6. Brind, S. H., Bianchine, J. R., and Levy, M. N., Am. J. Physiol. **185**, 483 (1956).

7. Edwards, A. W. T., Korner, P. E., and Thorburn, G. D., Quart. J. Exptl. Physiol. **44**, 309 (1959).

8. Groom, A. C., Loefving, B. M. A., Rowlands, S., and Thomas, H. W., Acta Physiol. Scand. **54**, 116 (1962).

9. Corcondilas, A., Donald, E. E., and Shepherd, J. T., J. Physiol. (London) **170**, 250 (1964).

10. Bayliss, W. M., J. Physiol. (London) **37**, 264 (1908).

11. Krayer, O. and Verney, E. B., Arch. Exptl. Pathol. Pharmakol. **180**, 75 (1936).

12. Engelking, R. and Willig, F., Arch. Ges. Physiol. **265**, 306 (1958).

13. Celander, O., Acta Physiol. Scand. **32**, Suppl. 116 (1954).

Received Dec. 18, 1968. P.S.E.B.M., 1969, Vol. 131.

Cholinergic Blockade and Growth Hormone Responsiveness to Insulin Hypoglycemia* (33837)

WILLIAM G. BLACKARD AND CAROLINE C. WADDELL[1]
(Introduced by G. S. Berenson)

Division of Endocrinology and Metabolism, Department of Medicine, Louisiana State University School of Medicine, New Orleans, Louisiana 70112

The observation of high concentrations of cholinergic and adrenergic neurons in the hypothalamus (1) raises the possibility that the neurotransmitters, acetylcholine and catecholamines may influence hypothalamic control of pituitary hormone secretion. Previous studies have indicated an adrenergic control mechanism for growth hormone secretion (2). The present study was performed to search for an antagonistic cholinergic control mechanism.

During the course of this investigation a possible pitfall in interpretation of paired data obtained from provocative tests of growth hormone secretion on consecutive days was uncovered. The initial experimental design for this project differed from previous growth hormone studies performed in our laboratory (2, 3) in that the control and experimental studies were carried out on consecutive days. The consistently lower growth hormone response on the second day might have led to a serious misinterpretation had not the order of performance of the control and experimental procedures been randomized.

Methods. Eight apparently healthy male medical students, 20–27 years old, were selected for this study. Two intravenous insulin tolerance tests were performed on consecutive days. Atropine was given during either the first or second test alternately to all subjects. The second test was repeated on each subject following an interval of 2–5 weeks.

The subjects were instructed to abstain from smoking and to take nothing by mouth except water after 10 p.m. the night before the study. Each subject reported to the experimental room at 7 a.m., was weighed, and then put to bed. A slow intravenous infusion of 0.85% NaCl was begun through a 20-gauge needle in an antecubital vein. The indwelling needle was used for withdrawal of blood samples as well as for injections of insulin and drugs. A sphygmomanometer was placed on the opposite arm and blood pressure and pulse rate were monitored every 15 min throughout the experiment.

After a 30-min base-line period, glucagon-free insulin (0.1 U/kg) was injected. Blood samples were taken every 15 min during the base-line period and for 90 min after the insulin had been given. In the cholinergic blockade experiments, 1 mg of atropine sul-

* Supported by a U.S. Public Health Service Grant (AM 10151) from the National Institute of Arthritis and Metabolic Diseases, Bethesda, Maryland.

[1] Research Fellow.

