Advances in

ENZYME REGULATION

Volume 8

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Proceedings of the eighth symposium on Regulation of Enzyme Activity and Synthesis in Normal and Neoplastic Tissues held at Indiana University School of Medicine Indianapolis, Indiana September 29 and 30, 1969

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FOREWORD

Advances in Enzyme Regulation is now in its eighth volume. The appreciative reception of this series reflected the need for such a source of information, inspiration, and laboratory and teaching companion.

Volume 8 concentrates on subjects which have reached the stage of productive summarization and critical evaluation in the light of extensive new results. This book also lives up to its goal of advancing a few steps ahead of the general front of mammalian enzyme regulation studies.

It has been my editorial policy to impose as few restrictions as possible, emphasizing, however, the objectives of excellence of contribution, perfection in presentation, and penetration and scope in interpretation. This principle gives a wide range of freedom to the participants to express their concepts. Thus, the responsibility for detail—accuracy of reporting, preciseness of references, allocations of priority, expressions of judgment and evaluation—lies with the individual authors.

The Editor, who enjoyed the advice of leaders in the field, has been organizing the Symposia and selecting new topics and speakers on the basis of immediate and long-range significance of the scientific contributions. It is hoped that the comments and suggestions of investigators and teachers in this field will continue to come to the Editor's office and contribute to shaping the course of forthcoming conferences and volumes.

Indiana University
1969

GEORGE WEBER, *Editor*

ACKNOWLEDGMENTS

THIS is the eighth in a series of Symposia dedicated entirely to problems and advances in regulation of enzyme activity and synthesis in mammalian systems.

I take great pleasure in expressing appreciation for the support and assistance I received in organizing and conducting this Conference. I wish gratefully to acknowledge that Indiana University School of Medicine, Burroughs Wellcome and Co., Hoffman LaRoche, Eli Lilly and Co., and the Squibb Institute for Medical Research provided the financial support for this Meeting.

In the planning of the program, selection of participants and arrangements for the Symposium the advice of the following was invaluable: J. Ashmore, G. F. Cahill, Jr., Sir H. A. Krebs, H. P. Morris, V. R. Potter and C. G. Smith.

I am very obliged to Drs. Cahill, Estabrook, Houck, E. G. Krebs, H. A. Krebs, LePage, Morris, Potter, Smith and Stadtman for serving as chairmen of the sessions, and to all contributing authors for their cooperation in the preparation of this volume.

At Indiana University School of Medicine in the local organization of the Symposium I had the kind assistance of Dean Glenn W. Irwin, Jr. The efficient and competent help of R. Dault in accommodation arrangements and the expert assistance of James Glore in the preparation of illustrations are very much appreciated.

Thanks are due to Delores Cameron, Cheryl Catt, Maureen Higgins, Freida Jones, Dr. Patrick C. Logan and Sarah Mertz, members of my staff, who assisted in the local arrangements and in the typing of the manuscripts.

My highest appreciation is due to my wife, Catherine E. Forrest Weber, whose contribution to the format and English style has been most valuable in the assembling of this volume.

GEORGE WEBER
Symposium Chairman

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HORMONES AND SUBSTRATES IN THE REGULATION OF GLUCONEOGENESIS IN FASTING MAN

E. MARLISS, T. T. AOKI, P. FELIG, T. POZEFSKY and G. F. CAHILL, JR.*

Joslin Diabetes Foundation and the Department of Medicine, Harvard Medical School and the Peter Bent Brigham Hospital, Boston, Massachusetts

INTRODUCTION

IN previous volumes of this series, data have been presented in which energy balance was quantified and aspects of its control demonstrated in man subjected to prolonged fasting (1, 2). Man adapts to the fasted state by an accelerated consumption of fat and its breakdown products as fuel and a sparing of body protein. That the protein conservation is the result of a marked attenuation of hepatic gluconeogenesis was suggested by the observation of diminished urinary nitrogen excretion (3), and confirmed by the measurement of splanchnic substrate exchange (4).

