Polydipsia Elicited by the Synergistic Action of a Saccharin and Glucose Solution

Abstract. *Rats consume significantly more of a solution combining saccharin and glucose than equivalent solutions of these substances presented in separate bottles. Fluid consumption may exceed body weight. Experiments helping to delineate the basis of this synergistic action are presented.*

The intake of sweet solutions, whether nutritive or nonnutritive, is limited. It is known that the 24-hour consumption of sugar solutions is a direct function of concentration up to a certain peak whereupon consumption levels off, then declines. However, with a brief-exposure two-choice method, animals will prefer a sweeter solution over a less-sweet solution throughout a range of concentration extended up to the saturation level (*1*). The difference in results with the two methods has generally been attributed to satiation in the 24-hour consumption test resulting from postingestional factors (see *2*).

Tests with saccharin solutions introduce other limitations. While postingestional factors such as osmolarity and caloric intake are not significant variables, it has been frequently observed that more-concentrated solutions of saccharin are experienced as bitter and unpleasant. Studies with rats indicate that the preferred concentration of saccharin, judged by 24-hour consumption tests, is approximately a 0.25 percent solution (*3*).

Recently we have been experimenting with a solution that combines the sweetness of a saccharin solution and the nutritive and mildly sweet properties of a dilute glucose solution. To a 0.125 percent (or 0.25 percent) saccharin solution we added the amount of glucose in a 3 percent solution. Distilled water was used to mix the solutions. Each 100 ml of fluid contained either 0.125 or 0.25 g of sodium saccharin (sodium-*o*-benzoic sulfimide) and 3 g of glucose. Previous work in this laboratory has established that rats normally consume between 40 and 65 ml of either a mildly sweet (as judged by humans) 3 percent glucose solution or a very sweet 0.25 percent or 0.125 percent saccharin solution. The 24-hour consumption of the saccharin-glucose mixture was found to be so unexpectedly high that we have initiated a more systematic investigation of the properties of this solution.

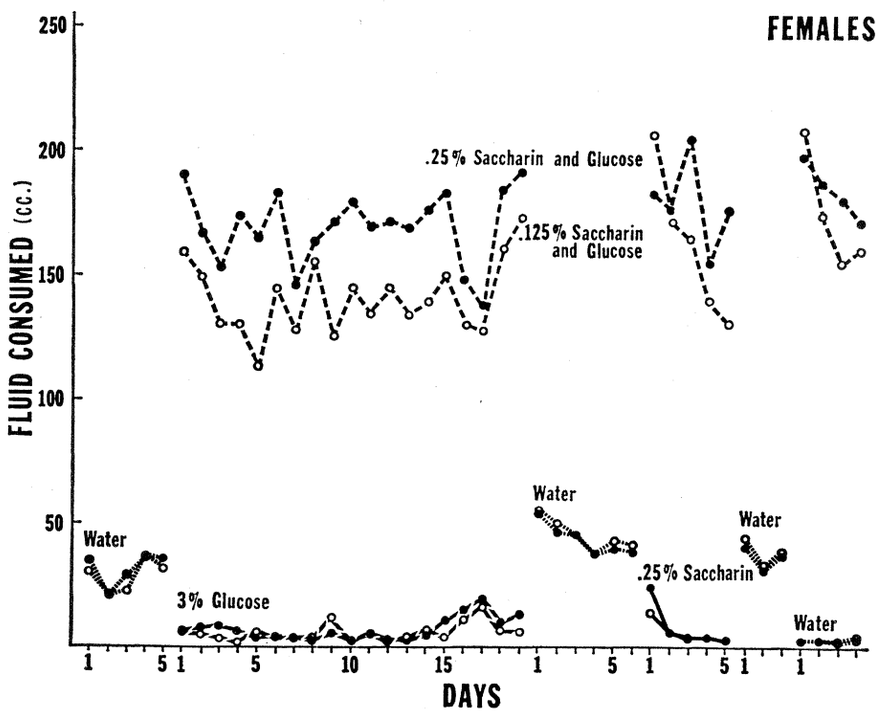

Fig. 1. Average daily fluid consumption. Open circles, seven female rats of the 0.125 percent saccharin + glucose solution group; filled circles, seven female rats of the 0.25 percent saccharin + glucose solution group.

