Altered Glucoregulatory Hormones During Acute Pneumococcal Sepsis in the Rhesus Monkey

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SUMMARY

To determine the effects of a *Diplococcus pneumoniae* infection upon the dynamics of glucose metabolism and the hormones responsible for its control, intravenous glucose tolerance tests (IVGTT's) were performed in monkeys during baseline control conditions and twenty-four hours after initiating infection when the monkeys were febrile. Along with plasma glucose, immunoreactive insulin and glucagon concentrations were measured sequentially through each IVGTT. Furthermore, fasting concentrations of plasma amino acids, immunoreactive growth hormone and cortisol were determined.

These data reveal no differences in the fasting plasma concentrations of growth hormone and glucose, or in the disappearance rates of glucose during the two IVGTT's. There was, however, a mild relative hyperinsulinemia ($p < 0.05$) (following glucose) along with fasting hyperglucagonemia ($p < 0.01$) during infection. Of the twenty-one amino acids measured, twenty were depressed during infection; only cysteine was elevated ($p < 0.05$) in the infected monkeys. Although plasma cortisol levels were significantly ($p < 0.05$) increased above preinfection values by the stress of infection, they remained within the control range.

The hyperglucagonemia and depression of the mean insulin: glucagon molar ratio are compatible with the catabolic events occurring during a mild bacterial infection in the monkey. DIABETES ***23*:544-49, June, 1974.**

Infection in humans is known to promote alterations in glucoregulatory hormone concentrations as well as in the carbohydrate metabolism of normal individuals or a worsening of the diabetic state.[1,2] During bacterial and viral illnesses, fasting hyperglycemia,[2,3] glucose intolerance associated with hyperinsulinemia,[2,4,5] hyperglucagonemia,[1,2] and elevated glucocorticoid concentrations[2,6] have been documented in man. Furthermore, alterations in the growth hormone responses following a glucose load have been observed in man during a viral illness[2] and in monkeys during bacterial infections.[7] The precise mechanism for the alterations of glucose tolerance and the role of glucoregulatory hormones in the host defensive mechanisms during an acute infection remain to be determined. Therefore, the rhesus monkey (*Macaca mulatta*) was adopted as a subhuman primate model for the study of glucose tolerance and the factors regulating hormonal control of insulin and glucagon during the stress of acute pneumococcal sepsis.

METHODS

Healthy male monkeys weighing from 2.5 to 4.3 kg. were fully conditioned and fed monkey chow ad libitum for several months prior to use in these studies. The monkeys were anesthetized using 1 ml. of 2 per cent sodium thiamytal and bilateral femoral vein catheters were surgically implanted. The catheters were connected by means of a three-way plastic stopcock and adapter to a syringe containing heparinized saline. Thermistor wires were implanted into the paraspinous muscles and connected to a Honeywell recorder for continuous temperature recordings. Upon completion of this minor surgery the monkeys were placed in restraining chairs and allowed seven days to adapt to their study environment. Ad libitum feeding was continued as before. During this equilibration period at least three baseline samples

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were obtained for complete blood counts, blood cultures, and fasting blood glucose measurements. Seventeen monkeys were used in the studies to be described.

The first glucose tolerance test (GTT-1) was begun eight days after surgery at 0800 hours following a sixteen-hour overnight fast. Each monkey received an intravenous infusion of glucose equal to 0.5 gm. of glucose per kilogram body weight, administered as a 10 per cent solution over two minutes and followed immediately by a 5-ml. flush with 0.9 per cent saline. Subsequent blood samples were obtained from the opposite catheter by a double syringe technic. At 0800 hours the following day the monkeys were inoculated intravenously with 10^8 virulent type I *Diplococcus pneumoniae.** The second glucose tolerance test (GTT-2) was performed twenty-four hours postinoculation at 0800 hours utilizing a procedure identical to that for GTT-1, including the sixteen-hour fast.