The reduced rate of gluconeogenesis is related to the adaptation of the brain to utilization of acetoacetate and β-hydroxybutyrate as principal substrates in place of glucose (5).

Recent studies of the individual amino acids as gluconeogenic substrates have shown that alanine is quantitatively the most important (6). The marked diminution in hepatic alanine uptake in prolonged fasting is due to diminished plasma concentration inasmuch as its fractional extraction remains unchanged. Furthermore, exogenous alanine administration results in a prompt hyperglycemic response due to its conversion to glucose (7), suggesting that hepatic gluconeogenic mechanisms remain intact.

Studies of forearm muscle metabolism from this laboratory have demonstrated that the decrease in plasma alanine concentration in starvation results from a marked decrease in its release from muscle, the body's principal protein store (8). Figure 1 demonstrates the forearm arteriovenous differences of amino acids in the postabsorptive and prolonged-fasted states. Of particular interest is that those amino acids showing significant forearm release in both states correspond in pattern and magnitude to the splanchnic uptake previously described (6).

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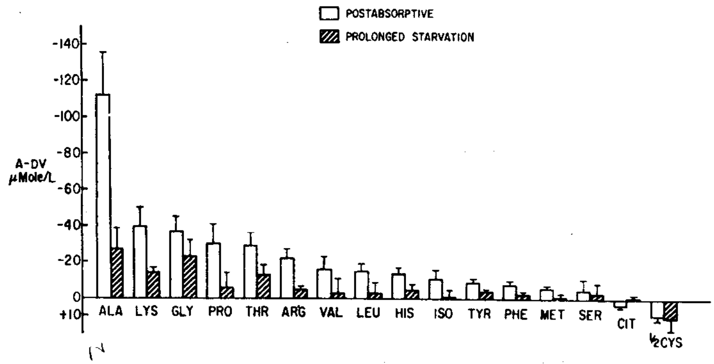

FIG. 1

Amino acid balance across forearm muscle tissue in the post-absorptive state and after 4–6 weeks starvation. A-DV=arterio-deep venous difference.

Thus, the rate-limiting step in the control of hepatic gluconeogenesis in prolonged starvation is seen to lie in the release of precursor substrate from the periphery. This occurs despite a substrate-hormone milieu well-documented as favoring hepatic gluconeogenesis: elevated free fatty acid levels, lowered insulin levels, and the associated increase in the activity of key gluconeogenic enzymes.

The roles of other hormones in influencing the flux of substrates and rate of gluconeogenesis have been investigated. Glucagon has long been known to augment hepatic gluconeogenesis in intact man (9) and in liver studied *in vitro* (10). Technical difficulties in the measurement of circulating levels have thus far yielded conflicting estimates of its response in the fasted state (11–13). No data have previously been obtained in prolonged fasting.

Similarly, growth hormone by virtue of its known adipokinetic, anabolic and glucoregulatory effects has been proposed as an important fasting hormone (14). Though no significant elevation in serum growth hormone levels occurs in fasted obese subjects, an increased sensitivity to its effects has not thus been excluded (15).

To further examine the possible physiological roles of glucagon and growth hormone in prolonged-fasted man, we have administered them to such subjects and have investigated the metabolic responses.

MATERIALS AND METHODS

Ten obese nondiabetic subjects between the ages of 23 and 52 years (five males and five females) were admitted to the Clinical Research Center of the Peter Bent Brigham Hospital for voluntary prolonged therapeutic starvation. All the patients fasted five to six weeks, during which time their daily oral intake was 1500 ml of water, 17 mEq NaCl, 13 mEq KCl and one multivitamin tablet. Techniques of urine collection, blood sampling and analysis are described elsewhere (4, 6).