In all experiments Holtzman albino rats were housed in individual cages and had no experience with sweet solutions prior to testing. Purina Lab Chow was available ad libitum unless otherwise specified. Each day the 24-hour fluid consumption was measured, bottles were washed, and solutions that had matured for 1 day in the refrigerator were provided. Fluid spilled was captured in plastic cylinders mounted under each drinking tube.

In the first experiment two groups of seven female rats (average weight 255 g, age 105 days) were given successive 24-hour two-bottle choice tests. After we obtained water consumption base lines for 5 days, each subject was provided with a 3 percent glucose solution and a saccharin-glucose (S+G) mixture as described above with either a 0.125 percent or 0.25 percent saccharin solution plus the glucose. These two solutions were presented for 19 days. Water base lines were obtained following the 19-day period, and subjects were then provided with a choice between a S+G solution and a 0.25 percent saccharin solution for 5 days. Once again water base lines were established and finally subjects were offered a choice between a S+G solution and tap water. This schedule and the results are summarized in Fig. 1.

In the initial condition where subjects were provided with S+G and glucose solutions, the subjects ignored the 3 percent glucose and consumed a daily average of 137 ml of the 0.125 percent S+G solution and 171 ml of the 0.25 percent S+G solution over the 19 days these solutions were available. Individual animals on some days consumed more than 250 ml of the S+G solutions, and one animal consumed over 350 ml in a 24-hour period. Daily fluid consumption occasionally exceeded body weight (*4*). Although comparisons are not simple, this would be equivalent to an average, adult, male human (75 kg) consuming more than 80 liters of fluid per day. After the 19 days of exposure to the S+G solutions the subjects were given tap water for 6 days to determine if the high fluid consumption, once established with the combined solutions, would persist with plain water. The water consumption was elevated the first day but dropped to com-

paratively normal levels thereafter. In subsequent test periods with only water available, consumption was not elevated even on the first day after exposure to the S+G solutions (see Fig. 1).

Since 3 percent glucose is only mildly sweet, we determined the preference for the S+G solutions when competing against a much sweeter solution, a 0.25 percent saccharin solution. The animals in both groups virtually ignored the plain saccharin solution after the first day (Fig. 1). It is important to note that even the subjects given a choice between the 0.125 percent S+G mixture and the 0.25 percent saccharin solution preferred the former. In view of the fact that a 0.25 percent saccharin solution is judged (by humans) to be considerably sweeter than a 0.125 percent S+G solution, it is unlikely that preference is based only on sweetness. Also pointing to the special properties of the S+G mixture is the finding that although at least initially either a 0.25 percent (*5*) or a 0.125 percent (*6*) saccharin solution is preferred over a 3 percent glucose solution, the addition of an equivalent amount of glucose to either saccharin solution produces a mixture that is highly preferred over the saccharin solutions.

Finally, after 3 days on tap water alone, the subjects were given a choice between the S+G solutions and tap water. The tap water was rejected and the S+G solutions were consumed in the large quantities already described (Fig. 1).

A second experiment tested male rats (average weight 350 g, age 75 days) with the S+G solution and a 3 percent glucose solution. Seven subjects were provided with the 0.125 percent S+G and seven subjects the 0.25 percent S+G solutions. As with the females in the first experiment, males in both groups virtually ignored the glucose solution and consumed a daily average of 187 ml of the 0.125 percent S+G and 129 ml of 0.25 percent S+G solutions (Fig. 2). The results of the second experiment were therefore essentially the same as those obtained with the female subjects except that the males consumed more of the 0.125 percent S+G solution while the females consumed more of the 0.25 percent S+G solution. This is consistent with our earlier reports that female rats consume more very sweet solutions than do males (*7*). The special properties of the S+G solution

