of tumor growth after transplantation to recipient mice (*10*). A kinetic analysis of cytidylate cyclase from these tissues has not yet been done. Preliminary data indicate that the K_m of cytidylate cyclase for CTP, with 0.3 m*M* Mn^{2+} as the cation, is 0.16 m*M* (obtained from a double-reciprocal plot of enzyme reaction velocity as a function of substrate concentration).

The data above represent the first demonstration of cytidylate cyclase activity and the formation of cyclic CMP in biologic tissues. These experimental findings are complementary to those reported by Bloch (*1*) in which cyclic CMP was identified as a natural constituent of malignant murine L-1210 cells in culture. Our findings on cytidylate cyclase activity in normal mammalian liver are supported by the report by Cailla and Delaage of the presence of cyclic CMP in rat liver (*5*). The experimental results reported from these three laboratories suggest that cyclic CMP is associated with stimulation of tissue growth and that this pyrimidine cyclic nucleotide may play a role in the bioregulation of cell proliferation in normal and malignant mammalian tissues.

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References and Notes

1. A. Bloch, *Biochem. Biophys. Res. Commun.* **58**, 652 (1974).
2. ———, G. Dutschman, R. Maue, *ibid.* **59**, 955 (1974).
3. S. Y. Cech, R. A. Gross, L. J. Ignarro, *Pharmacologist* **18**, 223 (1976); *Clin. Res.* **25**, 11 (1977); S. Y. Cech, L. J. Ignarro, *Pharmacologist* **19**, 202 (1977).
4. S. Y. Cech and L. J. Ignarro, Third International Conference on Cyclic Nucleotides, 17 to 22 July 1977, New Orleans, La. [the proceedings will be published in vol. 9 of *Advances in Cyclic Nucleotide Research*, W. J. George, L. J. Ignarro, G. A. Robison, P. Greengard, Eds. (Raven, New York, in press)].
5. H. Cailla and M. Delaage, in *ibid.*
6. Livers from BAGG-Swiss male mice (20 g) were homogenized (20 percent, weight to volume) in cold 10 m*M* tris-HCl (*p*H 7.4) containing 10 m*M* NaCl, 10 m*M* KCl, and 0.005 m*M* EDTA (disodium salt) with a Potter-Elvehjem tissue grinder equipped with a Teflon pestle. Homogenates were filtered through Nitex (No. 110) nylon filament bolting cloth (50 μm, pore size) and used 60 minutes after preparation.
7. Cytidylate cyclase assays were conducted in glass tubes at 37°C in a final volume of 1.0 ml. Incubation media consisted of 40 m*M* tris-HCl (*p*H 7.4), 0.1 m*M* CTP, [α-^{32}P]CTP (3×10^5 count/min; 13 to 25 c/mmole; New England Nuclear), 0.3 m*M* $Mn(C_2H_3O_2)_2$, and 0.1 ml of whole homogenate (2.8 to 3.4 mg of protein). Incubations were terminated by the addition of 0.1 ml of cold 60 m*M* EDTA (disodium salt) and cooling samples to 4°C. Cyclic [^{3}H]CMP ($\sim 3 \times 10^4$ count/min; 21 c/mmole: Amersham/Searle) was added to the samples, which were then chromatographed on neutral alumina columns. Alumina column chromatography retains more than 99.99 percent of added CTP and allows for a 60 percent recovery of cyclic [^{3}H]CMP. Chromatography of authentic CTP, CDP (cytidine diphosphate), CMP, and cyclic CMP indicates that only cyclic CMP is recovered in the first 3 ml of eluate after elution with neutral tris buffer. Eluates were added to scintillation fluid, and the radioactivity was measured (*8*). In most instances, neither [α-^{32}P]CTP nor cyclic [^{3}H]CMP required further purification prior to use.
8. L. J. Ignarro, R. A. Gross, D. M. Gross, *J. Cyclic Nucleotide Res.* **2**, 337 (1976).
9. Radioimmunoassays were performed by H. Cailla and M. Delaage from the Centre de Biochimie et Biologie Moleculaire in Marseille, France.
10. Myeloid leukemic tumors, which originated spontaneously in liver and spleen, were obtained as subcutaneous transplants in C57BL mice (Jackson Laboratory), and were transplanted in C57BL/6J or DBA/1J male mice.
11. Supported by a research grant from the Cancer Association of Greater New Orleans.