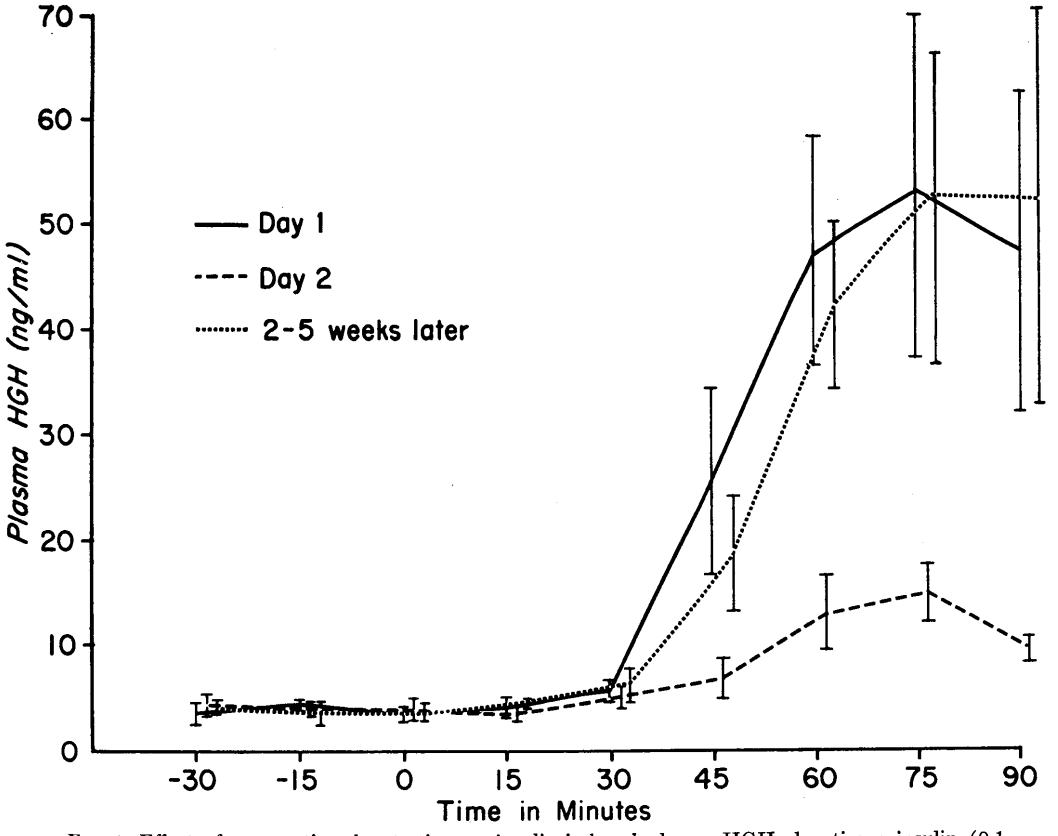

FIG. 1. Effect of consecutive day testing on insulin-induced plasma HGH elevations: insulin (0.1 U/kg) was administered at zero time; means ± SEM are shown (6 subjects).

fate was injected intravenously 5 min before insulin administration. Three subjects received atropine without insulin.

Blood samples were collected in chilled heparinized tubes. Protein-free filtrates for blood glucose were made from 0.2 ml of blood immediately after the blood had been obtained. Blood glucose was determined by a glucose oxidase method (4). Plasma human growth hormone (HGH) was determined by modification of the radioimmunoassay method of Schalch and Parker (5) utilizing ^{125}I-labeled growth hormone and separating free from bound hormone by the double antibody technique. Tracer and standards were prepared from highly purified HGH (Lot HS 968C) supplied by Dr. A. E. Wilhelmi. Glucagon-free insulin was kindly supplied by Eli Lilly and Company, Indianapolis, Indiana and atropine sulfate was purchased from Burroughs Wellcome and Company.

Results. In order to determine the effect of cholinergic blockade with atropine on growth hormone secretion, the plasma HGH response to insulin hypoglycemia was assessed on 2 consecutive days in 8 patients. Atropine (1 mg intravenously) was given 5 min prior to insulin injection on the first day to half the subjects and on the second day to alternate subjects. The HGH response was considerably less the second day regardless of whether atropine had been given or not (Fig. 1). The study which had been performed on day 2 was then repeated 2–5 weeks later and the HGH response was significantly greater than that in the same test performed earlier. Clearly, the short time interval between tests was a greater factor in the HGH response than any possible effect of atropine. The data on two of the eight patients are excluded as one subject did not exhibit a plasma HGH response on either

