*Journal of Clinical Investigation*
*Vol. 45, No. 9, 1966*

# Insulin Secretion in Response to Protein Ingestion *

JOHN C. FLOYD, JR.,† STEFAN S. FAJANS, JEROME W. CONN, RALPH F. KNOPF, AND JUAN RULL

*(From the Department of Internal Medicine, Division of Endocrinology and Metabolism and the Metabolism Research Unit, the University of Michigan, Ann Arbor, Mich.)*

We have reported previously that the oral or intravenous administration of the amino acid *l*-leucine to healthy subjects results in increases in plasma insulin and decreases in blood glucose and plasma free fatty acids (2, 3). After the administration of leucine to healthy subjects pretreated with either chlorpropamide or tolbutamide (2, 3), and also to some patients with functioning islet cell tumors of the pancreas (4), increments in plasma leucine caused increases in plasma insulin and decreases in blood glucose that were significantly greater than those observed in healthy subjects not pretreated with sulfonylurea drugs. We have also shown that increased release of insulin from the pancreatic beta cells is the mechanism by which leucine increases peripheral levels of insulin (5). We suggested that a rising plasma level of leucine is a physiologic stimulus for the release of insulin, and that the more pronounced sensitivity to leucine hypoglycemia produced experimentally by administration of sulfonylureas and observed in some patients with idiopathic hypoglycemia or insulin-secreting tumors of the pancreas represents a great exaggeration of a normal physiologic phenomenon (6, 7).

In an effort to determine the effect of leucine on insulin release under physiologic circumstances, protein meals rich in leucine were fed to healthy subjects, and the levels of plasma insulin, leucine, amino nitrogen, free fatty acids, and blood glucose were measured. It was surprising, indeed, to find, under these circumstances, that the increases of plasma leucine could not account for the sharp increments of plasma insulin that were observed. It was clear that protein ingestion was either intensifying the effect of the leucine contained therein or was acting to increase insulin secretion in another way.

## Methods

Twenty healthy males and 1 healthy female between the ages of 18 and 36 years served as subjects for this study. Each subject continued his usual unrestricted diet but took no food for at least 8 hours before the ingestion of the protein test meal. Before some of the protein tests, 7 subjects were treated with chlorpropamide [1] for 3 to 18 days. One g was given at noon of the first day and 0.5 g at the same time daily thereafter.

Test meals consisted of 500 g of either cooked ground beef or chicken liver calculated to contain 8.0 and 10.2 g of leucine, respectively. Beef was stripped of connective tissue and fat, ground twice, and then stored frozen in packages of 250 g each. It was broiled immediately before consumption. Chicken liver was prepared in a similar fashion. After a 30-minute control period, the subjects consumed the meals within 25 to 30 minutes. Beef was fed 23 times to 20 subjects. Chicken liver was fed 19 times to 19 subjects. In addition, after pretreatment with chlorpropamide, beef was fed 12 times to 7 subjects and chicken liver 8 times to 4 subjects. Peripheral venous blood, and in some cases simultaneously obtained peripheral arterial blood, were obtained 30, 15, and 1 minutes and immediately before ingestion of the meals. Thereafter, samples were usually obtained at 10-minute intervals for the first hour, 15-minute intervals for the second hour, and at 30-minute intervals for a total of 3 to 8 hours.

Concentrations of blood glucose were determined with the Technicon autoanalyzer with frequent verification by the Somogyi-Nelson technique (8). Levels of plasma insulin were determined by the immunoassay of Yalow and Berson (9). The details of the assay as performed in this laboratory have been described in earlier publications (3, 10). Concentrations of plasma leucine were determined by modification of the technique of Berry (11), plasma amino nitrogen by the method of Frame,

* Submitted for publication February 24, 1966; accepted June 9, 1966.

Supported in part by U. S. Public Health Service grants AM-02244, AM-00888, and TI-AM-5001 from the National Institute of Arthritis and Metabolic Diseases, and U. S. Public Health Service Career Research Award 5-K6-AM-14,237.

Presented in part before the Thirty-seventh Annual Meeting of the Central Society for Clinical Research, November 6 and 7, 1964 (1).

† Address requests for reprints to Dr. John C. Floyd, Jr., University Hospital, University of Michigan Medical Center, Ann Arbor, Mich. 48104.

[1] 1-Propyl-3-(*p*-chloro-benzenesulfonyl) urea.