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Hunger in Humans Induced by 2-Deoxy-D-Glucose: Glucoprivic Control of Taste Preference and Food Intake

Abstract. *Intracellular glucopenia induced by 2-deoxy-D-glucose (2DG) administration in man produces increased hunger ratings and magnitude estimates of pleasantness for sucrose solutions. Augmented food intake substantiates these changes in affective behavior and relieves experimentally induced hunger. Intracellular glucopenia activates counterregulatory mechanisms to raise plasma glucose concentrations. Inducing hunger experimentally with 2DG provides a useful method for studying appetitive behavior in humans. The neurohumoral control of pituitary hormone release and other hypothalamic functions may be examined after 2DG infusion.*

Hypoglycemia (*1*) or intracellular glucopenia (*2*) activates feeding behavior in most mammals. Previous studies have not described the hormonal, metabolic, and behavioral correlates of feeding during experimental hunger. In humans, manipulations of internal states by food loads (*3, 4*) or insulin administration (*5*) and by changes in body weight (*4, 6*) all modify affective behavior directed toward the sweet taste of sugar. In addition, insulin-induced hypoglycemia increases feelings of hunger in humans (*7, 8*).

Administration of 2-deoxy-D-glucose (2DG) competitively inhibits intracellular glucose utilization by inhibiting glucose transport (*9*) and glucosephosphate isomerase and hexokinase activity (*10*). The use of this glucose analog in humans has been limited to investigations of its antitumor effects in cancer patients (*11*) and to the study of metabolic and hormonal responses to glucopenia in several clinical models (*12*). In animal species, 2DG administration usually but not always increases food intake (*13*). Whether this is the case for humans is unknown. Since 2DG infusions increase plasma glucose but not insulin and glucagon concentrations in humans (*12*), the effects of intracellular glucopenia on hunger may be studied independently of these hormonal changes which may, by themselves, affect hunger and satiety (*14*). The use of 2DG facilitates a multidimensional evaluation of appetitive behavior in humans since 2DG induces metabolic, hormonal, and behavioral changes of longer duration than those produced by insulin (*15*).

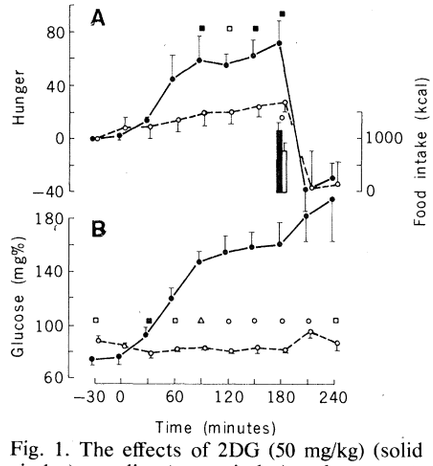

Fig. 1. The effects of 2DG (50 mg/kg) (solid circles) or saline (open circles) on hunger ratings from a visual analog scale (A) and on blood glucose concentrations (B). Bars (A) indicate food intake 180 minutes after 2DG infusion (solid bar) or normal saline infusion (open bar). Means and standard errors of the mean are plotted for a group of five males serving as their own controls. Levels of significance by paired *t*-tests are indicated as follows: $P < .05$, ■; $P < .01$, □; $P < .002$, ○; and $P < .001$, △.