During each GTT, sequential 4 ml. samples were taken at baseline prior to glucose administration and at 5, 10, 20, 30, 45, 60 and 120 minutes after beginning the glucose infusion. Additional blood was obtained at 0800 hours for a complete blood count, blood culture and fasting basal levels of plasma cortisol, immunoreactive growth hormone (GH) and plasma amino acids (AA) on the day of each GTT.† Sequential GTT blood samples were assayed for plasma glucose and immunoreactive insulin (IRI) and immunoreactive glucagon (IRG). The samples were transferred immediately from the syringes directly into heparinized chilled glass tubes for plasma glucose, AA, cortisol, IRI, and GH, whereas the IRG samples were placed in chilled glass tubes containing 1.5 mg. EDTA and 1,000 U. Trasylol/ml. of blood. All samples were centrifuged at 2,000 x g for twenty minutes in a refrigerated centrifuge and the plasma was stored at -20° C. until assayed.

Plasma glucose concentrations were determined in duplicate by a semiautomated enzymatic micromethod;[8] protein-free plasma filtrates were analyzed for AA content by an automated procedure in duplicate using an ion exchange resin technic.[9] Plasma IRI,[10] GH,[10] IRG[11] and total cortisol[12] concentrations were assayed in duplicate by radioligand assay technics. All the hormone concentrations of an individual monkey (GTT-1 and GTT-2) were determined in duplicate within the same assay. The glucagon radioimmunoassay was performed using antibody 30 K, purchased from R. H. Unger's Laboratory. This antibody is specific for pancreatic glucagon, and routinely detects concentrations as low as 75 pg./ml. Fasting human plasma glucagon levels determined under our conditions are in agreement with those reported elsewhere.[2] The monkey, however, appears to have fasting plasma glucagon levels which are approximately fourfold higher than man and other species. The mean fasting plasma glucagon concentrations of seventy-eight healthy chaired male monkeys measured in our laboratory was 408 ± 25 pg./ml., (Mean ± S.E.M.), which is in close agreement with previously published data on monkeys.[13] Glucose disappearance rates were determined from the formula $k = \frac{0.693}{t_{1/2}} \times 100$ per cent per minute.[14] The insulinogenic index (an expression of the magnitude of insulin output per unit of glycemic stimulus) was calculated by the method of Seltzer.[15] Statistical analyses were done using Student's t test for paired variates, the criterion for significance being $p < 0.05$.

RESULTS

Infection of the seventeen monkeys with 10^8 *D. pneumoniae* elicited a typical three-day febrile self-limited nonfatal illness, characterized by elevation of body temperature within six to eight hours postinoculation and positive *D. pneumoniae* blood cultures in concentrations from 1.5×10^2 to 1.6×10^6 organisms per milliliter.[16] The peak febrile response occurred approximately twenty-four hours postinoculation and coincided with GTT-2. At that time the body temperature was elevated to 40.6 ± 0.3° C. as compared with GTT-1, 39.1 ± 0.2° C. ($p < 0.05$).

Food intake did not diminish appreciably throughout the study period; the afternoon feeding on the day of inoculation, which coincided with the initial temperature elevation, was consumed normally. Since this was followed by the usual overnight fast, the alterations observed during GTT-2 could not be ascribed to decreased food or caloric intake.

Glucose intolerance did not occur in any of the monkeys and the kinetic disappearance of the infused glucose appeared unaltered (figure 1). Basal glucose values were within a normal range during GTT-1 (65

*Organisms were mouse-passed to maintain virulence and encapsulation, stored at -70° C. in fortified brain heart infusion broth and thawed immediately prior to inoculation.

†The cortisol, growth hormone and amino acid determinations were performed on ten of the seventeen monkeys. These ten monkeys are from a different group than was previously reported[7] but there was no significant change in mean fasting growth hormone level during infection in either group.