TABLE I

*Levels of plasma insulin after ingestion of*

| Subject | Food | Time in minutes: −30 | −15 | −1 | 0 | 5 | 10 | 15 | 20 | 30 | 40 | 50 | 60 | 75 | 90 | 105 | 120 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | | | | | | *μU/ml* | | | | | | | |
| J.B. | B | | | 13 | 13 | | | | | 26 | | | 49 | | 35 | | 42 |
| W.B. | B | 11 | 7 | 11 | 8 | | | 12 | 8 | 7 | 17 | 17 | 29 | 29 | 27 | 29 | 29 |
| B.E. | B | | | 9 | 10 | | | 12 | 14 | 24 | 33 | 32 | 31 | 25 | 27 | 17 | 20 |
| J.F. | B | 14 | 14 | 20 | 22 | | | 35 | 40 | 32 | 32 | 32 | 33 | 45 | 40 | 33 | 33 |
| M.G. | B | | | | 8 | | | | | 12 | | | 27 | | 26 | | 24 |
| L.H. | B | 18 | 18 | | 22 | | | 21 | 30 | 28 | 37 | 46 | 49 | 39 | 59 | 50 | 34 |
| J.V. | B | | | 8 | 11 | | | 11 | 6 | 10 | 11 | 21 | | 13 | 13 | 14 | 17 |
| J.W. | B | | | | 1 | | | | | 59 | | | 60 | | 71 | | 55 |
| J.W. | B | | | | 5 | | | | | 18 | | | 29 | | 12 | 7 | |
| R.F. | B | 9 | 7 | 9 | 8 | | 17 | | 13 | 14 | 21 | 23 | 20 | | 39 | | 37 |
| G.M. | B | | | 13 | 16 | | 11 | | 44 | 39 | 49 | 53 | 63 | | 80 | | 59 |
| B.E. | CL | | | 8 | 10 | | 15 | | 25 | 41 | 41 | 45 | 45 | | 42 | | 23 |
| J.C. | CL | | | 11 | 11 | | | 30 | 50 | 54 | 58 | 45 | 42 | 35 | 26 | 27 | 18 |
| J.F. | CL | 10 | 10 | 10 | 11 | | | 42 | 33 | 33 | 38 | 36 | 35 | 30 | 22 | 20 | 18 |
| M.G. | CL | | | 8 | 8 | | | | | 10 | | | 20 | | 17 | | 8 |
| L.H. | CL | 5 | 6 | | 5 | | | 12 | 23 | 28 | 35 | 34 | 30 | 30 | 43 | 28 | 27 |
| J.K. | CL | | | | 13 | | | | | | | | 20 | | 15 | | 14 |
| J.V. | CL | | | 10 | 9 | | | 17 | 12 | 20 | 32 | 26 | | 24 | 17 | 20 | 44 |
| J.W. | CL | | | 8 | 12 | | | | | 41 | | | 31 | | 12 | | 23 |
| D.H. | CL | | 4 | 2 | 1 | 12 | 5 | | 13 | 29 | 29 | 28 | 24 | | 29 | | 20 |
| J.A. | CL | | 8 | 4 | 4 | 14 | 33 | | 36 | 35 | 35 | 38 | 40 | | 35 | | 24 |
| G.M. | CL | | 7 | | 7 | | | | 32 | | 31 | | 31 | | 34 | | 12 |
| Mean | | 11 | 9 | 10 | 10 | | 16 | 21 | 25 | 28 | 33 | 34 | 35 | 30 | 33 | 25 | 28 |
| Range | | (5–18) | (4–18) | (2–20) | (1–22) | | (5–33) | (12–42) | (6–50) | (7–59) | (11–58) | (17–53) | (20–63) | (13–45) | (12–80) | (7–50) | (8–59) |

Russell, and Wilhelmi (12), and plasma FFA by the method of Dole (13).

## Results

### *Plasma insulin*

*Without chlorpropamide pretreatment.* Immunoassay was performed on samples from 22 tests. After the beginning of the meals, plasma insulin rose in each test (Table I). Maximal increases ranged from 11 to 70 μU per ml (mean 31 μU per ml) and occurred at 15 to 225 minutes (mean 76 minutes). Mean concentration of plasma insulin rose from a control value of 10 μU per ml to a peak of 35 μU per ml at 1 hour (Table I and Figure 1). Mean concentration of plasma insulin declined thereafter but was still elevated above control levels at the fourth hour. Actually, plasma insulin was elevated at the fourth hour in 12 tests (6 each of beef and chicken liver, Table I). In subject G.M. plasma insulin had not returned to control levels by the eighth hour (Figure 2). In subject B.E. (chicken liver) plasma insulin returned to control levels at 5½ hours.

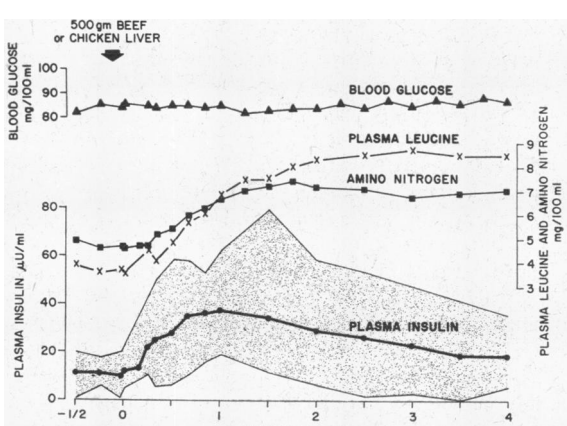


FIG. 1. EFFECT OF PROTEIN MEALS UPON MEAN PLASMA LEVELS OF INSULIN (22 TESTS), LEUCINE, AND AMINO NITROGEN AND UPON BLOOD GLUCOSE IN HEALTHY SUBJECTS.