Five healthy, college-age male volunteers of normal weight gave informed consent to receive 2DG (Grand Island Biological) or a sham intravenous infusion of normal saline. The subjects did not know the order of the infusions. They knew that they might experience such side effects as changes in mood, vigor, hunger, thirst, body temperature, and sweating; they did not know the direction of these changes. The subjects were asked to eat additional carbohydrates in the form of bread, potatoes, pasta, and other starches for 3 days before testing to ensure adequate glycogen stores and uniformity of metabolic status the day of infusion. No food, stimulants, or medications were permitted after 8 p.m. the evening before the infusions,

but water was allowed freely. All subjects reclined in bed upon arrival at the laboratory at 8 a.m. A butterfly needle was inserted in an antecubital vein, and intravenous saline was administered to keep a vein open for blood sampling and infusion of 2DG. At 30-minute intervals before and after 2DG infusion, the subjects rated mood, hunger, dryness of mouth, fullness, vigor, and thirst on a 7-point category scale (*16*). The subjects also gave magnitute estimates of hunger, thirst, and perceived body temperature on a visual linear analog scale (*17*). Water intake, pulse rate, and oral temperature were recorded every 30 minutes at the same time that blood was drawn for plasma glucose (*18*). At zero time, subjects received either 2DG (50 mg per kilogram of body weight) or normal saline delivered intravenously with a Harvard infusion pump over a 20-minute period. At 125 minutes after the start of 2DG infusion, subjects gave magnitude estimates of intensity and pleasantness for a series of sucrose concentrations (*19*). Subjects expectorated all solutions and rinses. Beginning at minute 185 (approximately 12 noon), the subjects ate a 25-minute lunch consisting of a chocolate-flavored, complete liquid diet (Nutri 1000, Syntex) containing 1.06 kcal/ml. Blood samples and behavioral ratings were again obtained 210 and 240 minutes after 2DG infusion or 25 and 55 minutes after starting lunch. Four subjects received 2DG infusions first and then returned one or more weeks later for a sham infusion of normal saline, and one subject received infusions in the reverse order. The subjects tolerated all the experimental procedures well and received normal saline to replace a 250-ml blood loss.

Hunger ratings obtained by a category scaling procedure (Table 1) or by a linear visual analog technique (Fig. 1) increased significantly at 30 minutes or 90 minutes, respectively, and remained elevated until food intake relieved hunger after 180 minutes. (All statistical comparisons were made with two-tailed *t*-tests.) The relief from hunger was complete as indicated by the markedly decreased ($P < .02$) hunger ratings at 210 and 240 minutes (Fig. 1). The visual analog scale appeared to be sensitive to changes in hunger for individuals but also produced variable responses between individuals, as indicated by the large standard errors of the mean (Fig. 1). These changes in hunger occurred in spite of a concomitant increase in plasma glucose concentrations. Category ratings of stomach fullness significantly decreased between 30 and 120 minutes after 2DG administration and corresponded in part with increased hunger ratings (Table 1). Ratings of vigor decreased ($P < .05$) between 30 minutes and 120 minutes after 2DG infusion. No significant changes in category ratings of thirst, dryness of mouth, or mood occurred, although thirst ratings tended to increase as long as 120 minutes after 2DG administration. Thirst ratings on a visual analog scale followed a similar pattern of change. Perceived body temperature on a visual analog scale did not change significantly. However, three of five subjects reported distinct feelings of warmth in spite of becoming hypothermic. In all subjects, oral temperatures significantly ($P < .05$) decreased 0.4° to 0.7°C between 90 and 210 minutes after 2DG infusion. Pulse rate significantly ($P < .05$) increased 15 to 20 beats per minute between 30 and 240 minutes and all subjects sweated visibly between 60 and 120 minutes after 2DG infusion. These changes indicate that hunger developed in the presence of sympathetic discharge and increased plasma catecholamines (*12*).

Table 1. Mean category ratings of hunger and fullness of stomach as a function of time in minutes before and after 20-minute infusions of normal saline (NS) or 2DG. The numerical values of hunger ratings are derived from those reported by the subject (*16*) so that increasing values represent increasing hunger. Statistical comparisons (*t*-tests) at 210 and 240 minutes are with the values at 180 minutes (before feeding). All other comparisons are with the saline control values.

