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The Effects of D-Penicillamine on Taste Preference and Volume Intake of Sodium Chloride by the Rat (33925)

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(Introduced by R. W. Berliner)

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Studies in man have demonstrated that D-penicillamine produces hypogeusia, a decrease in taste acuity, in 20–40% of the patients treated with this drug (1, 2). This decrease in taste acuity affected each of the four qualities of taste but was most noticeable subjectively for salt and sweet stimuli. The present experiments were carried out to determine the effect of D-penicillamine on preference behavior and fluid intake for salt solutions in the rat.

Methods. The studies were carried out in two parts, an initial experiment (A) and a final experiment (B). (A) In the initial experiment 8 weanling Holtzman male rats, 21 days of age, were individually caged. Four rats served as controls and were fed a diet of ground Purina chow diluted with 15% added dextrose to increase palatability. This diet contained adequate calories, vitamins and minerals necessary for normal rat growth. Four rats served as experimental animals and received the control diet to which D-penicillamine[2] was added at a rate of 1 g/100 g of mixed feed. Intake of food and distilled water, both of which were supplied *ad libitum*, was recorded daily and animal weights were recorded every third day. Daily weights were interpolated from these values by assuming a linear relationship of the weight change over each 3-day period. Nineteen days after the diets were begun, each rat was offered a choice between 0.15 *M* sodium chloride (NaCl), reagent grade, and water under standard conditions for preference testing, in a two-bottle-choice experiment (3). With these techniques evaporation and waste were negligible. For the purpose of the studies this day was considered day 0. Thirty-one days after the diets were begun (day 12 of the study), the experimental rats were given the control diet without added D-penicillamine and this diet was continued until the termination of Expt. A. Intake of NaCl solution was recorded daily and expressed as percentage preference; *i.e.*, (ml of NaCl solution

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[2] D-penicillamine (Cuprimine) was kindly supplied as the free base by Merck, Sharpe and Dohme from Dr. Elmer Alpert.

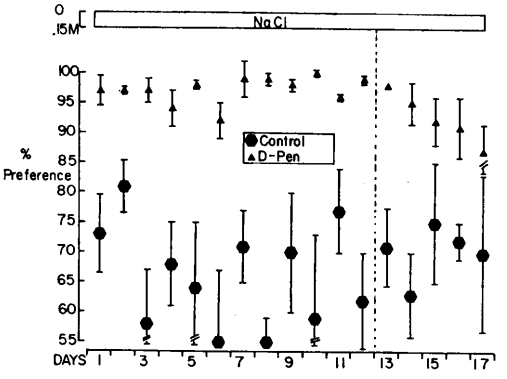

FIG. 1. Preference for 0.15 *M* NaCl in Control and D-penicillamine (D-Pen) treated rats in Expt. A. Each point represents the mean preference of four rats for each day; the line extending above and below the point the standard error of the mean (SEM). The percentage preference for 0.15 NaCl among the D-Pen-treated rats is significantly higher than among the controls. There is no overlap between the two groups of animals. The broken line at day 13 denotes that dietary D-Pen was stopped at this point and the control diet alone continued. There was then a gradual decrease of taste preference among the D-Pen-treated rats toward normal while a choice between NaCl and water was still offered.

consumed/ml of total fluid consumed) × 100. Volume intake was recorded daily as the total volume of fluid imbibed, both NaCl and water. This was expressed as the ratio of the total daily intake of fluid to the daily body weight of the animals. While the authors recognize that fluid intake increases slightly per gram of body mass in the smaller animal, the substantial changes in fluid intake encountered rendered these corrections of little consequence.

(B) In the final experiment, 16 weanling Holtzman male rats were individually caged. Eight were given the control diet while 8 received D-penicillamine as in (A). Feed, fluid intake, and body weight were measured as in (A). Twenty-three days after the diets were begun (day 0 of the study) each rat was offered a choice between 0.15 *M* NaCl and water for 6 days. Twenty-nine days after the diets were begun (day 6 of the study) they were offered a choice between 0.30 *M* NaCl and water for 7 days. At the completion of this presentation each animal received only water with continuation of his diet for 4 additional days. Data for preference and for fluid intake was recorded as indicated in (A).