### *Plasma leucine*

*Without chlorpropamide pretreatment.* Plasma levels of leucine, measured in 25 experiments, rose after ingestion of the meal and were still elevated at the fourth hour to levels above control (Table II). Maximal increases ranged from 3.8 to 10.3 mg per 100 ml (mean 5.8 mg per 100 ml) and occurred between 120 and 240 minutes (mean 193 minutes). Mean concentration of plasma leucine rose from a control value of 3.7 mg per 100 ml to

TABLE I

*500 g cooked beef (B) or chicken liver (CL)*

| Time in minutes | | | | | | | | | | | | | | | | Maximal increase | Time of maximal increase |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 135 | 150 | 165 | 180 | 195 | 210 | 225 | 240 | 270 | 300 | 330 | 360 | 390 | 420 | 450 | 480 | | |
| | | | | | | *μU/ml* | | | | | | | | | | *μU/ml* | *minutes* |
| 36 | 35 | 33 | 29 | 26 | 25 | 16 | 29 | | | | | | | | | 36 | 60 |
| | 31 | | 26 | | | | | | | | | | | | | 22 | 150 |
| | 22 | | 26 | | 24 | | 23 | | | | | | | | | 23 | 40 |
| | 24 | | 23 | | 18 | | 20 | | | | | | | | | 23 | 75 |
| 20 | 20 | 15 | 17 | 20 | 13 | 15 | 11 | | | | | | | | | 19 | 60 |
| | 38 | | 32 | | 29 | | 36 | | | | | | | | | 37 | 90 |
| | 20 | | | | 11 | | 10 | | | | | | | | | 11 | 50 |
| 39 | 54 | 48 | 45 | 25 | 15 | 18 | 13 | | | | | | | | | 70 | 90 |
| 26 | 21 | 21 | 13 | 19 | 11 | 14 | 6 | | | | | | | | | 24 | 60 |
| | 23 | | 15 | | 16 | | 6 | 7 | 6 | 8 | 13 | 8 | 8 | 13 | 14 | 30 | 90 |
| | 40 | | 31 | | 24 | | 27 | 26 | 31 | 35 | 37 | 28 | 27 | 23 | 29 | 65 | 90 |
| | 18 | | 23 | | 25 | | 18 | 19 | 14 | 10 | 20 | 21 | 8 | 7 | 10 | 36 | 60 |
| | 16 | | 7 | | 8 | | | | | | | | | | | 43 | 40 |
| | 14 | | 24 | | 20 | | 21 | | | | | | | | | 31 | 15 |
| 10 | 11 | 6 | 15 | 10 | 11 | 11 | 11 | | | | | | | | | 12 | 60 |
| | 26 | | | | | | 19 | | | | | | | | | 38 | 90 |
| 14 | 18 | 16 | 17 | 17 | 13 | 26 | 14 | | | | | | | | | 13 | 225 |
| | 28 | | | | 30 | | 20 | | | | | | | | | 34 | 120 |
| 1 | 2 | 2 | 3 | 0 | 0 | | 10 | | | | | | | | | 31 | 30 |
| | 9 | | 13 | | | | 11 | | | | | | | | | 27 | 30 |
| | 27 | | 26 | | | | 34 | | | | | | | | | 36 | 60 |
| | 14 | | 15 | | 18 | | 8 | 3 | 22 | 11 | 11 | 8 | | 9 | 14 | 27 | 90 |
| 21 | 23 | 20 | 21 | 17 | 17 | 17 | 17 | | | | | | | | | 31 | 76 |
| (1–39) | (2–54) | (2–48) | (3–45) | (0–26) | (0–30) | (11–26) | (6–36) | | | | | | | | | (11–70) | (15–225) |

levels between 8.3 and 8.7 mg per 100 ml from the second to the fourth hour. Figure 1 shows mean levels of plasma leucine for 16 of the 22 tests in which plasma insulin was measured. At 1 hour when plasma leucine had increased only 3.1 mg per 100 ml over control levels, mean plasma insulin was maximal. In 14 tests initial increases in plasma leucine (> 0.4 mg per 100 ml) and plasma insulin (> 6 μU per ml) were concurrent. In subjects B.E. and J.W. (beef) there were no detectable increases in plasma leucine at 30 minutes, when plasma insulin had increased 14 and 58 μU per ml, respectively, from control levels (Table I).

*With chlorpropamide pretreatment.* Plasma leucine was measured in 14 tests (Table II). Maximal increases ranged from 3.5 to 7.8 mg per 100 ml (mean 6.0 mg per 100 ml) and occurred at 60 to 300 minutes (mean 173 minutes). Mean concentration of plasma leucine rose from a control value of 3.8 mg per 100 ml to levels between 8.1 and 8.6 mg per 100 ml from the second to the fourth hour. These values are not significantly different from those obtained without pretreatment with chlorpropamide.