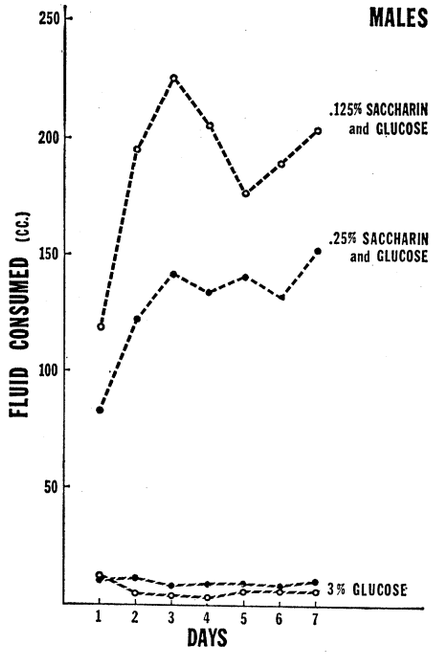

Fig. 2. Average daily fluid consumption. Open circles, seven male rats of the 0.125 percent saccharin + glucose solution group; filled circles, seven male rats of the 0.25 percent saccharin + glucose solution group.

are emphasized by comparing these results with those obtained from 13 comparable male subjects provided with a 0.125 percent saccharin solution and a 3 percent glucose solution in separate bottles. When the saccharin and glucose were not combined, total fluid consumption never exceeded 65 ml. In contrast, when the saccharin and glucose were combined, consumption increased 300 percent.

The S+G solution could be a useful tool for any experimental purpose for which it would be desirable to have animals consume large volumes of fluid. There does not seem to be any danger of water intoxication as animals apparently do not retain abnormal amounts of fluid. The average daily gain in body weight of the female subjects in the first experiment, for example, was slightly lower during the period they were drinking the S+G solution than during the control base line period (0.79 compared to 1.54 g). This difference in weight gain is probably attributable to the lower average daily food consumption (15.7 compared to 19.9 g).

These experiments indicate that animals will drink more of a saccharin + glucose mixture than they will drink of both solutions when they are presented either alone or simultaneously, but in separate bottles. The basis of this synergistic action is not clear, but several possibilities can be considered. The S+G solution has a qualitatively different taste, as judged by humans, than either solution alone. The bitter after-taste of the saccharin solution, for example, is reduced by the addition of a small amount of glucose. It is possible, therefore, that the exaggerated fluid consumption (polydipsia) may be elicited by the palatability of the solution. Another possibility is that a saccharin + glucose combination may increase thirst as a result of polyuria. Combining these two hypotheses it could be suggested that initially the palatability of the S+G solutions induces excessive drinking, resulting in a plasma volume increase triggering an inhibition of antidiuretic hormone. The consequent diuresis may produce thirst and the animals may perpetuate the process by then drinking large quantities of the more palatable S+G solution.

Several additional experiments bear on these possible interpretations. In previous studies, with 3 percent glucose and 0.25 percent saccharin solutions provided simultaneously, but in separate bottles, total consumption has never been observed to approach the levels seen with the S+G solution (*8*). Also, two naive animals were provided with a diet consisting of Purina Lab Chow and saccharin and glucose combined in a powdered form. Animals ate their customary 20 g of food and thereby consumed the same amount of glucose (6 g) and saccharin (0.5 g) as would be ingested in 200 ml of the 0.25 percent S+G solution. The animals given this diet for 7 days consumed the same amount of bulk as before, but actually drank less water than with the undiluted chow. Thus ingestion of saccharin and glucose in separate solutions or in powdered form does not seem to produce diuresis, as increases in consumption of fluids were not observed.

In addition, we determined that animals do not gradually build up their preference for the S+G solutions, as would be expected if these solutions had a polyuric effect. Six naive male rats (average weight 320 g, age 90 days) that were satiated for water and food were tested with the 0.125 percent S+G solution and tap water. Animals consumed an average of 10.3 ml of the S+G solution and only 0.5 ml of tap water during the initial

30 minutes of exposure to these fluids. Apparently there is no time delay in either the preference for the S+G solution or the high rate of consumption. To compare preference differences between thirsty and nonthirsty animals, six comparable male animals treated identically, except that they were deprived of water for 48 hours prior to the test, consumed approximately equal quantities (9.5 ml) of the 0.125 percent S+G solution and tap water during the first 30 minutes. To the extent that deprivation-induced thirst simulates diuresis-induced thirst, these findings would argue against any explanation based upon a diuresis-induced thirst, as animals have different preferences when thirsty and when allowed water ad libitum.