Results. The preference responses of Expts. A and B are shown in Figs. 1 and 2. In A the mean daily preference of the control rats for 0.15 *M* NaCl varied between 55 ± 3 and 81 ± 4% (Fig. 1); in B the mean daily preference of the control rats for 0.15 *M* NaCl was essentially the same, varying between 48 ± 5 and 81 ± 6% (Fig. 2). The mean daily preference for 0.15 *M* NaCl among the D-penicillamine-treated rats was

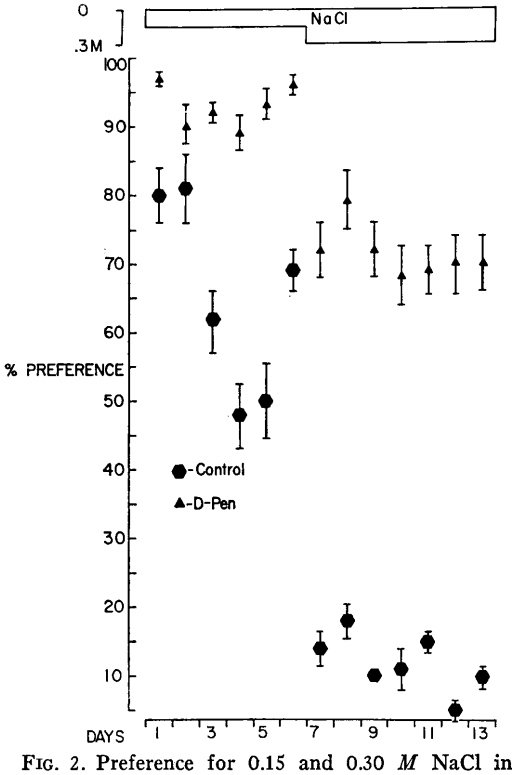

FIG. 2. Preference for 0.15 and 0.30 *M* NaCl in control and D-Pen-treated rats in Expt. B. Each point represents the mean preference of four rats for each day, the line extending above and below the point, the SEM. The SEM for the mean intake of the control rats on the ninth day of the experiment was too small to extend outside the width of the legend symbol. The percentage preference for both 0.15 and 0.30 *M* NaCl among the D-Pen-treated rats is significantly higher than among the controls. The controls reject 0.30 *M* NaCl and prefer water. There is no overlap between the two groups of animals.

