the ileal and cecal contents indicates transport of water from the blood into the cecal lumen presumably through the cecal mucosal membrane(10). The cause of this transport is unclear but it is of interest to note the distinct difference in the content and functional state of the mucous membrane between the small intestine and cecum. Of further interest is the consistency of solute concentrations in the ileal content with or without bacterial contamination. This indicates very little, if any, influence on small intestinal absorption by intestinal flora. It must be concluded that any change observed in cecal content must be accounted for by the functional change in the cecum itself.

Discussion. The most salient characteristic of the germfree cecal content is the relative scarcity of electrolytes, especially an extreme paucity of Cl ion. This situation resembles very closely the terminal stage of the "chloride impoverishment" experiment by Visscher and co-workers(11), in which they placed an isotonic solution containing NaCl and Na₂SO₄ at equal concentration in the intestinal loop of a dog. They found that the Cl concentration fell to an extremely low value. Further they observed that the change in Cl concentration observed during an experiment parallels the change in volume of intestinal solution. That is to say that with a very low concentration of Cl in the lumen, no volume change or absorption of water occurs.

The mechanism of active transport of ions

TABLE IV. Total Amount of Ions in Cecal Content. (Mean \pm S.E.)

Status	No. of rats	Na, m mols	K, m mols	Cl, m mols
Germfree	12	1.148 \pm .062	.265 \pm .011	.039 \pm .008
<i>C. difficile</i> contaminated	12	.717 \pm .050	.115 \pm .014	.102 \pm .018

TABLE V. Water Content and Concentrations in Contents of Terminal Ileum in Germfree and *C. difficile* Monocontaminated Rats. (Mean \pm S.E.)

Status	No. of rats	% of water	Osmolarity, mOsmol/l	Conductivity, mM NaCl	Na, mM	K, mM	Cl, mM
Germfree	11	74.6 \pm .8	423 \pm 18	146 \pm 4.3	132 \pm 6.4	9.20 \pm .45	5.20 \pm 1.27
<i>C. difficile</i> contaminated	12	75.8 \pm .4	446 \pm 8	153 \pm 1.7	124 \pm 5.2	9.86 \pm .42	4.12 \pm .40

and water in the intestine is still controversial in that the question of whether Na or Cl is actively transported primarily is not settled(12). But from either point of view, the very low concentration of Cl observed in the germfree cecal content is enough to explain very low water absorption and hence a very large cecum.

The reduction of cecal size and the increase in ion concentration provoked by *C. difficile* contamination are in concordance with this view. The higher ionic concentrations are definitely a favorable factor for water absorption. Furthermore there is a definite relationship between the cecal size and the ionic concentrations in monocontaminated condition as shown in Fig. 1. In this Figure individual results are depicted so that the conductivity, concentrations of Na, Cl and carbon dioxide in the cecal contents are plotted against the cecal size expressed in percent body weight. The results with germfree rats are shown with closed circles and those with contaminated rats are shown with open circles. Despite the large scatter of points it may be pointed out that the points representing the contaminated condition seem to cluster around a straight line and those representing germfree condition do not seem to belong to this grouping. With results for contaminated condition, a regression equation and a correlation coefficient are calculated and shown in the Figure. Even with this correlation between the cecal size and ion concentrations it is impossible to deduce a causal relationship starting from bacterial in-

fection leading to cecal shrinkage through intervention of ion concentration change. The fact that only Cl showed an increase in the total amount may suggest that availability of this ion plays a key role in the production of enlarged cecum in germfree rats.

It has been reported that the germfree cecum contains a high concentration of mucin or "hexosamine containing substance"(4). Since mucin is an acidic polyelectrolyte, the anion deficit observed in the germfree cecal content may be explained with the high concentration of this substance. Since its molecular weight is very high, it is natural to expect that the abundance of mucin provokes the depletion of Cl and retention of water. Further it was reported that by bacterial infection the fecal output of hexosamine increases greatly thus accounting for the relative increase of concentrations of Cl and bicarbonate ion.

The germfree intestine does not contain enzymes which degrade mucin(4). Therefore, the accumulation of mucin may be attributed to a lack of destruction. However, *C. difficile* was shown not to attack mucin in the germfree cecal content *in vitro*(4). The fact that this microorganism can induce cecal shrinkage in spite of this enzymatic inability suggests a still unobserved phenomenon which links the activity of *C. difficile* to changes in ion concentration and volume of the cecal content.

Summary. The cecal content of germfree rats was analyzed for osmolarity, conductivity and concentrations of Na, K, Cl and