Treatment	Time (minutes)									
	−30	0	30	60	90	120	150	180	210	240
					Hunger					
NS	3.6	3.2	3.2	3.4	4.2	4.2	4.6	4.8	1.6§	1.8§
2DG	3.6	3.6	5.0*	5.2*	5.4†	5.4†	5.2	5.4*	3.0†	2.5§
					Fullness					
NS	2.8	2.4	2.4	2.2	2.2	2.2	2.2	2.2	5.0†	4.5†
2DG	2.0*	2.0	1.6‡	1.6*	1.6*	1.6*	1.8	1.8	4.8†	4.5†

*$P < .05$. †$P < .025$. ‡$P < .01$. §$P < .005$.

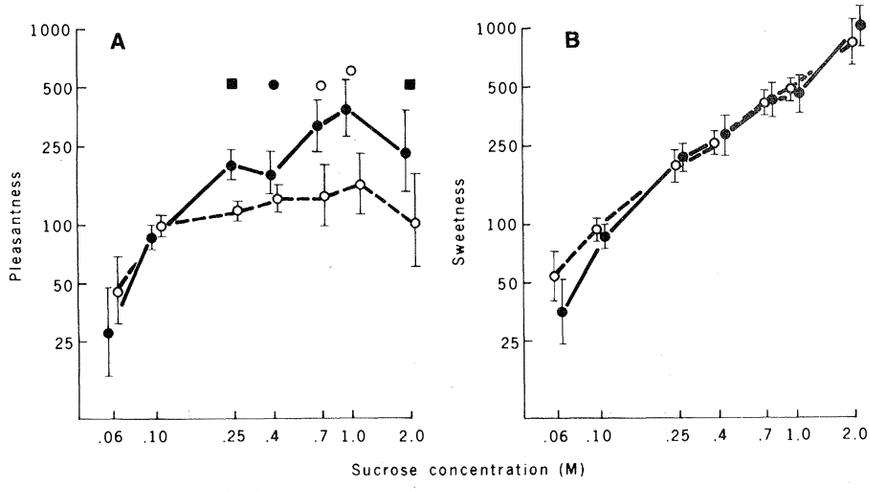

Fig. 2. The effects of 2DG (50 mg/kg) (solid circles) or saline (open circles) on magnitude estimates for sucrose pleasantness (A) and sucrose intensity (B). Geometric means and standard errors of the mean are plotted in log-log coordinates. Levels of significance by paired *t*-tests are indicated as follows: $P < .05$, ■; $P < .02$, ●; and $P < .002$, ○.

Because preliminary experiments indicated that hunger increased maximally around 120 minutes (*15*), all subjects were tested then to determine the effects of intracellular glucopenia on intensity and pleasantness ratings for different sucrose concentrations (Fig. 2). At the five highest concentrations, magnitude estimates of pleasantness significantly increased while intensity ratings remained unchanged during 2DG-induced experimental hunger.

Water intake significantly ($P < .02$) increased from 19 ml after normal saline infusion to 728 ml in the 180-minute period after 2DG infusion. During this period of free drinking, thirst ratings did not significantly increase. Food intake significantly ($P < .002$) increased from 797 ml after the sham infusion to 1170 ml 180 minutes after the 2DG infusion (Fig. 1).

Some of the hormonal correlates of these behavioral responses to 2DG infusion include significant elevations in prolactin, cortisol, and growth hormone (*12*). Plasma glucose concentrations, however, rise more consistently (Fig. 1), than do pituitary hormone and cortisol levels (*12*). In fact, the plasma glucose elevation after 2DG infusion is primarily dependent on sympathetic innervation of the adrenal medulla and, to a lesser extent, the liver (*12*). Plasma glucose concentrations increased significantly 30 minutes after food intake in both the normal saline control ($P < .025$) and in the 2DG-stimulated ($P < .025$) groups (Fig. 1).