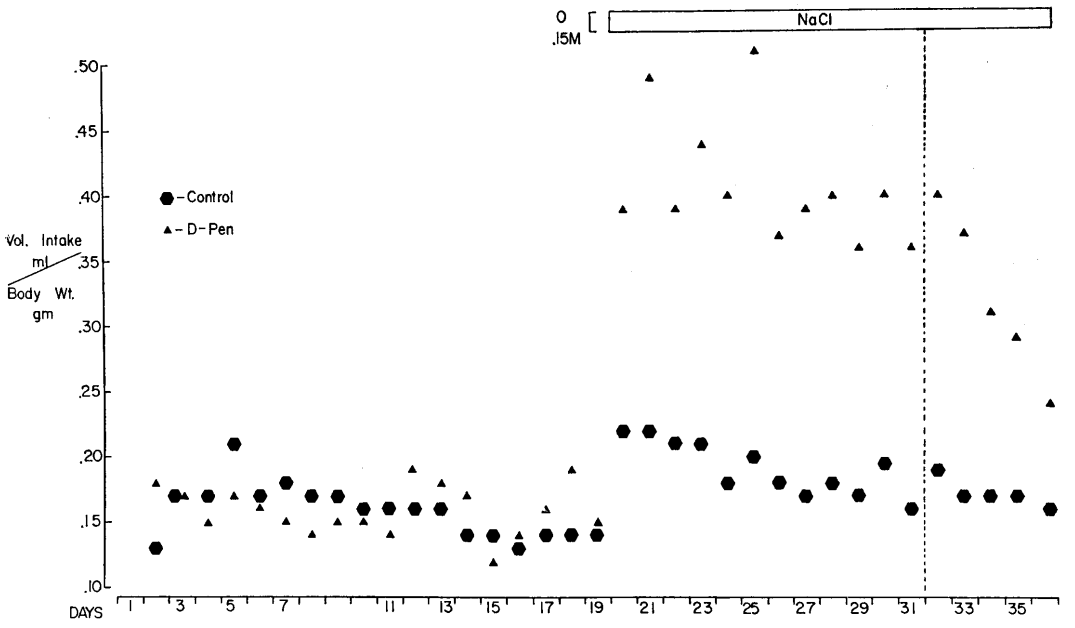

FIG. 3. Total fluid intake of 0.15 *M* NaCl and water in control and D-Pen-treated rats in Expt. A. Fluid intake is expressed as total volume intake (ml/g of animal body weight). The symbols used are the same as those in Fig. 1 and represent the mean of the daily fluid intake of eight rats. No SEM are evident since they were less than 0.01 ml/g of body weight for each measurement. The intake of water for the first 19 days of the experiment for each group was variable but essentially the same; from day 16 on the intake of the D-Pen-treated rats was slightly greater than the controls. From day 20 on, after offering a choice between 0.15 *M* NaCl and water, this difference increased such that total fluid intake of the D-Pen-treated rats was 2.5 times that of the controls. This represented an increase from a mean of 0.15 ml/g of body weight to 0.40 ml/g of body weight after the introduction of the NaCl. The broken line at day 32 denotes that D-Pen was stopped at this point and the control diet alone was continued. This was followed by a gradual decrease in total fluid intake among the D-Pen-treated rats while a choice between NaCl and water was still offered.

significantly greater than that of the control rats. In A, preference among the D-penicillamine-treated rats varied between 93 ± 3 and 99 ± 1% (Fig. 1) while in B it varied between 89 ± 3 and 96 ± 2% (Fig. 2). There was no overlap in mean preference for NaCl between treated and control rats for any day in which data were collected. Discontinuation of the D-penicillamine on day 13 of Expt. A along with the intake of the control diet produced a gradual return of mean taste preference toward normal within 5 days. In B the mean daily preference of the control rats for 0.30 *M* NaCl varied between 1 ± 1 and 17 ± 3% while the preference of the D-penicillamine-treated rats was significantly higher, varying between 67 ± 4 and 78 ± 4% (Fig. 2). Again there was no overlap in the mean daily preference between the D-penicillamine-treated and the control rats for any day during which data were collected.

The results of the volume intake in A and B are shown in Figs. 3 and 4. In A and in B the mean daily volume intake of water prior to the offering of a choice of fluids to the control rats was essentially the same, 0.15 and 0.13 ml/g of body weight for A and B, respectively. With the offering of a choice between 0.15 *M* NaCl and water the total fluid intake of the control rats increased significantly to a level between 0.20 and 0.16 ml/g of body weight in A and B, respectively. The standard error of the means was less than 0.01 ml/g of body weight for each of these measurements. This increase in fluid