*Plasma amino nitrogen*

*Without chlorpropamide pretreatment.* Plasma concentrations of amino nitrogen were measured in 22 tests (Table II). Levels rose in each test and were still elevated at the fourth hour. Maximal increases ranged from 2.2 to 4.6 mg per 100 ml (mean 3.2 mg per 100 ml) and occurred at 50 to 240 minutes (mean 129 minutes). Mean concentration of plasma amino nitrogen rose from a

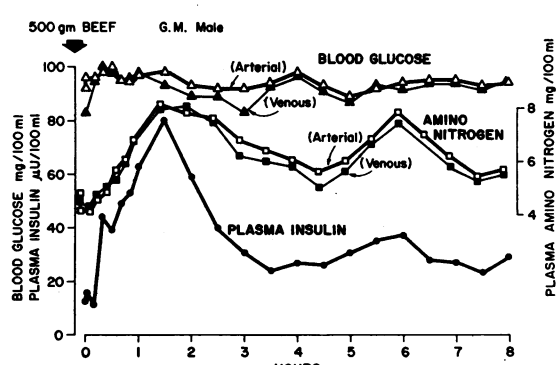


FIG. 2. EFFECT OF A PROTEIN MEAL UPON VENOUS PLASMA INSULIN AND VENOUS AND ARTERIAL PLASMA AMINO NITROGEN AND BLOOD GLUCOSE IN A HEALTHY SUBJECT.

TABLE II

*Levels of plasma leucine and amino nitrogen, blood glucose, and plasma*

| | No. tests | | No. days of chlorpropamide pretreatment | Time in minutes | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | −30 | −15 | −1 | 0 | 10 | 15 | 20 | 30 | 40 | 50 |
| | | | | *mg/100 ml* | | | | | | | | | |
| Plasma leucine | 25 | Mean | | 3.9 | 3.6 | 3.7 | 3.6 | 3.3 | 4.5 | 4.1 | 4.8 | 5.7 | 6.1 |
| | | Range | | (2.8–4.7) | (2.6–4.7) | (2.3–5.4) | (2.1–5.2) | (2.1–4.1) | (3.5–6.2) | (3.2–5.3) | (2.8–8.0) | (3.9–7.1) | (4.7–6.9) |
| | | No. samples | | 11 | 11 | 20 | 25 | 6 | 10 | 12 | 24 | 12 | 12 |
| | 14 | Mean | 7.5 | | | | 3.8 | | | | 4.9 | | |
| | | Range | (3–18) | | | | (3.0–4.6) | | | | (2.6–9.2) | | |
| | | No. samples | | | | | 12 | | | | 14 | | |
| Plasma amino nitrogen | 22 | Mean | | 4.9 | 4.6 | 4.7 | 4.6 | 4.7 | 4.7 | 5.2 | 5.4 | 6.0 | 6.3 |
| | | Range | | (4.1–6.1) | (3.6–5.4) | (4.3–5.1) | (3.7–6.0) | (4.1–5.4) | (3.8–5.3) | (4.3–6.2) | (4.6–6.6) | (4.8–7.1) | (5.1–7.2) |
| | | No. samples | | 9 | 12 | 8 | 18 | 9 | 7 | 16 | 20 | 15 | 18 |
| Blood glucose | 42 | Mean | | 80 | 85 | 85 | 86 | 86 | | 87 | 88 | 87 | 86 |
| | | Range | | (73–91) | (79–96) | (76–96) | (77–101) | (78–95) | | (80–100) | (79–98) | (78–95) | (78–97) |
| | | No. samples | | 21 | 21 | 42 | 42 | 19 | | 28 | 42 | 28 | 28 |
| | 20 | Mean | 6.3 | | | 79 | 80 | | | | 80 | | |
| | | Range | (3–18) | | | (68–94) | (68– 93) | | | | (65–98) | | |
| | | No. samples | | | | 16 | 19 | | | | 20 | | |
| | | | | *μEq/L* | | | | | | | | | |
| Plasma free fatty acids | 33 | Mean | | 621 | 657 | 571 | 610 | | | 583 | 595 | 573 | 471 |
| | | Range | | (283–1,083) | (286–1,022) | (206–955) | (286–1,079) | | | (354–951) | (174–1,166) | (256–905) | (266–850) |
| | | No. samples | | 18 | 18 | 27 | 32 | | | 21 | 31 | 23 | 19 |
| | 10 | Mean | 6.8 | | | | 561 | | | | 516 | | |
| | | Range | (3–18) | | | | (185–1,187) | | | | (271–897) | | |
| | | No. samples | | | | | 9 | | | | 10 | | |

control value of 4.7 mg per 100 ml to a peak value of 7.4 mg per 100 ml at 105 minutes (Table II). Figure 1 shows mean levels of plasma amino nitrogen for 17 of the 22 tests in which plasma insulin was measured. One hour after the beginning of ingestion of protein meals, when mean plasma amino nitrogen had increased 2 mg over control levels, mean plasma insulin was maximal. In 1 test a significant initial increase in plasma amino nitrogen (> 0.4 mg per 100 ml) preceded the increase in plasma insulin. In 8 tests the increases were concurrent. In 5 tests there was no significant increase in plasma amino nitrogen that either immediately preceded or was concurrent with the increase in plasma insulin. However, in 4 of these 5 tests an increase of at least 0.1 mg per 100 ml in plasma amino nitrogen had occurred before or at the time of initial increase in plasma insulin. (In 3 of the 17 tests plasma samples for the control period were not available for measurement of amino nitrogen.) Figure 2 shows the changes in levels of plasma amino nitrogen in peripheral arterial and venous blood and plasma insulin in peripheral venous blood after the ingestion of 500 g ground beef by subject G.M. Plasma amino nitrogen and plasma insulin increased, whereas changes in levels of blood glucose, especially in arterial blood, were small. During and subsequent to the major elevations of plasma insulin in the second hour, there was the development of a positive arteriovenous difference in blood glucose and subsequently in plasma amino nitrogen. Similar results were seen in subject B.E.