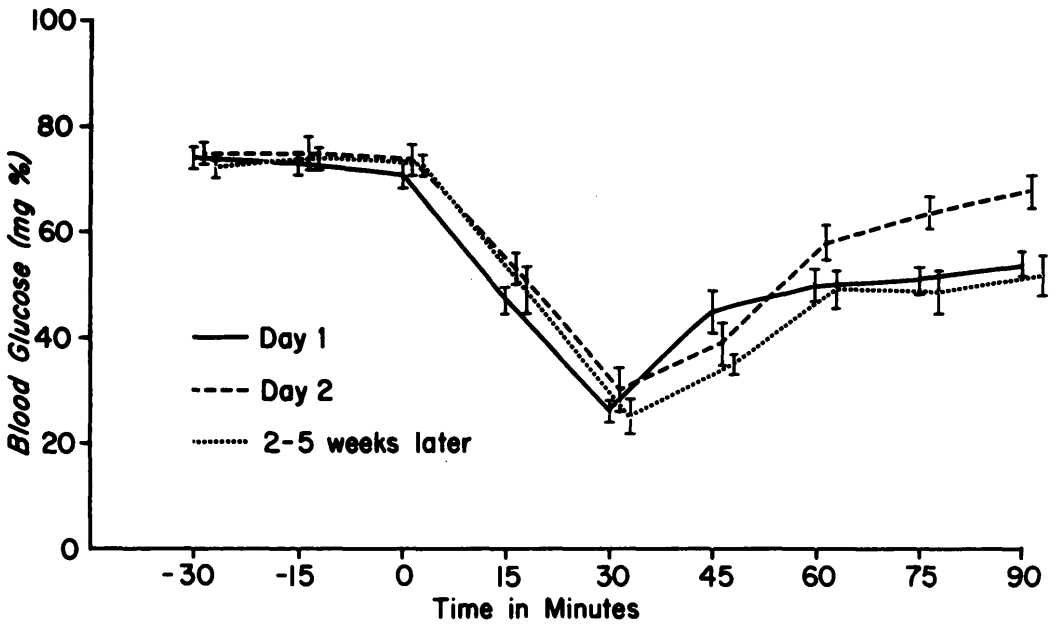

FIG. 2. Effect of consecutive day testing on insulin hypoglycemia: insulin (0.1 U/kg) was administered at zero time (6 subjects).

day of insulin hypoglycemia and the second subject's blood glucose nadir varied by 24 mg/100 ml in the two tests.

The blood glucose values during the several insulin hypoglycemia tests on subjects included in this study were not significantly different (Fig. 2). Thus, it is unlikely that the variation in HGH response could be attributed to blood glucose differences.

Only data from the first test and the third test (2–5 weeks later) were used to assess a possible effect of atropine on hypoglycemia-induced plasma HGH elevations. Two of the six subjects received atropine the first day and the remaining four received atropine during the second and third tests. Figure 3 shows that the HGH response to insulin hypoglycemia was not significantly altered by cholinergic blockade with atropine. The hypoglycemic response to insulin was similar in the control and atropine experiments (data not shown). Three subjects received atropine without insulin and no plasma HGH response was detected (data not shown).

Four of the 6 subjects reported that the hypoglycemic symptoms were milder when atropine was given. Atropine also caused a dry mouth, inhibition of sweating, and tachycardia which began within 15 min in contrast to the insulin-induced tachycardia which began at approximately 30 min.

Discussion. Cholinergic and adrenergic pathways exist in the hypothalamus and have been the subject of a recent review (1). Although the ventromedial nucleus which has been shown to be an important site of hypothalamic control of growth hormone release (6) is low in both cholinergic and adrenergic neurons, an adrenergic control mechanism for growth hormone secretion has been indicated in two separate laboratories (2, 7). An antagonistic cholinergic control mechanism was therefore suspected but could not be demonstrated by the present experiments. Atropine in quantities large enough to produce significant signs and symptoms (inhibition of sweating, dry mouth, and tachycardia) did not alter basal plasma HGH values or influence hypoglycemia-induced growth hormone elevations. Although atropine in large doses produces central nervous system effects, the possibility can not be excluded that hypothalamic tissue levels of atropine were insuffi-