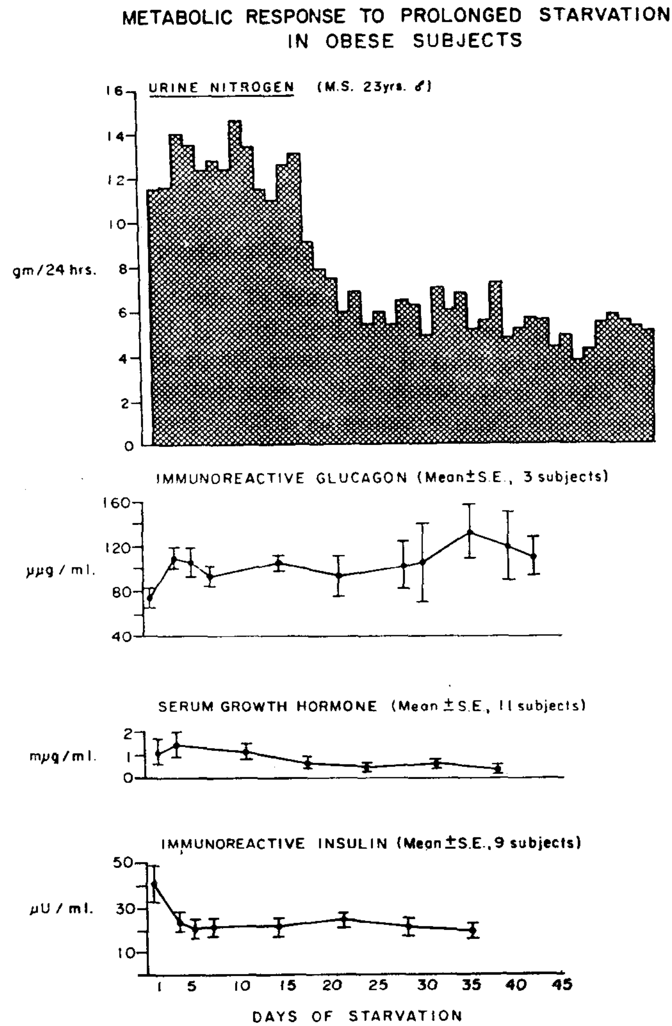

FIG. 2

Metabolic response to prolonged starvation in obese subjects. Daily urine nitrogen excretion is shown for a typical patient. Values for immunoreactive glucagon and growth hormone during fasting are not significantly different from post-absorptive. (Immunoreactive glucagon determinations performed by Dr. R. H. Unger.)

Six patients received glucagon (crystalline, Eli Lilly and Company) by constant intravenous infusion in an albumin-containing solution over 48 hr during the fifth week of fasting. Daily dose was 10 mg in three patients, 1 mg in two patients, and 0.1 mg in one patient. Immunoreactive glucagon determinations were kindly performed by Dr. Roger H. Unger, by a recent procedure developed to exclude non-pancreatic glucagon immunoreactivity (16).

Four further patients received human growth hormone (generously supplied by Dr. M. S. Raben) for three days in a daily dose of 10 mg, administered as 5 mg intramuscularly every 12 hr.

RESULTS AND DISCUSSION

Figure 2 shows the metabolic response of obese patients to prolonged fasting. The urine nitrogen excretion drops from 11–15 g/day to a plateau of 4–6 g/day, representing marked curtailment of protein mobilization from 70–90 g to 25–35 g daily. Serum immunoreactive glucagon shows a small increase from postabsorptive values ($p < 0.05$, paired t). Serum growth