### *Blood glucose*

*Without chlorpropamide pretreatment.* Blood glucose was measured in all 42 tests. It increased after ingestion of the meals in 40 tests and decreased below control levels in 29 tests. Maximal increases ranged from 0 to 15 mg per 100 ml (mean 6 mg per 100 ml), exceeded 4 mg per 100 ml in 27 of 42 tests, and occurred at 20 to 240 minutes (mean 125 minutes) (Table II). In those 22 tests in which plasma insulin was measured, increases in blood glucose ranged from 0 to 7 mg per 100 ml (mean 2 mg per 100 ml) before or at the time of initial increases in plasma insulin.

TABLE II

*free fatty acids after ingestion of 500 g of cooked beef or chicken liver*

| Time in minutes | | | | | | | | | Maximal increase | Time of maximal increase | Maximal decrease | Time of maximal decrease |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 60 | 75 | 90 | 105 | 120 | 150 | 180 | 210 | 240 | | | | |
| *mg/100 ml* | | | | | | | | | *mg/100 ml* | *minutes* | | |
| 6.8 | 7.5 | 7.5 | 8.0 | 8.3 | 8.5 | 8.7 | 8.5 | 8.5 | 5.8 | 193 | | |
| (3.9–10.3) | (5.9–11.4) | (4.6–11.8) | (6.6–11.1) | (5.1–11.9) | (6.3–11.2) | (6.6–13.0) | (6.1–11.9) | (4.3–11.9) | (3.8–10.3) | (120–240) | | |
| 25 | 12 | 24 | 12 | 25 | 25 | 24 | 24 | 24 | | | | |
| 7.2 | | 7.6 | | 8.1 | 8.3 | 8.0 | 8.4 | 8.6 | 6.0 | 173 | | |
| (4.0–11.3) | | (5.8–9.4) | | (6.4–11.1) | (6.4–10.5) | (4.6–11.3) | (6.2–11.3) | (6.6–10.5) | (3.5–7.8) | (60–300) | | |
| 14 | | 14 | | 13 | 14 | 14 | 11 | 10 | | | | |
| 6.7 | 7.0 | 7.2 | 7.4 | 7.2 | 7.1 | 6.7 | 6.9 | 7.0 | 3.2 | 129 | | |
| (5.5–7.6) | (5.9–9.8) | (6.1–8.8) | (6.1–8.4) | (5.9–8.6) | (5.4–10.5) | (4.5–9.6) | (5.8–8.5) | (5.6–8.8) | (2.2–4.6) | (50–240) | | |
| 16 | 12 | 16 | 13 | 14 | 16 | 14 | 15 | 14 | | | | |
| | | | | | | | | | | | *mg/100 ml* | *mniutes* |
| 87 | 84 | 87 | 85 | 87 | 87 | 87 | 88 | 89 | 6 | 125 | 4 | 104 |
| (75–98) | (74–94) | (70–101) | (73–93) | (63–100) | (77–97) | (77–98) | (79–94) | (72–97) | (0–15) | (20–240) | (0–23) | (10–240) |
| 42 | 23 | 42 | 23 | 42 | 42 | 42 | 41 | 41 | | | | |
| 80 | | 78 | | 76 | 76 | 78 | 78 | 78 | 5 | 90 | 6 | 161 |
| (59–101) | | (51–97) | | (43–99) | (46–97) | (48–96) | (48–97) | (49–99) | (0–13) | (30–210) | (0–32) | (90–240) |
| 20 | | 20 | | 20 | 20 | 20 | 18 | 17 | | | | |
| | | | | *μEq/L* | | | | | | | *μEq/L* | |
| 496 | 406 | 390 | 386 | 395 | 366 | 373 | 425 | 429 | | | 307 | 132 |
| (178–940) | (204–690) | (214–982) | (190–609) | (200–1,072) | (152–1,029) | (114–1,217) | (152–1,147) | (228–1,109) | | | (0–731) | (30–210) |
| 33 | 19 | 33 | 19 | 32 | 32 | 29 | 30 | 28 | | | | |
| 486 | | 412 | | 410 | 422 | 370 | 378 | 512 | | | 231 | 123 |
| (178–877) | | (249–802) | | (224–948) | (224–786) | (170–521) | (240–551) | (255–1,017) | | | (0–666) | (60–180) |
| 10 | | 10 | | 9 | 9 | 10 | 8 | 8 | | | | |

Maximal decreases for all tests ranged from 0 to 23 mg per 100 ml (mean 4 mg per 100 ml), exceeded 4 mg per 100 ml in 14 of 42 tests, and occurred between 10 and 240 minutes (mean 104 minutes) (Table II). There were no significant changes in mean blood glucose (Table II). Figure 1 illustrates this finding in those tests in which plasma insulin was measured. An exceptional decrease in blood glucose was observed in subject R.F. After an increase in plasma insulin of 30 μU per ml (Table I, beef) blood glucose fell from 86 to 63 mg per 100 ml.