Previous attempts at scaling experimental hunger (*7, 15*) have relied on descriptive category ratings of appetite without independent measures of hunger, such as preference ratings for sugar, records of food intake, or determinations of stomach contractions. The poor relationship frequently found between hunger and gastric motility (*20*) demonstrates the importance of considering multiple independent measures of hunger. Our experiments bring together three commonly used variables in the assessment of hunger in man: hunger ratings, hedonic judgments for sweet solutions, and food intake. Direct comparison of a visual linear analog scale with a category rating scale of hunger revealed the former to be more sensitive and the latter to be less variable between individuals, which probably accounts for the earlier detection of significant hunger changes with the category rating scale. The linear visual analog scale has been introduced to circumvent three problems associated with the use of category scales (*21*). (i) One does not know whether categories or differences between adjoining categories are the same. (ii) Discrete scores are not additive. (iii) Resolution tends to be limited since only the central few categories are commonly used. With regard to 2DG-induced experimental hunger, category scales in this study provided adequate resolution for differences, and there was no limitation to the central few categories.

The behavioral and metabolic responses of the single subject receiving normal saline first and 2DG later did not differ from those of the four subjects receiving the infusions in the reverse order. Affective processes change dramatically after 2DG infusion. One subject spontaneously commented that he had visions of eating hamburgers approximately 90 minutes after starting a 2DG infusion. Experimental hunger induced by 2DG increased the pleasantness of sucrose. Mayer-Gross and Walker (*5*) have made similar observations in patients receiving insulin. Most important, in our study, intracellular glucopenia increased food intake significantly.

That experimental hunger induced by 2DG infusion in humans is accompanied by several behavioral, metabolic, and hormonal changes indicates a more general hypothalamic activation not limited to stimulation of feeding behavior. Water intake increases and category ratings of vigor decrease. The increased intake of a liquid diet after 2DG infusion cannot be attributed to increased thirst since the subjects relieved their thirst before being given the food. Paradoxically, decreases in oral temperature are usually accompanied by increased feelings of body warmth. Therefore, the subjects did not eat to keep warm since they already felt warm. However, the rate of intracellular glucose utilization is reduced below a set point or if the set point is above the prevailing rate of utilization, behavioral and physiological mechanisms would be activated to reinstate glucose utilization to the set point. Thus, preferences for sweets, hunger, and food intake are increased, and endogenous glucose is released from glycogen stores through catecholamine-mediated glycogenolysis (*12*) and may be produced by growth hormone, cortisol, and prolactin-mediated gluconeogenesis.

The hypothalamic neurotransmitter system or systems involved in these behaviorally and hormonally mediated processes are unknown in humans, but central alpha adrenergic blockade reduces the feeding response to 2DG while not affecting the hyperglycemic response (*22*). The dose of 2DG necessary to stimulate food intake in most mammals is greater than 50 mg/kg; however, in humans this dose stimulates appetite (*23*). The hormonal and metabolic responses to this dose of 2DG include increased secretion of catecholamines, growth hormone, prolactin, and cortisol along with elevations of free fatty acids, lactate, and β-hydroxybutyrate (*12*).

Although our results are consistent with the glucostatic theory of food intake control (*24*) they do not demonstrate a physiological role for glucose in the normal control of food intake. Nonetheless, the data support a glucoprivic control of food intake in humans (*25*). Moreover, 2DG-induced experimental hunger should be a useful tool for the study of behaviorally related phenomena in humans, that is, preference for sweets and the control of food intake. The measurement of pituitary hormones may clarify the role of some of the neurotransmitter systems associated with human feeding behavior. The effectiveness of various drugs in stimulating or suppressing appetite in anorectic cancer patients or obese patients, respectively, may be evaluated with methods developed with 2DG-induced experimental hunger. Since 2DG produces hyperglycemia and a state of relative insulin deficiency or resistance similar to that observed in diabetes mellitus, it may be used as an experimental model in humans. Other hypothalamic functions, including the regulation of water balance and temperature regulation, may be studied under conditions imposed by 2DG.