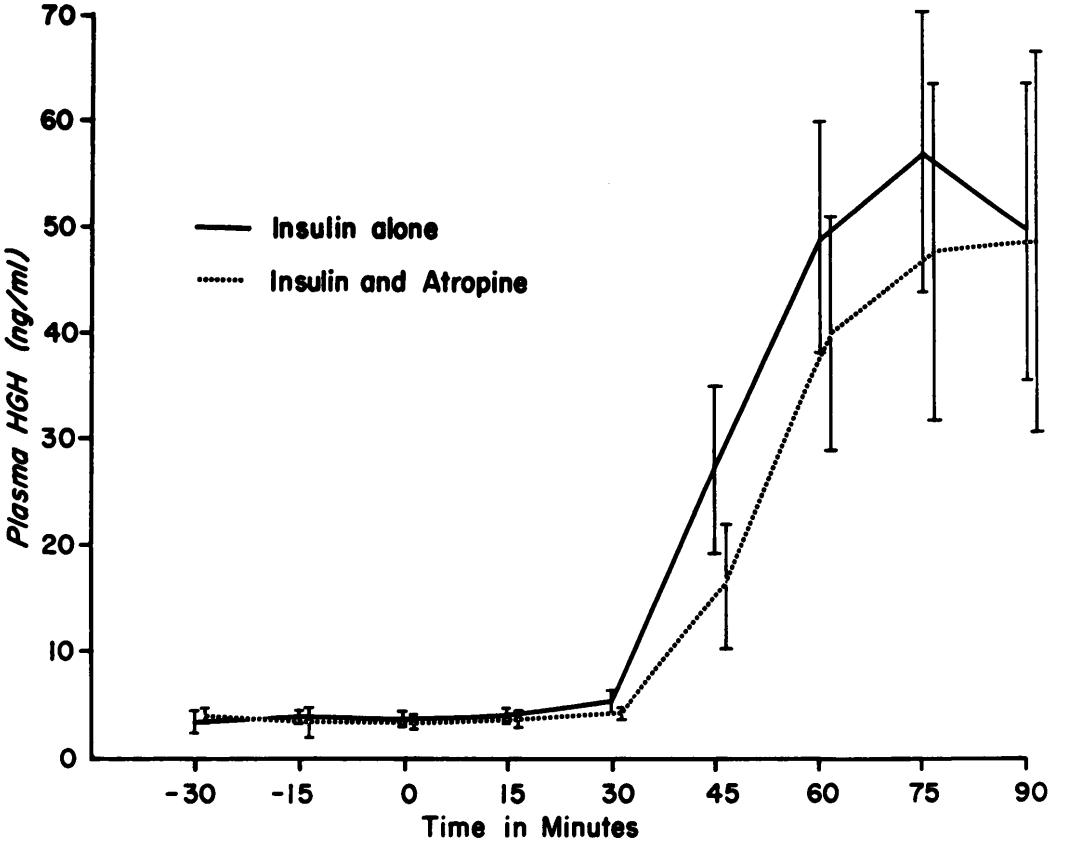

FIG. 3. Effect of atropine on insulin-induced plasma HGH elevations: insulin (0.1 U/kg) was administered at zero time; in the cholinergic blockade experiments 1 mg of atropine sulfate was given 5 min prior to insulin injection; means ± SEM are shown (6 subjects).

cient to produce cholinergic blockade in these experiments.

An important variable in growth hormone testing was identified in this study. When provocative tests of growth hormone secretion with insulin hypoglycemia were performed on consecutive days, the second day's response was considerably less than that on the first day. Too short a time interval between tests may be one of the factors causing the poor reproducibility of the plasma growth hormone response to insulin hypoglycemia reported by others (8, 9). Our studies did not establish the duration of this relative refractory period of the growth hormone secretory process to insulin hypoglycemia although the effect seems clearly dissipated in 2 weeks. Investigators performing paired biological studies on growth hormone secretion must take this refractory period into consideration. Previous growth hormone studies from our laboratory (2, 3) have utilized paired biological data but these tests were not done on consecutive days. Furthermore, our practice of randomizing the order in which the control and experimental tests were performed would detect any effect due to refractory growth hormone secretion. Whether a relative refractory period exists when two different stimuli such as insulin and arginine are given on consecutive days has not been established.