*With chlorpropamide pretreatment.* Blood glucose was measured in all 20 tests. Maximal decreases ranged from 0 to 32 mg per 100 ml (mean 6 mg per 100 ml), exceeded 4 mg in 6 of 20 tests, and occurred at 90 to 240 (mean 161) minutes (Table II). Again, there were no significant changes in mean blood glucose.

### *Plasma FFA*

*Without chlorpropamide pretreatment.* Plasma levels of free fatty acids were measured in 33 tests (Table II) and fell more than 100 μEq per L in 25. Maximal decreases ranged from 0 to 731 μEq per L (mean 307 μEq per L) and occurred at 30 to 210 minutes (mean 132 minutes). Mean concentration fell from a control value of 610 to 366 μEq per L at 150 minutes (Table II).

*With chlorpropamide pretreatment.* Plasma levels of free fatty acids decreased more than 100 μEq per L in 8 of 10 tests. Maximal decreases ranged from 0 to 666 μEq per L (mean 231 μEq per L) and occurred at 60 to 180 minutes (mean 123 minutes) (Table II). Mean concentration fell from a control value of 561 to 370 μEq per L at 180 minutes (Table II).

## Discussion

After healthy subjects had ingested protein meals, plasma levels of insulin increased consistently and significantly. These acute changes in insulin levels may be interpreted to reflect increases in the secretory rate of insulin, since *1*) the fractional rate of degradation of the hormone is independent of hormonal concentration over wide limits (14, 15), and *2*) we have demonstrated that intravenous administration of leucine and of

TABLE III

*Effect of the ingestion of leucine and protein meals upon plasma levels of insulin and leucine in healthy subjects*

| Test procedure | Mean of maximal increases | |
|---|---|---|
| | Plasma insulin | Plasma leucine |
| | *μU/ml* | *mg/100 ml* |
| Ingestion of 14 to 16 g leucine | 12* | 16.7† |
| Ingestion of protein meal containing 8.0 or 10.2 g leucine | 31 | 5.8 |

* Reference 3.
† Reference 2.

a mixture of 10 essential amino acids evokes increases in plasma insulin due to increased release of insulin from the pancreas (5, 16). It is to be emphasized, however, that the stimulation of insulin secretion in response to protein ingestion is a phenomenon that cannot be attributed solely or even largely to the effect of the leucine contained in the meals. It will be observed in Table III that after the ingestion of 14 to 16 g of leucine (200 mg per kg) the mean of maximal increases in plasma insulin was 12 μU per ml (3), and the mean of maximal increases in plasma leucine was 16.7 mg per 100 ml (2). On the other hand, after the ingestion of protein meals containing 8.0 or 10.2 g of leucine, the mean of maximal increases in plasma insulin was 31 μU per ml, whereas the mean of maximal increases in plasma leucine was only 5.8 mg per 100 ml. At 1 hour the increase in mean concentration of plasma leucine was only 3.2 mg per 100 ml, whereas mean plasma insulin had reached a peak value of 35 μU per ml (Figure 1 and Tables I, II). Increases in plasma insulin of this magnitude cannot be accounted for by these modest elevations of plasma leucine. Also, pretreatment with chlorpropamide, which accentuates leucine-induced changes in plasma insulin and blood glucose (2, 3), failed to accentuate the changes in blood glucose and plasma free fatty acids that occurred after the protein meals. In addition, levels of plasma insulin were measured on samples from 3 tests performed in chlorpropamide-pretreated subjects. Maximal increases in plasma insulin were 44, 40, and 29 μU per ml. These results are within the range and near the mean of the results for the subjects who were not pretreated with chlorpropamide. All of these observations suggest that amino acids in addition to leucine, or amino acids in combination with leucine, induce the large increases in plasma insulin observed after ingestion of the protein meals (7). In addition, Berger, Joondeph, and Rachmeler have reported that the ingestion of 400 calories of gelatin by healthy human subjects caused a significant increase in plasma insulin (17). They found a maximal increase in mean plasma insulin of 13 μU per ml, an increase that could be explained only in part by the leucine content of that protein (17). In other studies that have evolved from the present ones, we have been able to show that a series of essential amino acids other than leucine, when administered intravenously singly or in combination, produces significant increases in plasma insulin that exceed those produced by leucine (1, 18, 19). The results of the present study, which show that levels of amino acids (including leucine) and plasma insulin increase during the first half hour after the beginning of ingestion of a protein meal, suggest that an increase in plasma levels of certain amino acids constitutes a physiologic stimulus for insulin release. The parallelism between increases in plasma levels of amino nitrogen and plasma levels of insulin observed when these amino acids are administered intravenously (19) supports this view.