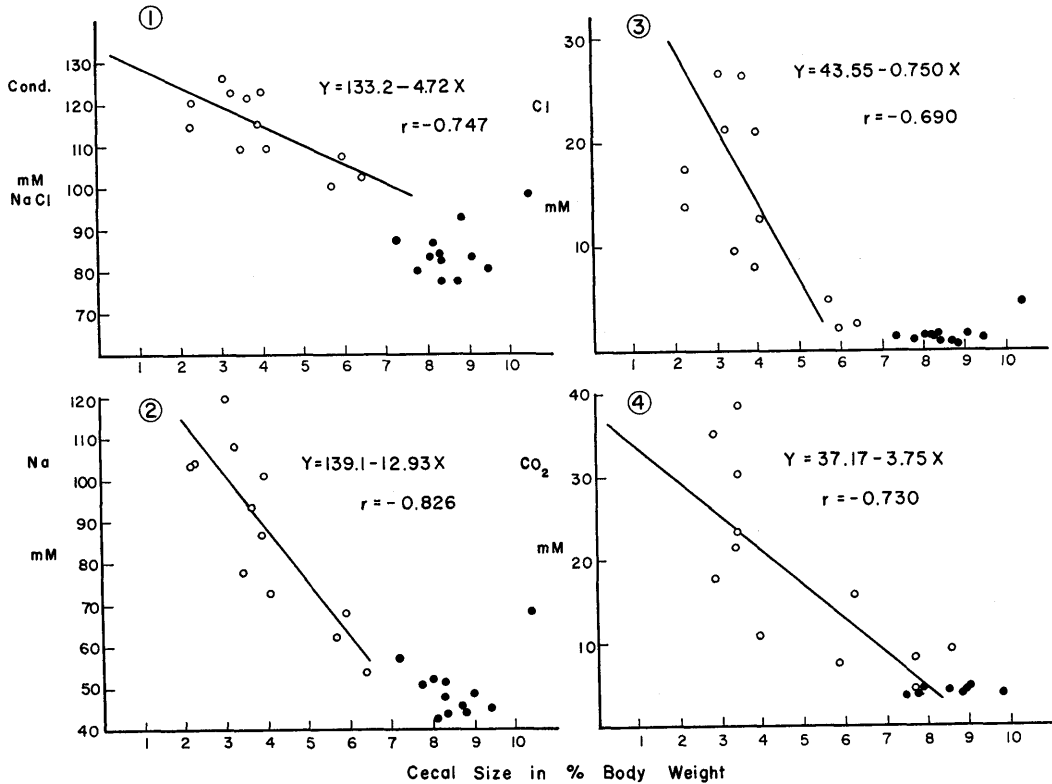


FIG. 1. Relationship between cecal size and the conductivity, concentrations of Na, Cl and carbon dioxide in the cecal content under germfree and *C. difficile* monocontaminated conditions. Abscissa indicates cecal size expressed in per cent body weight. Ordinate indicates (1) conductivity in mM NaCl, (2) concentration of Na in mM, (3) concentration of Cl in mM and (4) concentration of carbon dioxide in mM. Closed circles indicate results with germfree cecum and open circles indicate results with monocontaminated cecum. Regression equation and correlation coefficient are shown.

carbon dioxide with the following results: referred to blood plasma, the osmolarity was slightly hypertonic, the conductivity was low and the concentrations of Na, Cl and carbon dioxide were low. The K concentration was about twice the plasma concentration. Monocontamination with *Clostridium difficile* for 24 hours induced a reduction of the cecal size and changes in the composition of the cecal content. The conductivity, concentrations of Na, Cl and carbon dioxide increased proportional to the cecal size reduction. The osmolarity and K concentration remained unchanged. The content of terminal ileum of germfree rats showed a distinctly different ionic composition from that of the cecum and the concentrations were not affected by monocontamination.

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Activity of Repository Sulfones Against *Mycobacterium leprae* In Mice.* (31757)

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It was reported(1) recently that the growth of *Mycobacterium leprae* is suppressed in mice by very small dosages of 4,4'-diaminodiphenylsulfone (DDS). This drug, the most effective one in the treatment of leprosy and against which drug-resistance is very rare(2), has ordinarily been given in doses of 50 to 100 mg a day. Such intake produced 1-3 $\mu\text{g}/\text{ml}$ blood and tissue(3) (with daily intake the drug level is relatively constant throughout the 24 hours). The result in mice indicated that much lower blood levels might be effective, and raised the possibility of innovation in the drug regimen.

One such possibility lies in repository forms. The drug 4,4'-diacetyldiaminodiphenylsulfone (DADDS) has recently been studied as a repository injection against malaria parasites(4), and a number of other repository sulfones have been synthesized for the same purpose(5). All these repository forms have one or both of the amino groups combined with other radicals. They are all poorly soluble in aqueous solution, and in the vehicle in which they are suspended. Antibacterial activity apparently depends upon cleavage by tissue enzymes to release DDS, or other possibly active compounds with only one of the amino groups free.

Materials and methods. The general pro-

cedure was to infect mice with a few thousand *M. leprae* and then to follow the growth curve in untreated mice until the bacterial population passed 1×10^6 . This event signaled the onset of the plateau phase(6), so counts were then made from each treated and control group. Counts were repeated 3 months later. The methods for working with *M. leprae* in mice are described elsewhere(1,7). All counts were made on pools of the foot pad tissue of 4 mice, except for a few instances in the second harvest in Table I (at ca. 300 days) where only 2 mice remained.

The injected drugs (Tables I and II) were suspended in 40% benzyl benzoate and 60% castor oil. DADDS was supplied as a sterile suspension; the other compounds were heated to 150°C for 1¼ hours just before use and the sterile vehicle added. In the experiments listed in Table III the drugs were mixed in the powdered diet as described(1).

The mice were of the CFW strain of the Communicable Disease Center. They were fed commercial chow in the experiments of Tables I and II. The same diet, but unpelleted, was used for the experiments of Table III.

Results. Activity of 7 repository sulfones and DDS: In this experiment (Table I) all of the repository sulfones suppressed growth completely even when given every 2 months. DDS was completely effective when given twice a month, but allowed partial growth when given once a month, and nearly complete growth when given every 2 months. In other words DDS had a duration of activity

* The drugs were suggested by Dr. Paul E. Thompson on the basis of their repository activity against *Plasmodium berghei* in mice and were supplied by Dr. E. F. Elslager and Mr. D. F. Worth, all of the Research Division of Parke, Davis and Co., Ann Arbor, Mich.