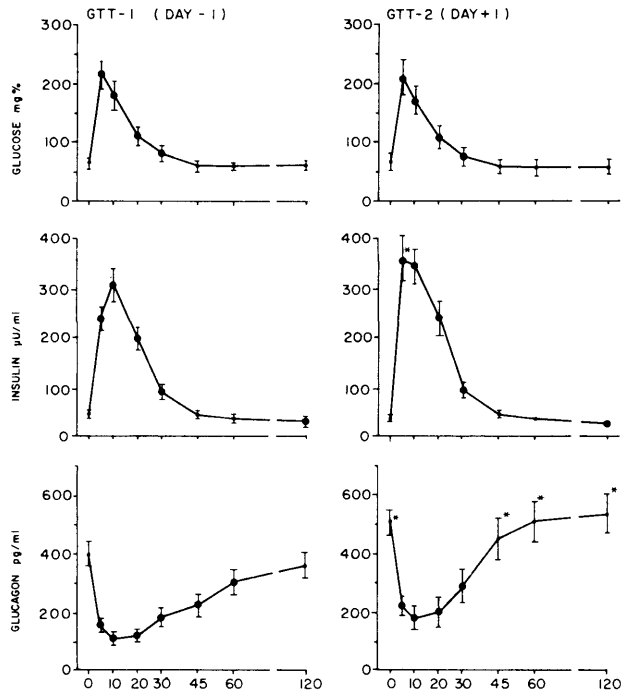

FIG. 1. Glucose, insulin, and glucagon responses following intravenous glucose tolerance tests during a control period (day −1) and twenty-four hours after inoculation (day +1) with *D. pneumoniae* in seventeen monkeys (mean ± S.E.M.). The large dots represent statistically significant ($p < 0.05$) differences relative to time 0; the asterisks represent statistically significant ($p < 0.05$) differences relative to GTT-1 at the specific times by paired *t* test.

± 2 mg./100 ml.) and GTT-2 (67 ± 2 mg./100 ml.). The disappearance rate, k, during GTT-1 (2.49 ± 0.06) was not significantly different from GTT-2 (2.66 ± 0.16).

The fasting levels of insulin were comparable prior to both GTT-1 (47.2 ± 3.9 μU./ml.) and GTT-2 (46.7 ± 4.1 μU./ml.). However, the insulin responses to glucose loading were significantly ($p < 0.05$) increased at the five-minute time, when the infected monkeys' (GTT-2) values of 358.5 ± 49.9 μU./ml. were elevated above the five-minute control values (GTT-1) of 238.8 ± 27.2 μU./ml. (figure 1). Furthermore, there was a relative hyperinsulinemia in response to glucose loading during infection as reflected by the significant ($p < 0.02$) change of the insulinogenic index from 2.2 ± 0.2 during GTT-1 to 3.2 ± 0.4 during GTT-2.

The basal glucagon concentrations were significantly ($p < 0.01$) elevated during infection (GTT-2, 512 ± 41 pg./ml.) when compared to the controls (GTT-1, 399 ± 39 pg./ml.). Following glucose administration there was a significant decrease ($p < 0.001$) at five minutes during both the control GTT and during fever. Glucagon concentrations were suppressed during GTT-1 until 120 minutes, whereas during GTT-2 glucagon returned to the elevated basal values by forty-five minutes (figure 1). The mean fasting insulin:glucagon molar ratios were not significantly depressed before GTT-2 (3.0 ± 0.6) when compared with the control values before GTT-1 (4.4 ± 1.0).

The 0800 hours plasma cortisol concentrations were slightly but significantly elevated ($p < 0.05$) when control values were compared to those of infected monkeys (table 1). However, with the exceptions of monkeys 4 and 8 the response to infection remained within the range of the preinfection values.

TABLE 1

Plasma cortisol levels in μg./100 ml. of ten monkeys at 0800 hours on day -1 (control) and day +1 (twenty-four-hour infected febrile monkeys).