Increases in plasma amino nitrogen after ingestion of protein meals and increases in plasma insulin were not always concurrent, however. In 5 tests plasma insulin rose before plasma amino nitrogen had increased significantly ($> 0.4$ mg per 100 ml), although increases of at least 0.1 mg per 100 ml were detected in 4 of the 5. Perhaps the method employed to measure amino nitrogen is not sensitive enough to detect small changes in plasma levels. However, in 1 of these 5 tests plasma leucine was measured and was found to have increased concurrently with plasma insulin. The increase in plasma leucine, another index of absorption of amino acids, was concurrent with increases in plasma insulin in 17 of 19 tests, although its magnitude appears to have been insufficient to provoke the insulin response. The initial increases in plasma insulin found in the presence of minimal increases in plasma amino nitrogen and small increases of plasma leucine can be explained by assuming a potent insulin-releasing effect of particular amino acids other than or in combina-

tion with leucine (18, 19). Such amino acids may make only a small contribution of total amino nitrogen. Nevertheless, failure to detect consistent increases in levels of plasma amino nitrogen before or at the time of initial increases in plasma insulin in some tests as well as the lack of a temporal correlation between the mean plasma levels of amino nitrogen and insulin from the first to the fourth hour (Figure 1) suggests that something besides the increase in plasma concentration of amino acids acts as the stimulus for the release of insulin after the ingestion of protein meals. It has been shown that the intrajejunal administration of glucose evokes greater increments in plasma insulin than does the intravenous administration of the same amount of glucose at the same rate, even though levels of blood glucose are higher in the latter circumstance (20). It is possible that liberation of amino acids into the gastrointestinal tract during digestion of proteins, or their absorption, may similarly enhance insulin release by a mechanism not mediated directly by the plasma concentration of the absorbed amino acids.

The increases in blood glucose occurring after the ingestion of the protein meals are most likely derived from the metabolism of glucogenic amino acids. It is unlikely that the increases in blood glucose either caused or contributed importantly to the increases in plasma insulin observed. The onset of increases in plasma insulin either occurred without increases in blood glucose or were preceded or accompanied by increases in blood glucose of only up to 7 (mean 2) mg per 100 ml. Berger and co-workers also suggested that the ingestion of protein by normal human subjects increases insulin secretion by a nonglucose-mediated mechanism (17).

A metabolic effect of the hyperinsulinemia is observed in the decline in levels of free fatty acids. The decreases in free fatty acids were preceded and accompanied by appropriate increases in plasma insulin. Failure to observe a decrease in mean blood glucose deserves comment. That the hyperinsulinemia was affecting glucose metabolism is suggested by the finding that decreases in blood glucose (5 to 23 mg per 100 ml) occurred in one-third of the tests. The development of a positive arteriovenous difference in blood glucose as well as in plasma amino nitrogen concomitant with the increases in plasma insulin (Figure 2) is consonant with a peripheral effect exerted by the latter. One wonders why greater and more consistent decreases in blood glucose were not observed. Continuing glucogenesis from amino acids derived from the meals may be one factor. Another contributing factor may be the rising plasma levels of growth hormone observed after the ingestion of proteins (21) although significant changes (as presently measured) usually occur well after the first hour after the ingestion of the protein meals. The latter finding is consonant with our demonstration that intravenous administration of certain amino acids to normal subjects induces a release of immunodetectable growth hormone into the blood (22), a finding confirmed with respect to arginine by Merimee, Lillicrap, and Rabinowitz (23). Other metabolic and hormonal influences on glucose metabolism induced by ingestion of protein meals are not excluded.

Finally, it is interesting to consider the possible biologic significance of the relationship between rising levels of certain amino acids after protein meals and increased insulin release. It is well established that insulin increases the transfer of certain amino acids across the cell membrane of muscle and increases protein synthesis. Thus, rising plasma levels of insulin in response to rising plasma levels of certain amino acids after a protein meal would be expected to facilitate disposal and utilization of absorbed amino acids in a relationship analogous to that which exists between rising blood glucose levels and stimulation of insulin release after ingestion of a carbohydrate load. The later participation of growth hormone in the over-all response to protein ingestion (21) and amino acid infusion (22) makes all the more intriguing the physiologic processes that follow protein alimentation.

## Summary

Protein meals were fed to healthy subjects, and concentrations of plasma insulin, leucine, amino nitrogen, free fatty acids, and blood glucose were determined. Plasma insulin, leucine, and amino nitrogen increased significantly during each test in which they were measured. Mean concentrations of plasma free fatty acids decreased. Mean blood glucose concentration did not change, although decreases were observed in one-third of the tests. Pretreatment of some subjects with chlor-

propamide failed to modify the changes in blood glucose and plasma free fatty acids observed in the absence of such pretreatment.

The increases in plasma insulin after the ingestion of the protein meals were considerably greater than those that would have been expected to result from the modest increases observed in plasma leucine. These findings suggest that amino acids other than leucine, or amino acids in combination with leucine, induced the release of insulin.

We conclude that *1*) ingestion of protein meals results in significant increases in plasma insulin, *2*) these increases in plasma insulin cannot be accounted for solely or even largely by the effect of *l*-leucine contained in the meals, and *3*) ingestion of protein and rising plasma levels of certain amino acids appear to be associated with a physiologic stimulus for insulin release.