The results indicate that a large part of the unique motivating properties of the S+G solution can be explained by the fact that the combination of saccharin and a relatively small amount of glucose produces a mixture that is very palatable, and possesses a minimum of postingestional inhibiting factors. A 0.25 percent saccharin solution is very sweet, but also is judged by most people to have a bitter "off-taste." A glucose solution equal in sweetness to the saccharin solution would be above a 60 percent solution. It is known that animals cannot consume much of such a high concentration of sugar during a 24-hour period, although they exhibit a preference for such a concentration in brief exposure tests. The addition of a small amount of glucose to a very sweet saccharin solution may eliminate the bitter taste component without appreciably adding to the postingestional inhibiting factors such as caloric intake and osmolarity.

ELLIOT S. VALENSTEIN
VERNE C. COX
JAN W. KAKOLEWSKI
Department of Psychophysiology-Neurophysiology, Fels Research Institute, Yellow Springs, Ohio

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Leukotactic Factor Elaborated by Mixtures of Genetically Dissimilar Cells

Abstract. *Mixtures of immunologically competent cells, from genetically dissimilar donors from inbred strains of mice, rats, or Syrian hamsters, cultivated in vitro elaborate into the medium a factor capable of attracting polymorphonuclear cells when it is injected into the skin of irradiated hamsters. Supernatant medium of cultured node cells from genetically identical donors contains no leukotactic activity. The degree of the cell accumulation seemed to parallel closely the degree of histoincompatibility.*

The role played by lymphoid cells in homograft reactions has been considered both in its immunological (*1*) and possibly nonimmunological aspects (*2*). When immunologically competent cells, like lymph node cells, from two strains of inbred animals of a given species are mixed in vitro and inoculated in the skin of lethally irradiated hamsters, delayed inflammatory reactions develop (*3*). These are absent when the components of the cell mixtures are injected separately, or when cells unable to react with each other for genetic reasons are mixed and inoculated. Irradiated hamsters might therefore be used for the study of cellular mechanisms in homograft reactions. In this model, the cutaneous inflammatory reactions are of equal intensity whether lymphoid cells from specifically sensitized animals are mixed with cells of donor-type origin, or with mixtures of lymphoid cells from normal, immunogenetically dissimilar animals. Furthermore, the reactions are independent of the species used, since mixtures of genetically incompatible lymphoid cells from inbred strains of mice, rats, guinea pigs, Syrian hamsters, or even mixtures of blood lymphocytes from two unrelated human individuals, may provoke reactions. Finally, the intensity of the reactions seems to reflect the degree of histocompatibility differences existing between the cells used in the mixtures. Thus these inflammatory reactions are seemingly an expression of cellular homograft reactivity developing in the integument of irradiated animals serving as immunologically neutralized vehicles. Their nature is, however, completely unknown.

An indication of the cellular events was obtained by impression smears prepared from skin reactions. These showed a striking accumulation of polymorphonuclear (PMN) cells at sites where node cell mixtures were injected, but not at sites where the components of the mixtures were injected separately.

This finding suggested that the interaction of genetically incompatible node cells in the skin of irradiated hamsters might lead to the formation of a leukotactically active mediator. To test this hypothesis, immunologically competent cells were confronted in vitro with cells carrying alien transplantation antigens, and the culture fluid was tested for a leukotactic factor.

Lymph nodes from animals of various inbred strains of mice, rats, or Syrian hamsters were harvested, and viable suspensions of node cells were prepared in tissue culture medium 199 (TCM 199). The medium contained 5 percent heat-inactivated calf serum, 100 international units of penicillin per milliliter and 50 μg of streptomycin per milliliter. Portions of 10×10^6 node cells from each of the donors were mixed and cultivated in plastic culture dishes (Falcon, 60 × 15 mm) in 5 ml TCM 199 for 48 hours at 37°C in a humid atmosphere of 95 percent air to 5 percent CO_2. Controls were provided by cultivating 20×10^6 node cells from one or from the other of the donors, or by incubating TCM 199 without cells under identical conditions. At the end of the incubation period, culture fluids were harvested and centrifuged (450 g for 10 minutes). The cell-free supernatants were concentrated to one-tenth their volume by lyophilization and reconstitution with physiological saline and dialyzed overnight against 1/15 molar phosphate buffer (*p*H 7.8) in the cold. Volumes of 0.1 ml were then inoculated with 28-gauge hypodermic needles in