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References and Notes

1. C. M. McKay, S. W. Calloway, H. Barnes, *J. Nutr.* **20**, 59 (1940); H. L. Jacobs, *J. Comp. Physiol. Psychol.* **51**, 304 (1958).
2. M. Rezek and E. A. Kroeger, *J. Nutr.* **106**, 143 (1976).
3. D. A. Thompson, H. R. Moskowitz, R. G. Campbell, *J. Appl. Physiol.* **4**, 77 (1976); G. A. Bray, R. E. Barry, J. Benfield, P. Castelnuovo-Tedesco, J. Rodin, in *Hunger: Basic Mechanisms and Clinical Implications*, D. Novin, W. Wyrwicka, G. Bray, Eds. (Raven, New York, 1976), p. 431; J. Grinker and J. Hirsch, *Ciba Symp.* **8**, 349 (1973); O. Wooley, S. Wooley, R. Dunham, *Physiol. Behav.* **9**, 765 (1972).
4. M. Cabanac, R. Duclaux, N. H. Spector, *Nature (London)* **229**, 125 (1971).
5. W. Mayer-Gross and J. W. Walker, *Br. J. Exp. Pathol.* **27**, 297 (1946).
6. G. A. Bray, R. E. Barry, J. R. Benfield, P. Castelnuovo-Tedesco, J. Rodin, *Am. J. Clin. Nutr.* **29**, 779 (1976).
7. H. D. Janowitz and A. C. Ivy, *J. Appl. Physiol.* **1**, 643 (1949).
8. J. T. Silverstone and M. Besser, *Postgrad. Med. J.* **47** (Suppl.), 427 (1971).
9. J. Brown and H. L. Bachrach, *Proc. Soc. Exp. Biol. Med.* **100**, 641 (1959); T. Bidder, *J. Neurochem.* **15**, 867 (1968); R. W. Cutler and J. C. Sipe, *Am. J. Physiol.* **220**, 1182 (1971); H. S. Bachelard, *Biochem. J.* **127**, 836 (1972).
10. F. B. Cramer and G. E. Woodward, *J. Franklin Inst.* **253**, 354 (1952); G. E. Woodward and M. T. Hudson, *Cancer Res.* **14**, 599 (1954); A. Sols and R. K. Crane, *J. Biol. Chem.* **210**, 581 (1954).
11. B. R. Landau, J. Laszlo, J. Stengle, D. Burk, *J. Natl. Cancer Inst.* **21**, 485 (1958).
12. P. D. Woolf, L. A. Lee, W. Leebaw, D. A. Thompson, U. Lilavivathana, R. G. Brodows, R. G. Campbell, *J. Clin. Endocrinol. Metab.* **45**, 377 (1977); W. R. Harlan, J. Laszlo, M. D. Bogdonoff, E. H. Estes, Jr., *ibid.* **23**, 41 (1963); S. G. Grasso, J. H. Karam, L. C. Wegienka, *ibid.* **28**, 535 (1968); J. Laszlo, W. R. Harlan, R. F. Klein, N. Kirshner, E. H. Estes, Jr., M. D. Bogdonoff, *J. Clin. Invest.* **40**, 171 (1960); R. G. Brodows, F. X. PiSunyer, R. G. Campbell, *ibid.* **52**, 1841 (1973); *Metabolism* **24**, 617 (1975); L. C. Wegienka, G. M. Grodsky, J. H. Karam, S. G. Grasso, P. H. Forsham, *ibid.* **16**, 245 (1967); A. Peytremann, L. Favre, M. B. Vallotton, *Eur. J. Clin. Invest.* **2**, 432 (1972).
13. J. E. Jalowiec, J. Panksepp, H. Shalshelomitz, A. Zolovick, W. Stern, P. J. Morgane, *Physiol. Behav.* **10**, 805 (1973).
14. G. C. Kennedy, *Br. Med. Bull.* **22**, 216 (1966); S. B. Penick and L. E. Hinkle, Jr., *New Engl. J. Med.* **264**, 893 (1961).
15. Insulin (0.2 unit per kilogram of body weight) given intravenously to 13 healthy volunteers increased category ratings of hunger maximally 40 minutes after injection, and blood glucose de-