Summary. Atropine in amounts sufficient to produce moderate signs and symptoms did not influence base-line plasma HGH values or the plasma HGH rise following insulin hypoglycemia. A relative refractory period in HGH responsiveness to insulin hypoglycemia was observed when insulin tolerance tests

were performed on consecutive days.

1. Shute, C. C. D. and Lewis, P. R., Brit. Med. Bull. **22**, 221 (1968).

2. Blackard, W. G. and Heidingsfelder, S. A., J. Clin. Invest. **47**, 1407 (1968).

3. Heidingsfelder, S. A. and Blackard, W. G., Metabolism **17**, 1019 (1968).

4. Saifer, A. and Gerstenfeld, S., J. Lab. Clin. Med. **51**, 448 (1958).

5. Schalch, D. S. and Parker, M. L., Nature **203**, 1141 (1964).

6. Frohman, L. A., Bernardis, L. L., and Kant, K. J., Science **162**, 580 (1968).

7. Imura, H., Kato, Y., Ikeda, M., Morimoto, M., and Yawata, M., Program of Third Intern. Congr. Endocrinol., **1968**, 17.

8. Mintz, D. H., Finster, J. L., Taylor, A. L., and Fefer, A., Am. J. Med. **45**, 187 (1968).

9. Greenwood, F. C., Landon, J., and Stamp, T. C. B., J. Clin. Invest. **45**, 429 (1966).

Received Dec. 20, 1968. P.S.E.B.M., 1969, Vol. 131.

Properties of Mycobacteriophage DS6A
I. Immunogenicity in Rabbits* (33838)

B. U. BOWMAN[1] (Introduced by Henry G. Cramblett)

The Ohio State University College of Medicine, Department of Medical Microbiology, Columbus, Ohio 43210

Mycobacteriophage DS6A was found by Redmond and Cater (1) and Redmond (2) used it to identify human strains of mycobacteria in phage typing of the genus. In edition, Mankiewicz and Béland (3) reported producing experimental sarcoid lesions by injecting phage DS6A and *Mycobacterium tuberculosis*, strain $H_{37}Rv$, into guinea pigs. They found neutralizing activity in 15 of the 16 sera tested (94%). The 16 sera had neutralizing activity against a heterologous unnamed phage in the same proportion (94%); and the neutralizing activity against a second heterologous unnamed phase was reported as 80%. Since Mankiewicz and Béland's neutralization experiments did not yield quantitative data (3), it seemed desirable to perform quantitative immunogenicity and neutralization studies with this phage. In addition, since the results of studying the immunogenicity of phages R1, D29, and Leo in rabbits were recently presented (4), a need existed for comparing the immunogenicity of phage DS6A with the three former phages. Accordingly, this report gives the results obtained after injecting rabbits with mycobacteriophage DS6A, and the results of some cross neutralization experiments are presented also.

Materials and Methods. TB broth. For liquid cultures, the medium used contained the following ingredients: TB broth base (Difco), 11.6 g; glycerol, 10 ml; bovine serum albumin, fraction V (Armour and Company) 0.15 g; water 1000 ml. The pH was adjusted to 7.0 before autoclaving.

TB bottom agar. The TB broth was solidified with agar (Difco), 10 g/liter. After autoclaving, the medium was distributed in 30 ml amounts in sterile plastic petri plates.

TB soft agar. This consisted of 1 liter of TB broth with 7.0 g of agar. Before autoclaving, the medium was distributed in 3-ml amounts in test tubes. For use, the TB soft agar was melted at 100° and held at 44° for inoculation with phage and bacteria.

Mycobacteria. Mycobacterium tubercu losis, strain $H_{37}Rv$, was obtained from Dr. W. B. Redmond.[2] The BCG was obtained from a commercial vaccine preparation (Eli Lilly and Company). Stocks of each strain were maintained on Lowenstein-Jensen slants, with

* Supported by an American Thoracic Society-National Tuberculosis Association Grant.

[1] Recipient of an NIH Research Career Development Award.

[2] Chief, Tuberculosis Research Laboratory, Veteran's Administration Hospital, Atlanta, Georgia.