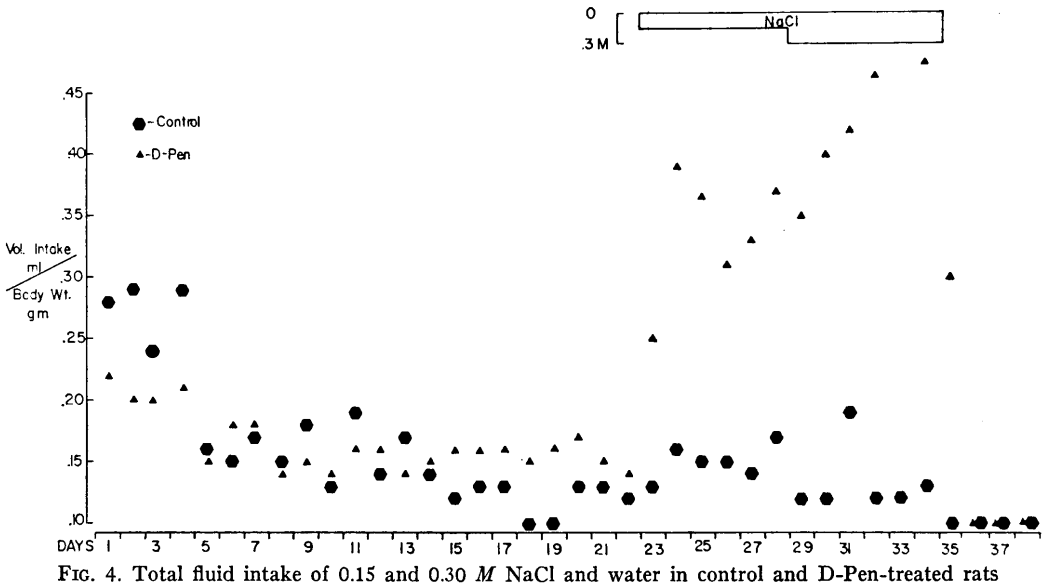

FIG. 4. Total fluid intake of 0.15 and 0.30 *M* NaCl and water in control and D-Pen-treated rats in Expt. B. Each symbol is the same as in Fig. 3. The results are essentially the same for similar parts of the experiment. However, after the offering of a choice between 0.30 *M* NaCl and water the control rats exhibited a slight decrease in fluid intake compared to their intake with 0.15 *M* NaCl, while the D-Pen-treated rats exhibited a further increase in fluid intake such that the difference between the two groups was almost 4-fold. After discontinuation of the choice there was a rapid decrease in total fluid intake among both groups of rats. The symbols for the mean fluid intake of the D-Pen-treated rats are left of the symbols for the mean intake of the control rats during this period of the study, days 37–39. Among the control rats this decrease reached the lowest intake recorded in the experiment, as if a "rebound" had occurred. This decrease was precipitous among the D-Pen-treated rats and total fluid intake returned to the level of intake of the controls within 24 hr.

intake began as soon as the NaCl was offered and persisted as long as the choice between 0.15 *M* NaCl and water was available. The mean daily volume intake of water for the D-penicillamine-treated rats prior to the offering of a choice between NaCl and water was similar to that encountered in the control rats (Figs. 3 and 4). Upon presentation of a choice between 0.15 *M* NaCl and water there was an immediate and significant increase in the mean daily volume intake of both NaCl and water in the D-penicillamine-treated rats compared with their intake of water alone prior to choice and compared with the intake of the control rats after a choice was offered. The intake of the D-penicillamine-treated rats had a median value of 0.40 ml/g of body weight which is more than 2.5 times the intake of the control animals. Since the treated rats were approximately half the weight of the controls this could influence the volume intake measurements when calculated with respect to body weight. However, the D-penicillamine-treated rats were significantly smaller than the controls from day 3 on until the end of the experiments yet significant differences in fluid intake were evident mainly after the introduction of the taste stimulant. In addition, this difference in fluid intake is significant even if intake is not expressed in terms of body weight. In B the mean daily volume intake for the control rats varied about 0.12 ml/g of body weight after offering them a choice between 0.30 *M* NaCl and water. This intake is significantly lower than that after 0.15 *M* NaCl was offered but is not significantly different from that recorded when only water alone was offered. The mean daily volume intake of the D-penicillamine-treated rats offered 0.30 *M* NaCl was not only significantly higher than that of the control rats but was significantly higher than

their fluid intake when 0.15 *M* NaCl was offered. The mean intake of the treated rats was about 0.44 ml/g of body weight which approaches 4 times the comparable intake of the control rats. This increase in volume intake occurred despite the fact that the D-penicillamine-treated rats weighed approximately one half as much as the control rats. In some rats total daily fluid intake comprised a volume equal to their own weight.

Upon discontinuation of the D-penicillamine from the diet of the treated rats there was a fall in their daily total volume intake even though the choice between NaCl and water persisted (Fig. 3). This fall was gradual and after 5 days of control diet fluid intake was still significantly above control values but significantly below values obtained during intake of D-penicillamine. Discontinuation of the choice between NaCl and water with the continuation of the D-penicillamine-treated diet produced a rapid and precipitous fall in the daily total volume intake of the treated rats to control levels within 24 hr after the choice was stopped (Fig. 4).