creased to a nadir of 29 mg percent at 30 minutes. Venous glucose rose and hunger ratings decreased toward the baseline over the next 60 minutes (D. A. Thompson, in preparation). These results confirm those reported by Janowitz and Ivy (*7*). In preliminary experiments, 2DG (50 mg/kg) was infused for 20 minutes into six healthy volunteers who rated their hunger highest at 120 minutes (a time when plasma glucose was maximally elevated). Both plasma glucose concentrations and hunger ratings remained elevated for at least another 60 minutes.

16. Category rating scales of subjective feelings consisted of seven categories for each attribute. Decreasing values for ratings of hunger or thirst represent increasing hunger or thirst with rating 1 referring to extreme hunger or thirst, 4 being neutral, and 7 referring to no desire to eat or drink even on request. Two scales appeared on each page, but related scales (such as hunger and fullness of stomach, thirst and dryness of mouth, and mood and vigor) were on different pages.
17. Subjects rated hunger, thirst, and perceived body temperature by marking a vertical line on a 300-mm horizontal line anchored in the center by a vertical line labeled "standard" to represent the subjects' feelings at the beginning of an experiment. Deviations in millimeters from the standard line in positive (increasing) or negative (decreasing) numbers were recorded.
18. A. Kadish, R. Little, J. Sternberg, *Clin. Chem.* **14**, 116 (1968).
19. Subjects assigned positive numbers proportional to the intensity and pleasantness of each sweet test stimulus. A moderately sweet solution of sucrose served as a fixed standard with which each test solution was compared. A modulus value of 100 was assigned to the standard for both intensity and pleasantness. If a test solution was twice as strong and half as pleasant as the standard, values of 200 and 50 would represent intensity and pleasantness.
20. A. J. Stunkard and S. Fox, *Psychosom. Med.* **33**, 123 (1971).
21. R. C. B. Aitken, *Proc. R. Soc. Med.* **62**, 989 (1969).
22. E. E. Müller, D. Cocchi, P. Mantegazza, *Am. J. Physiol.* **223**, 945 (1972); E. E. Müller, L. A. Frohman, D. Cocchi, *ibid.*, **224**, 1210 (1973).
23. R. G. Jones and D. A. Booth, *Physiol. Behav.* **15**, 85 (1975). The lowest dose reported to have an effect on feeding was 100 mg/kg. Latency of feeding was more sensitive than meal size as a measure of the effect of 2DG on feeding.
24. J. Mayer and D. Thomas, *Science* **156**, 328 (1967).
25. R. R. Miselis and A. N. Epstein, *Am. J. Physiol.* **229**, 1438 (1975).

26. Supported by PHS award F32AM 05184-01 to D. T. and by grants from Weight Watchers Foundation, Inc., and Sandoz Pharmaceuticals. R.G.C. is the recipient of NIH academic career development award 5K07AM-70290-5. We thank F. Bogardus, T. Thompson, H. Stefano, and B. Johnson for their technical assistance. We also thank Dr. D. Lockwood for helpful advice in preparing this report.

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Hepatitis B "e" Antigen: An Apparent Association with Lactate Dehydrogenase Isozyme-5

Abstract. *Serums containing the "e" antigen of hepatitis B virus were subjected to electrophoresis in polyacrylamide gel. An extra band appeared in the lactate dehydrogenase isozyme pattern, but this band was undetectable in serums containing antibodies to the e antigenic determinant. Prior separation of the lactate dehydrogenase isozyme-5 fraction by chromatography of serum on minicolumns of diethylaminoethyl-Sephadex-A50 improved electrophoretic identification of the extra band. Neutralization with antibodies to the e antigen as well as by antibodies to the homologous d or y component of the hepatitis B surface antigen removed the extra band; antibodies to the lactate dehydrogenase isozyme-5 removed both the normal and the extra enzymatic band of isozyme-5. This feature of the e antigen provides an assay system for laboratory diagnosis of potential clinical usefulness and suggests its possible role in pathogenesis of hepatocellular injury.*