Monkey Number	Day -1	Day +1
1	19.5	18.5
2	10.5	15.5
3	19.0	25.5
4	24.5	30.0
5	13.0	19.0
6	20.5	27.5
7	15.5	13.5
8	23.0	30.5
9	28.5	27.5
10	23.0	26.5
Mean*	19.7	23.4
S.E.M. ±	1.7	± 1.9

*$p < 0.05$

Total venous plasma amino acid levels were significantly ($p < 0.002$) depressed when baseline control values (3.86 ± 0.38 mmoles/L.) were compared with values during fever (2.35 ± 0.10 mmoles/L.). Only one (cysteine) of the twenty-one amino acids measured was significantly ($p < 0.05$) increased when control values (54 ± 0.5 μmoles/L.) were compared with those from febrile monkeys (76 ± 0.6 μmoles/L.). All the twenty remaining individual amino acids measured were decreased, nine significantly ($p < 0.05$) and the other eleven were all below the mean control values. Furthermore, alanine was among the nine that were significantly ($p < 0.05$) depressed by the infection (figure 2).

As is demonstrated in table 2, the basal concentrations of GH were not significantly changed by a pneumococcal infection when the fasting control val-

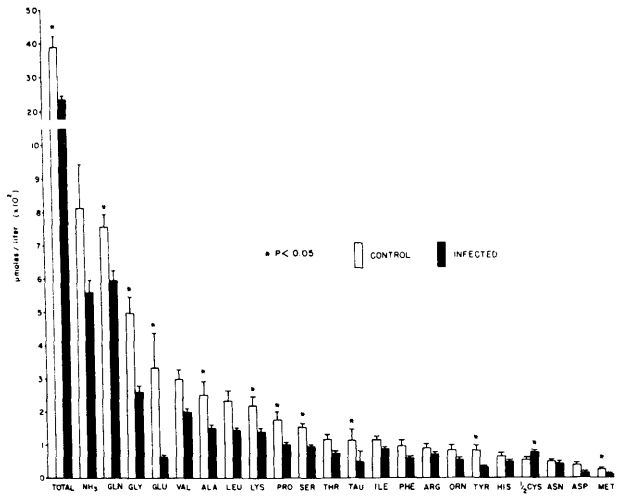

FIG. 2. Fasting plasma amino acid concentrations determined on ten of the seventeen monkeys, (mean ± S.E.M.) of twenty-one individual amino acids, total amino acids and plasma ammonia (NH_3) during a control period (day −1) and pneumococcal infection (day +1). The asterisks represent statistically significant ($p < 0.05$) differences between control and infected values in the ten monkeys, as determined by paired *t* test.

ues (2.4 ± 0.5 ng./ml.) on day -1 were compared with the fasting febrile values (2.3 ± 0.7 ng./ml.) on day +1.

DISCUSSION

This study clearly demonstrates elevated circulating concentrations of glucagon and insulin although glucose tolerance remained normal during the stress of acute pneumococcal sepsis, which was a relatively mild infection in these rhesus monkeys. Elevated plasma glucagon concentrations have also been reported during infection in man[1,2] and dogs[1] as well as in endotoxemic monkeys.[13] Since there were no changes in the antecedent food or caloric intake in this study, the finding of significant fasting hyperglucagonemia with a decrease in the insulin:glucagon molar ratio during acute pneumococcemia is compatible with what one would expect in a situation of "glucose need" and a comparatively catabolic set of the bihormonal glucagon insulin response.[17]

TABLE 2

Plasma growth hormone levels in ng./ml. at 0800 hours on day −1 (control) and day +1 (twenty-four-hour infected febrile monkeys).

Monkey	Day -1	Day +1
1	1.6	0.9
2	0.5	0.3
3	0.5	0.4
4	5.1	5.2
5	0.3	0.9
6	3.8	4.5
7	3.9	1.8
8	2.2	0.8
9	2.7	2.6
10	4.2	5.8
	Mean* 2.48	2.32
	S.E.M. ± 0.54	±0.66

*$p > 0.50$

Lack of differences in fasting plasma glucose concentrations or in the disappearance rates of glucose during either GTT-1 or GTT-2 contrasted with earlier studies in nondiabetic volunteers in which the stress of a mild bacterial[5] or viral illness[2] was sufficient to cause glucose intolerance and mild fasting hyperglycemia. However, the hyperinsulinemia reflected by the increased insulinogenic index between GTT-1 and GTT-2 was similar to data previously studied in man.[2,5] As in the human studies, the fasting values of insulin in infected monkeys were unaltered.