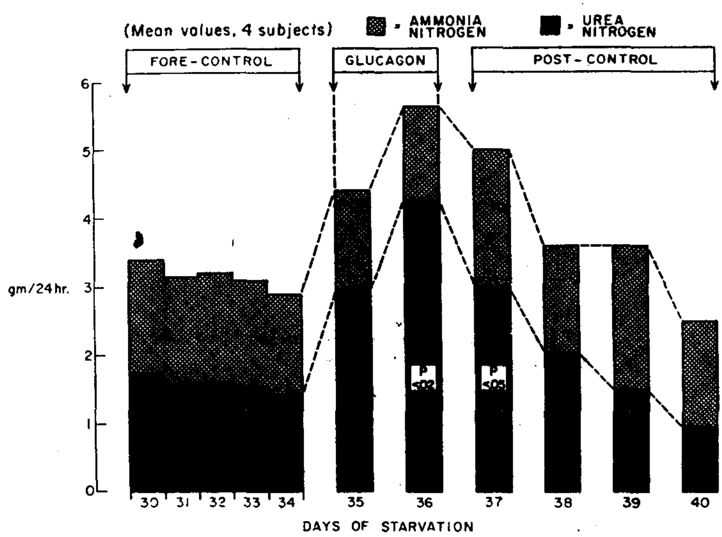

FIG. 3

Influence of glucagon on urinary ammonia and urea nitrogen excretion during prolonged starvation. The increase in total nitrogen is due to a significant increase in the urea nitrogen fraction only (days 35 through 40 vs. mean of fore-control, paired t).

hormone concentrations do not change, but immunoreactive insulin falls significantly, as previously documented (4).

In the glucagon-infused subjects a steady-state elevation of glucose and insulin was induced, which in the 10 mg/day studies was 42 ± 9 mg/100 ml for glucose and 27 ± 5 μU/ml for insulin. Figure 3 demonstrates the marked increase in nitrogen excretion which occurred due entirely to the urea fraction, with no alteration in ammonia. That the increment in gluconeogenesis thus induced is due to augmented hepatic gluconeogenic activity is further suggested by the striking fall in plasma amino acid concentrations. All amino acids measured showed a decrease from baseline at one or more of the sampling intervals during glucagon infusion. Figures 4 and 5 show those amino acids for which a significant decrease ($p < 0.05$, paired t) in concentration was found. Most showed a trend toward baseline values by 48 hr. Figure 4 shows those amino acids whose nadir in concentration was at 24 hr, Fig. 5 those at 8 hr. These alterations are similar to those recently reported after subcutaneous glucagon injection in the postabsorptive state (17).

To be noted in Fig. 5 is that with the exception of taurine, lysine and histidine, the remainder are the amino acids which are known to be most sensitive to ambient insulin concentration (18). With the smaller glucagon doses no

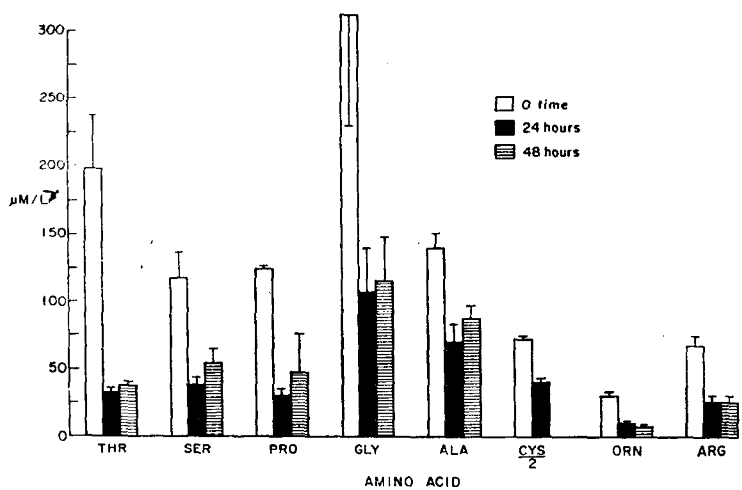

FIG. 4

Plasma amino acid response to continuous glucagon infusion over 48 hr (10 mg/24 hr). 0 time values are typical of those reported in prolonged-fasted man (6). Amino acids whose maximum decrease in concentration occurred at 24 hr are shown. (Mean ±SE.)