We speculate that the purpose of the insulogenic response to protein ingestion is to aid both in the utilization of absorbed amino acids and in their synthesis to protein.

## References


1. Floyd, J. C., Jr., S. S. Fajans, R. F. Knopf, J. Rull, and J. W. Conn. Postprandial aminoacidemia and insulin secretion, a physiologic relationship (abstract). J. Lab. clin. Med. 1964, **64**, 858.
2. Fajans, S. S., R. F. Knopf, J. C. Floyd, Jr., L. Power, and J. W. Conn. The experimental induction in man of sensitivity to leucine hypoglycemia. J. clin. Invest. 1963, **42**, 216.
3. Floyd, J. C., Jr., S. S. Fajans, R. F. Knopf, and J. W. Conn. Evidence that insulin release is the mechanism for experimentally induced leucine hypoglycemia in man. J. clin. Invest. 1963, **42**, 1714.
4. Floyd, J. C., Jr., S. S. Fajans, R. F. Knopf, and J. W. Conn. Plasma insulin in organic hyperinsulinism: comparative effects of tolbutamide, leucine, and glucose. J. clin. Endocr. 1964, **24**, 747.
5. Floyd, J. C., Jr., S. S. Fajans, J. Rull, R. F. Knopf, M. M. Kirsh, and J. W. Conn. Direct evidence that leucine induces release of pancreatic insulin (abstract). Diabetes 1965, **14**, 439.
6. Fajans, S. S., R. F. Knopf, J. C. Floyd, Jr., and J. W. Conn. On the physiologic significance of leucine hypoglycemia in healthy subjects (abstract). J. Lab. clin. Med. 1962, **60**, 873.
7. Fajans, S. S., J. C. Floyd, Jr., R. F. Knopf, and J. W. Conn. Secretion of insulin induced by leucine in healthy subjects *in* Ciba Foundation Colloquia on Endocrinology. Aetiology of Diabetes Mellitus and Its Complications, M. P. Cameron and M. O'Connor, Eds. London, Churchill, 1964, vol. 15, pp. 99–106.
8. Nelson, N. A photometric adaptation of the Somogyi method for the determination of glucose. J. biol. Chem. 1944, **153**, 375.
9. Yalow, R. S., and S. A. Berson. Immunoassay of endogenous plasma insulin in man. J. clin. Invest. 1960, **39**, 1157.
10. Fajans, S. S., J. C. Floyd, Jr., R. F. Knopf, and J. W. Conn. A comparison of leucine- and acetoacetate-induced hypoglycemia in man. J. clin. Invest. 1964, **43**, 2003.
11. Berry, H. K. Paper chromatographic method for estimation of phenylalanine. Proc. Soc. exp. Biol. (N. Y.) 1957, **95**, 71.
12. Frame, E. G., J. A. Russell, and A. E. Wilhelmi. The colorimetric estimation of amino nitrogen in blood. J. biol. Chem. 1943, **149**, 255.
13. Dole, V. P. A relation between non-esterified fatty acids in plasma and the metabolism of glucose. J. clin. Invest. 1956, **35**, 150.
14. Yalow, R. S., S. M. Glick, J. Roth, and S. A. Berson. Plasma insulin and growth hormone levels in obesity and diabetes. Ann. N. Y. Acad. Sci. 1965, **131**, 357.
15. Yalow, R. S., and S. A. Berson. Dynamics of insulin secretion in hypoglycemia. Diabetes 1965, **14**, 341.
16. Floyd, J. C., Jr., S. S. Fajans, C. Thiffault, E. M. Guntsche, and J. W. Conn. Unpublished observations.
17. Berger, S., H. C. Joondeph, and B. Rachmeler. The effect of protein ingestion on plasma insulin concentration (abstract). J. Lab. clin. Med. 1964, **64**, 841.
18. Floyd, J. C., Jr., S. S. Fajans, R. F. Knopf, J. Rull, and J. W. Conn. Stimulation of insulin secretion by amino acids (abstract). Clin. Res. 1965, **13**, 322.
19. Floyd, J. C., Jr., S. S. Fajans, J. W. Conn, R. F. Knopf, and J. Rull. Stimulation of insulin secretion by amino acids. J. clin. Invest. 1966, **45**, 1487.
20. McIntyre, N., C. D. Holdsworth, and D. S. Turner. New interpretation of oral glucose tolerance. Lancet 1964, **2**, 20.
21. Knopf, R. F., J. W. Conn, J. C. Floyd, Jr., S. S. Fajans, J. A. Rull, E. M. Guntsche, and C. A. Thiffault. The normal endocrine response to ingestion of protein and to infusion of amino acids. Sequential secretion of insulin and growth hormone. Trans. Ass. Amer. Phycns 1966, **79**, in press.
22. Knopf, R. F., J. W. Conn, S. S. Fajans, J. C. Floyd, Jr., E. M. Guntsche, and J. A. Rull. Plasma growth hormone response to intravenous administration of amino acids. J. clin. Endocr. 1965, **25**, 1140.
23. Merimee, T. F., D. A. Lillicrap, and D. Rabinowitz. Effect of arginine on serum-levels of human growth hormone. Lancet 1965, **2**, 668.