The hyperglucagonemia of infection[1,2] in man and dogs is also seen in monkeys. Bloom et al.[13] reported elevation of fasting glucagon levels from 378 ± 143 to 1,272 ± 615 pg./ml. following endotoxin injection in rhesus monkeys, and fasting hyperglucagonemia also occurred in our monkeys during this bacterial illness. The administration of an intravenous glucose bolus resulted in an abrupt diminution in plasma IRG within five minutes. After thirty minutes, glucagon values returned toward the elevated levels observed prior to the glucose infusion.

There are several possible explanations for fasting hyperglucagonemia during sepsis. Although repeated pharmacologic doses of prednisolone appear to increase glucagon,[18] cortisol values did not correlate with fasting glucagon elevations in this study. Gerich et al.[19] have reported hyperglucagonemia in association with acromegaly and postulated that GH may increase plasma glucagon. A "nonspecific stress" may also cause elevated glucagon concentrations. When conscious monkeys were startled by a loud noise or when an electric current passed through their heads, there was a rapid rise in plasma glucagon.[13] Furthermore, stimulation of mixed autonomic nerves to the pancreas in anesthetized dogs has led to virtual doubling of pancreato-duodenal vein glucagon output, which was not altered by atropine administration.[20] Others have also given evidence of adrenergic influences on glucagon secretion.[21]

Hyperaminoacidemia may stimulate an increased secretion of glucagon by the pancreatic alpha cell.[22-25] Although elevated fasting plasma glucagon concentrations in various infections have been ascribed to a release of amino acids from a localized site of inflammation,[1] experimental data do not support this view. Rather, hypoaminoacidemia is observed in sequential samples from volunteers with sandfly fever,[9] typhoid fever,[26] or rats exposed to virulent *D. pneumoniae*.[27] A similar general hypoaminoacidemia is noted in the current study.

While infection-elevated plasma glucagon concentrations do not result from hyperaminoacidemia, it can be argued that the resultant infection-related hypoaminoacidemia is, in part, caused by an effect of glucagon excess. Glucagon reduces the concentration of certain plasma amino acids in the fed or postabsorptive states.[28-30] This effect is associated with increased splanchnic amino acid uptake.[31-33] In contrast, insulin inhibits the release of amino acids from forearm muscles.[34] Thus, elevated concentrations of plasma glucagon during infection would tend to stimulate an increased splanchnic uptake of amino acids.

The insulin:glucagon molar ratio may be useful in assessing hepatic glucose balance and amino acid utilization.[17,35] Low values are indicative of a catabolic state such as occurs after burns, trauma, infection, or starvation.[17,35] In the present study, the mean fasting insulin:glucagon molar ratio decreased from a control value of 4.4 to 3.0 during mild sepsis in monkeys.

Since infection is a catabolic state,[36] hyperglucagonemia, decrease in the insulin:glucagon molar ratio, and decreased plasma amino acid values all appear to be directed toward stimulating a greater synthesis of glucose at the expense of muscle protein and body nitrogen.

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In conducting the research described in this report, the investigators adhered to the "Guide for Laboratory Animal Facilities and Care," as promulgated by the Committee on the Guide for Laboratory Animal Facilities and Care of the Institute of Laboratory Animal Resources, National Academy of Sciences-National Research Council. The facilities are fully accredited by the American Association for Accreditation of Laboratory Animal Care.

REFERENCES

[1]Rocha, M. D., Santeusanio, F., Faloona, G. R., and Unger, R. H.: Abnormal pancreatic alpha-cell function in bacterial infections. N. Engl. J. Med. *288*:700-03, 1973.

