7. Cate, T. R., Couch, R. B., Johnson, K. M., J. Clin. Invest., 1964, v43, 56.
8. Phillips, C. A., Riggs, S., Melnick, J. L., Grim, C. A., J.A.M.A., 1965, v192, 277.

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## Gastric Acid Response to 2-Deoxy-D-Glucose in Chronic Fistula Rats.* (31455)

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Hirschowitz and co-workers(1,2) recently established the usefulness of 2-deoxy-D-glucose (2-D.G.) as a gastric secretory stimulant in dog and man. Acid production after 2-D.G. equalled that after augmented histamine stimulation and exceeded that after insulin, while pepsin secretion was greater with 2-D.G. than after either histamine or insulin. The secretory response was abolished by bilateral vagotomy or atropine. The mechanism of the stimulatory action was not clear but it depended upon the competition of 2-D.G. with glucose at an intracellular site(3) in the brain. On the basis of these results 2-D.G. was proposed as a single stimulus which could be used to evaluate central neural mechanisms involved in acid and pepsin secretion, and to estimate the maximum secretory capacity of the stomach. We were attracted by the possibility that 2-D.G. might have similar gastric secretory effects in the rat and thus would be useful in studies of the central nervous control of gastric secretion. This paper is a report of the acid response of the chronic gastric fistula rat to varying doses of 2-D.G. (25-400 mg/kg). No dose of 2-D.G. in this range produced a significantly greater acid response than that produced by insulin (0.4 U/kg).

*Materials and methods.* Seven Sprague-Dawley rats, weighing 300-500 g, were equipped with chronic gastric fistulas. Gastric contents were collected according to the method of Brodie *et al*(4). Hourly volumes were measured directly. Acid concentration was determined by electrometric titration to pH 7.0. Animals were deprived of food for at least 18 hours before each experiment. All experiments had the following design: 1 hour control gastric collection followed by a subcutaneous injection of 0.9% saline, or U/40 regular insulin§ (0.4 U/kg) or 2-D.G.‖ (25-400 mg/kg), followed by 4 hourly gastric collections. Acid outputs were computed for each hour. The statistical significance of the differences in acid responses to the various stimuli was determined using the two-sample rank test.

*Results.* The gastric acid response to 2-D. G. was "all or none" over the range of 50-400 mg/kg (Table I). The peak response occurred in the second or third hour following the subcutaneous injection in most of the experiments. The response usually lasted longer than 4 hours; no attempt was made to measure the duration of the response more precisely.

Four rats were tested with both 2-D.G.

TABLE I. Acid Response to 2-D.G.

| Treatment | No. of rats | "N" | Acid output ($\mu$Eq/4 hr) |
|---|---|---|---|
| .9% Saline | 3 | 9 | 40.1 ± 11.8 |
| 2-D.G. ( 25 mg/kg) | 2 | 6 | 49.2 ± 15.9 |
| 2-D.G. ( 50 " ) | 7 | 8 | 184.9 ± 48.1 |
| 2-D.G. (100 " ) | 7 | 11 | 296.8 ± 80.4 |
| 2-D.G. (200 " ) | 7 | 10 | 136.5 ± 36.0 |
| 2-D.G. (400 " ) | 5 | 10 | 216.1 ± 77.7 |

Using the two-sample rank test, there is no significant difference between the acid responses to doses of 2-D.G. $\geq$ 50 mg/kg.

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§ Iletin, Eli Lilly & Co.

‖ Mann Research Laboratories, Inc., New York, and Sigma Chemical Co., St. Louis, Mo.

(100 mg/kg) and U/40 regular insulin (0.4 U/kg). There was no significant difference in the acid response to these two stimuli (U = 17, $p>0.05$).

Atropine sulfate (U.S.P., 1.5 mg/kg), injected simultaneously with 2-D.G. (100 mg/kg) in 6 experiments, abolished the gastric response.

*Discussion.* Our results indicate that the rat gastric acid response to 2-D.G. is "all or none" over the dose range of 50-400 mg/kg and that it is not larger than the response obtained with insulin. Hirschowitz and coworkers(1,2), however, have demonstrated a graded dose-response relationship in the range of 25-100 mg/kg when the drug is injected intravenously into conscious dogs; they also reported that the acid response to 2-D.G. in the dog and man exceeded that produced by insulin. Species difference and/or the difference in route of administration may account for the lack of agreement between the results of Hirschowitz *et al* and our own.

The inhibition of the response by atropine indicates that a cholinergic mechanism is critically involved. This was previously reported in the dog(1).

The response is mediated, at least in part, over the vagi because Hirschowitz *et al*(2) observed no response in vagotomized human subjects and Brodie(5) recently demonstrated that vagotomy abolished the response in esophageal-ligated and pylorus-ligated rats.

*Summary.* The gastric acid response to 2-D.G. (25-400 mg/kg) was studied in 7 chronic gastric fistula rats. The gastric response was "all or none" and the threshold dose was approximately 50 mg/kg. Atropine abolished the response. No dose of 2-D.G. produced a significantly larger acid response than insulin.

1. Hirschowitz, B. I., Sachs, G., Am. J. Physiol., 1965, v209, 452.

2. Duke, W. W., Hirschowitz, B. I., Sachs, G., Lancet, 1965, v ii, 871.

3. Tower, D. B., J. Neurochem., 1958, v3, 185.

4. Brodie, D. A., Marshall, R. W., Moreno, O. M., Am. J. Physiol., 1962, v202, 812.

5. Brodie, D. A., Knapp, P. G., Gastroenterology, 1966, in press.

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## Preparation of Inactivated St. Louis Encephalitis Virus Vaccine from Hamster Kidney Cell Culture.* (31456)

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The possibility of developing a vaccine against Japanese B encephalitis (JBE) and St. Louis encephalitis (SLE) was explored by Sabin *et al* in 1943(1). They developed mouse brain formalin inactivated vaccines which were capable of producing neutralizing antibodies in about 50% of the adult vaccinees. The SLE vaccine was never produced and used on a large scale for field testing as was the JBE vaccine. With tissue culture techniques available and the apparent recent success in developing an improved inactivated JBE vaccine in hamster kidney cell (HKC) monolayers(2), the possibility of developing a similar vaccine for SLE was explored.

*Materials and methods. Virus strain used.* (1) The large plaque variant(3) of P-15 strain, isolated from *Culex nigripalpus* mosquitoes of Pinellas County, Florida(4) was used. The first plaque picking (chick embryo tissue) was made from mouse passage 6, followed by 7 serial selections of large plaques. A pool was then prepared from this seed which was used for preparing the vaccine and for neutralization tests. (2) A 20% suspension of suckling mouse brain infected with mouse

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