Immunologic and enzymatic markers of infection with the hepatitis B virus (HBV) include the hepatitis B surface antigen (HBsAg) with mutually exclusive *adw*, *ayw*, *adr*, and *ayr* subtypes, antibodies to HBsAg (anti-HBs), hepatitis B core antigen and antibodies (HBcAg and anti-HBc, respectively), and HBV specific DNA polymerase (*1*). Magnius and Epsmark (*2*) defined a new immunologic marker, the "e" and anti-e system, by gel diffusion analyses of precipitin reactions between various serums containing HBsAg, and suggested that the e antigen may be an indicator of contagiousness (*2, 3*). This marker has been designated HBeAg, and its antibodies, anti-HBe (*4*). Numerous investigators have found a positive correlation between the e antigen and DNA polymerase, Dane particle counts, infectivity, and chronicity of hepatitis (*5*), suggesting that the clinical outcome of the disease is more favorable in the presence of anti-HBe. The HBeAg has been characterized by Magnius (*6*) as a soluble protein distinct from HBsAg, with electrophoretic mobility in the fast gamma region and an estimated molecular weight of 300,000; Neurath and Strick (*7*) have postulated that HBeAg is a dimer of immunoglobulin G. Williams and Le Bouvier (*8*) have reported antigenic heterogeneity and thermolability of the HBeAg. We report here that HBeAg is associated with an isozyme of serum lactate dehydrogenase.

Serum lactate dehydrogenase (LDH, L-lactic acid NAD oxidoreductase, E.C. 1.1.1.27) consists of five isozymes (LDH-1, -2, -3, -4, and -5) of identical molecular weight but different charge, which permits their separation by electrophoresis; being the least negatively charged isozyme, LDH-5 separates in the gamma region (*9*). The LDH isozymes represent tetramers consisting of various combinations of two different polypeptide chains (M and H) each of 35,000 daltons (*9*). The isozyme LDH-5, composed of four noncovalently bound M chains, is the major component of the LDH synthesized by the liver cells (*10*). In serums of HBsAg positive individuals, Damle and co-workers (*11*) found an "anomalous" or extra band between LDH-4 and LDH-5, and also observed the removal of this anomalous band by anti-HBs (*11*). Complexing of immunoglobulins with LDH is known to produce abnormal isozyme patterns (*12*).

Because this extra band was present in all of the 90 HBsAg positive serums but in none of a series of HBsAg negative samples, it was suggested that this new finding might serve as a convenient method for detection of HBsAg (*11*). We confirmed this observation and further detected that this extra band is absent in serums of individuals containing anti-HBe. Moreover, this extra band was removed by immunochemical neutralization with specific antibodies against HBeAg, LDH-5, and the *d* or *y* subtypes of HBsAg. We designated this extra band as LDH-5ex.

The HBsAg was identified by a solid-phase radioimmunoassay (Ausria II, Abbott). Plasma units from apparently healthy carriers of HBsAg were obtained from blood banks in northern California and kept frozen at −20°C as a research

Table 1. HBsAg, LDH-5, and LDH-5ex of serum or plasma from hepatitis patients and asymptomatic HBsAg carriers who were accepted as blood donors.

Source of serum	Number of specimens	Number positive for		
		HBsAg	LDH-5	LDH-5ex
Acute non-B hepatitis	20	0	20	0
Acute hepatitis B	30	30	30	30
Asymptomatic HBsAg carriers with HBeAg	5	5	5	5
Asymptomatic HBsAg carriers with neither HBeAg nor anti-HBeAg	13	13	13	10
Asymptomatic HBsAg carriers with anti-HBe	14	14	14	0
Normal human donors	10	0	10	0