[2]Rayfield, E. J., Curnow, R. T., George, D. T., and Beisel, W. R.: Impaired carbohydrate metabolism during a mild viral illness. N. Engl. J. Med. *289*:618-21, 1973.

[3]Fox, M. J., Kuzma, J. F., and Washam, W. T.: Transitory diabetic syndrome associated with meningococcic meningitis. Arch. Intern. Med. *79*:614-21, 1947.

[4]Williams, J. L., and Dick, G. F.: Decreased dextrose tolerance in acute infectious diseases. Arch. Intern. Med. *50*:801-18, 1932.

[5]Shambaugh, III, G. E., and Beisel, W. R.: Insulin response during tularemia in man. Diabetes *16*:369-76, 1967.

[6]Beisel, W. R., Bruton, J., Anderson, K. D., and Sawyer, W. D.: Adrenocortical responses during tularemia in human subjects. J. Clin. Endocrinol. Metab. *27*:61-69, 1967.

[7]Rayfield, E. J., Curnow, R. T., and George, D. T.: Mechanistic studies in rhesus monkeys (RM) to explain paradoxical growth hormone (GH) secretion during infection. Program, The Endocrine Society: A-257, 1973.

[8]Asrow, G.: Semiautomated enzymatic micromethods for blood glucose and lactic acid on a single filtrate. Anal. Biochem. *28*:130-38, 1969.

[9]Wannemacher, Jr., R. W., Pekarek, R. S., Bartelloni, P. J., Vollmer, R. T., and Beisel, W. R.: Changes in individual plasma amino acids following experimentally induced sandfly fever virus infection. Metabolism *21*:67-76, 1972.

[10]Juan, C., and AvRuskin, T. W.: A combined immunoassay of human growth hormone and insulin: cumulative assessment of assay performance. J. Clin. Endocrinol. Metab. *33*:150-52, 1971.

[11]Aguilar-Parada, E., Eisentraut, A. M., and Unger, R. H.: Pancreatic glucagon secretion in normal and diabetic subjects. Am. J. Med. Sci. *257*:415-19, 1969.

[12]Murphy, B. E. P.: Some studies of the protein-binding of steroids and their application to the routine micro and ultramicro measurement of various steroids in body fluids by competitive protein-binding radioassay. J. Clin. Endocrinol. Metab. *27*:973-90, 1967.

[13]Bloom, S. R., Daniel, P. M., Johnston, D. I., Ogawa, O.,

and Pratt, O. E.: Release of glucagon induced by stress. Q. J. Exp. Physiol. *58*:99-108, 1973.

[14]Lundbaek, K.: Intravenous glucose tolerance as a tool in definition and diagnosis of diabetes mellitus. Br. Med. J. *1*:1507-13, 1962.

[15]Seltzer, J. S., Allen, E. W., Herron, Jr., A. L., and Brennan, M. T.: Insulin secretion in response to glycemic stimulus: relation of delayed initial release to carbohydrate intolerance in mild diabetes mellitus. J. Clin. Invest. *46*:323-35, 1967.

[16]Woeber, K. A.: Alterations in thyroid hormone economy during acute infection with *Diplococcus pneumoniae* in the rhesus monkey. J. Clin. Invest. *50*:378-87, 1971.

[17]Unger, R. H.: Glucagon and the insulin: glucagon ratio in diabetes and other catabolic illnesses. Diabetes *20*:834-38, 1972.

[18]Marco, J., Calle, C., Roman, D., Diaz-Fierros, M., Villanueva, M. L., and Valverde, I.: Hyperglucagonism induced by glucocorticoid treatment in man. N. Engl. J. Med. *288*:128-31, 1973.

[19]Gerich, J. E., Langlois, M., Schneider, V., Noacco, C., and Gustafson, G.: Abnormal pancreatic alpha cell function in acromegaly. Program, The Endocrine Society: A-173, 1973.