The total amount of D-penicillamine consumed by the rats to produce these effects was approximately 2 g/kg. Every rat treated with the drug developed an increase both in preference and in volume intake. However, only 20–40% of the patients given D-penicillamine developed hypogeusia. One possible explanation for this difference is that the total dose given to the patients was approximately 0.4 g/kg, or about ⅛ the dose given to the rats.

Discussion. There were two major effects of D-penicillamine on the preference behavior of rats for various fluids. First, after consuming the drug there was a significant increase in preference for NaCl. Second, there was a consistent increase in fluid intake of both water and salt solutions. This polydipsia was magnified by the ratio of weight of animal to fluid imbibed since the D-penicillamine-treated animals generally weighed one half as much as the controls.

Both of these effects occurred in rats given D-penicillamine from the time of weaning. It is not known whether these effects would occur if D-penicillamine were administered to adult rats although hypogeusia was produced in patients of all ages and related only to length of administration and dosage.

Both increased preference and polydipsia disappeared gradually after D-penicillamine was discontinued; however, this was accompanied by the continued intake of a diet which contained adequate minerals, including copper. This suggests that the correction of a metabolic abnormality occurred during this period.

The rats treated with D-penicillamine exhibited significant decreases in food intake. This decrease in food intake had no effect on either taste preference or fluid intake. Rats who were pair-fed with that amount of the control diet equivalent to the amount of D-penicillamine treated diet taken by the rats in A or B did not show any difference in taste preference or fluid intake from the controls. In other studies those patients who developed hypogeusia after D-penicillamine administration stated that they had no interest in food since it was tasteless. However, no gastrointestinal toxicity such as nausea, vomiting, or diarrhea was observed during the administration of the drug to either rats or patients.

No anatomical abnormalities were apparent in the D-penicillamine-treated rats for examination by light microscopy of serial sections of their tongues revealed papillae and taste buds which could not be distinguished from normal.

The mechanism by which D-penicillamine produced the alteration in taste preference is not clear. However, in humans with hypogeusia following D-penicillamine administration, withdrawal of the drug or administration of copper was associated with a gradual return of taste acuity to normal (1). Similar results were obtained in rats who were similarly treated (4).

These data could be interpreted to mean that D-penicillamine administration produces a decrease in taste acuity in rats. Rats normally prefer 0.15 *M* NaCl and reject 0.30 *M* NaCl. The D-penicillamine-treated rats in these studies preferred 0.15 *M* NaCl even more than did normal rats. Although preference for 0.30 *M* NaCl was less than that for

0.15 *M* NaCl, the D-penicillamine-treated rats preferred this concentration to the same extent as the normal rat preferred 0.15 *M* NaCl. Since rats normally prefer to drink 0.15 *M* NaCl rather than water a decrease in taste acuity might be expected to cause the rat to drink more of a more concentrated solution of NaCl to satisfy its innate drive for salt. We might hypothesize that a significantly higher concentration than 0.30 *M* NaCl would have to be offered to the D-penicillamine-treated rats before they would reject NaCl. Similarly, rats increase their total fluid intake when presented with preferred fluids. Thus, if D-penicillamine-treated rats showed a decrease in taste acuity they might persist in their polydipsia, drinking more fluid as if it were a fluid of lower concentration presented to a normal animal. The direct relationship to taste of the D-penicillamine effect in the production of polydipsia is emphasized by the fact that treated animals increased total fluid intake only when a taste stimulant was administered. Further emphasis that D-penicillamine-treated rats do indeed demonstrate hypogeusia is found in preliminary data which suggest that increased preference and polydipsia occurred after they were offered a choice between 0.5% sucrose and water (5).

The mechanism by which D-penicillamine produced the observed alteration in fluid intake behavior is unclear. Previously we, and subsequently other investigators, have reported that simply offering rats a choice between water and sugar solutions increases the total intake of fluid (6, 7). This was indeed the case in our studies for some fluid choices and this behavior persisted as long as these fluids were presented. However, this polydipsia appeared to be carefully regulated in normal rats and limited by the concentration of fluid presented. Thus, normal rats when offered a choice between 0.15 *M* NaCl and water developed polydipsia, but when offered a choice between 0.30 *M* NaCl and water they decreased their total fluid intake. In the case of the D-penicillamine-treated animals the polydipsia occurred with presentation of both 0.15 *M* and 0.30 *M* NaCl; in fact, it was increased upon presentation of the more concentrated solution of NaCl. This phenomenon appears dependent upon the continued administration of D-penicillamine, for after the drug was discontinued there was a gradual decrease in polydipsia toward normal over a 5-day period despite the continued presentation of a choice between 0.15 *M* NaCl and water. However, this phenomenon is also dependent upon the presentation of a taste stimulant, since removal of the NaCl while the D-penicillamine was still added to the diet produced a precipitous decrease in fluid intake to normal levels within 24 hr of the removal of the taste stimulant.

D-Penicillamine has effects on many tissue systems other than taste. This includes collagen metabolism (8, 9), cystine metabolism (10), and metal metabolism (11). The manner by which this drug affects each of these systems is the subject of active investigation. How it affects taste acuity may offer important clues to the manner by which taste behavior is controlled at the biochemical level.

Summary. D-Penicillamine administration to rats produced significant increases in preference for salt solutions. It also produced a consistent increase in fluid intake of both water and salt solutions. Discontinuation of the D-Penicillamine, along with continued administration of a diet consisting of an adequate mineral content gradually returned taste preference and fluid intake toward normal.

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Gastric Mucosal Antibodies and Iron Absorption* (33926)

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Circulating antibodies to parietal cells have been found in the serum of patients with iron deficiency anemia, especially if such patients have achlorhydria. Dagg *et al.* (1) reported 11 positive antibody tests in 33 patients with iron deficiency anemia and histamine fast achlorhydria, but only two in 31 iron-deficient patients with acid. The observations of Shearman *et al.* (2) were similar. They found 7 positive tests in 8 anemic patients with total achlorhydria. None of these 7 had circulating antibodies to intrinsic factor. With these observations in mind we decided to explore the possibility that antiparietal cell antibodies might inhibit iron absorption. Since we had no human clinical material available, we chose the following experimental model in rats: we studied the effect of (i) antigastric mucosal antibody on iron absorption by normal rats and rats made anemic by iron depletion, (ii) antigastric mucosal antibody on the secretion of hydrochloric acid by rats in the unlikely event that depression of acid secretion would decrease iron absorption, and (iii) antigastric mucosal antibody on the binding of iron by gastric juice *in vitro*.

Methods. Preparation of antigastric mucosal antibody (AGMS). This was prepared by a modification of the technique of Walder and Lundseth (3) for the production of antiparietal cell antibody. Mucosa was scraped from the stomachs (except the antral region) of 110 male Sprague-Dawley rats in five batches. The tissues were minced, rinsed, and the cells were extracted after incubation with collagenase. The material was then centrifuged at 5° for 15 min at 800 rpm and washed with buffer several times. The deposits of each batch were pooled and disrupted with ultrasound at 38 kcps for 3 min and then resuspended. After centrifugation at 5° for 1 hr at 1000 rpm the supernatant was collected; and had a protein content of 7 mg/ml by the Folin-Ciocalteau method. Four New Zealand white rabbits were immunized as follows with this material. Each received four injections, once weekly, of 5 mg of protein made up to a volume of 2 ml with 30% aluminum hydroxide. The serum was harvested 1 week later from the rabbits and the presence of antigastric mucosal antibodies was demonstrated by reacting the serum against the original antigen on Ouchterlony double gel diffusion plates. Control antigens were prepared from pooled rat spleen, kidney, and serum using the technique of Gold and Freedman (4). The control antigens were adjusted to a protein concentration of 10 mg/ml and reacted against antigastric mucosal serum on Ouchterlony plates. Reactions were noted with all antigens but many more lines developed with gastric antigen than with the others.

Preparation of gastric juice for binding studies. Gastric juice was collected from 20 normal and 20 anemic mature male Sprague-Dawley rats by the technique of Shay *et al.* (5). Each specimen was measured and filtered through cheesecloth. The pH was determined, the specimen was titrated to pH 10–11 for 30 min with 10% potassium hydroxide and then back titrated to pH 7 with

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