[20]Marliss, E. B., Girardier, L., Seydoux, J., Wallheim, C. B., Kanazawa, Y., Orci, L., Renold, A. E., and Porte, Jr., D.: Glucagon release induced by pancreatic nerve stimulation in the dog. J. Clin. Invest. *52*:1246-59, 1973.

[21]Gerich, J. E., Karam, J. H., and Forsham, P. H.: Stimulation of glucagon secretion by epinephrine in man. J. Clin. Endocrinol. Metab. *37*:479-81, 1973.

[22]Ohneda, A., Parada, E., Eisentraut, A. M., and Unger, R. H.: Characterization of response of circulating glucagon to intraduodenal and intravenous administration of amino acids. J. Clin. Invest. *47*:2305-22, 1968.

[23]Pek, S., Fajans, S. S., Floyd, Jr., J. C., Knopf, R. F., and Conn, J. W.: Effects upon plasma glucagon of infused and ingested amino acids and of protein meals in man. Diabetes *18*:328(Abst), 1969.

[24]Unger, R. H., Ohneda, A., Aguilar-Parada, E., and Eisentraut, A. M.: The role of aminogenic glucagon secretion in blood glucose homeostasis. J. Clin. Invest. *48*:810-22, 1969.

[25]Rocha, D. M., Faloona, G. R., and Unger, R. H.: Glucagon-stimulating activity of 20 amino acids in dogs. J. Clin. Invest. *51*:2346-51, 1972.

[26]Wannemacher, Jr., R. W., DuPont, H. L., Pekarek, R. S., Powanda, M. C., Schwartz, A., Hornick, R. B., and Beisel, W. R.: An endogenous mediator of depression of amino acids and trace metals in serum during typhoid fever. J. Infect. Dis. *126*:77-86, 1972.

[27]Wannemacher, Jr., R. W., Powanda, M. C., Pekarek, R. S., and Beisel, W. R.: Tissue amino acid flux after exposure of rats to *Diplococcus pneumoniae*. Infect. Immun. *4*:556-62, 1971.

[28]Bondy, P. K., and Cardillo, L. R.: The effects of glucagon on carbohydrate metabolism in normal human beings. J. Clin. Invest. *35*:494-501, 1956.

[29]Marliss, E. B., Aoki, T. T., Unger, R. H., Soeldner, J. S., and Cahill, Jr., G. F.: Glucagon levels and metabolic effects in fasting man. J. Clin. Invest. *49*:2256-70, 1970.

[30]Bromer, W. W., and Chance, R. E.: Zinc glucagon depression of blood amino acids in rabbits. Diabetes *18*:748-54, 1969.

[31]Kibler, R. F., Taylor, W. J., and Meyers, J. D.: The effect of glucagon on net splanchnic balances of glucose, amino acid nitrogen urea, ketones, and oxygen in man. J. Clin. Invest. *43*:904-15, 1964.

[32]Felig, P., Brown, W. V., Levine, R. A., and Klatskin, G.: Glucose homeostasis in viral hepatitis. N. Engl. J. Med. *283*:1436-40, 1970.

[33]Shoemaker, W. C., and Van Itallie, T. B.: The hepatic response to glucagon in the unanesthetized dog. Endocrinology *66*:260-68, 1960.

[34]Pozefsky, T., Felig, P., Tobin, J. D., Soeldner, J. S., and Cahill, Jr., G. F.: Amino acid balance across tissues of the forearm in postabsorptive man. Effects of insulin at two dose levels. J. Clin. Invest. *48*:2273-82, 1969.

[35]Muller, W. A., Faloona, G. R., and Unger, R. H.: The influence of the antecedent diet upon glucagon and insulin secretion. N. Engl. J. Med. *285*:1450-54, 1971.

[36]Beisel, W. R.: Interrelated changes in host metabolism during generalized infectious illness. Am. J. Clin. Nutr. *25*:1254-60, 1972.