Response of Plasma Glucose and Fatty Acids to Hypermetabolism* (32775)

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Over the past 30 years several investigations have been made concerning the behavior of the supply of nutrients in the blood during periods of exercise. Considerable evidence in support of the increased metabolism of both glucose and non-esterified fatty acids (Nefa) has been obtained; however, reports describing the behavior of blood concentrations of these substances provide inconsistent data. Many of these inconsistencies are no doubt due to variation in the severity of exercise, the site of blood sampling, and the amount of previous physical training of the subject. Moreover, concentrations of plasma nutrient measured at various times during the transient period following initiation of exercise may differ considerably. Heretofore, the experimental periods of continuous elevated metabolic rate have been of such brief duration as to preclude attainment of steady state, thereby not only eliminating steady state information, but also introducing a "time error" among experimenters and rendering comparison of results difficult. The following experiments were therefore designed to examine the time course of response of plasma glucose and Nefa levels to an increase in metabolic rate and to determine whether steady state plasma concentrations of these nutrients could be observed.

Having characterized this response, it was then of interest to investigate the possible involvement of catecholamines. The glycogenolytic effect of epinephrine has long been implicated in the sympathetic response to stress, although its role in the regulation of glucose supply during an increase in metabolic rate has not been delineated. Norepinephrine has been shown to be a potent fatty acid releasing agent, both when present in blood and via adrenergic innervation of adipose tissue tissue (1, 2). In an attempt to determine if the plasma nutrient responses to an increase in metabolic rate are dependent on the action of these catecholamines, a second set of experiments was performed on animals in which catecholamine stores had been depleted by reserpine administration.

Because of the various technical problems encountered in maintaining constant exercise for periods of several hours, hypermetabolic states were achieved in anesthetized dogs by injection of 2,4-dinitrophenol (DNP). Previous studies of plasma nutrient response to DNP have revealed a marked hyperglycemia with depletion of hepatic and muscle glycogen stores (3), and an apparent increase in glucose (4) and Nefa (5) uptake by muscle tissue.

Methods and Materials. Dogs fasted overnight were anesthetized with intravenous sodium diethylbarbiturate, 200 mg/kg, preceded 0.5 hour by intraperitoneal morphine sulfate, 4 mg/kg. Such a combination was capable of maintaining anesthesia for at least 5 hours without supplemental anesthetic. Each animal was fitted with a tracheal cannula and attached to a Benedict-Roth metabolimeter; a Cournand needle was inserted in the left external jugular vein. Following the completion of all surgical procedures, the animal was permitted a 1-hour stabilization period. Blood samples of 5 ml were drawn into heparinized syringes every 15 min during a 0.5 hour pre-DNP basal period and a 3 hour post-DNP period. The DNP, 5 mg/kg, was injected intravenously as a 3.16% solution of Na dinitrophenoxide in water, pH = 9.4. Duplicate determinations of plasma glucose and Nefa concentrations were made using the glucose-oxidase and a modified Dole's method (6) respectively. The catecholamine depletion experiments were identical with the exception that reserpine, 0.1 mg/kg, was injected intraperitoneally as a 0.5% solution in 20% ascorbic acid on each of two afternoons preceding the day of the experiment.

Results. Our preliminary interest is the long-term behavior of plasma glucose and Nefa concentrations in normal, anesthetized animals (Fig. 1). Average glucose concen-

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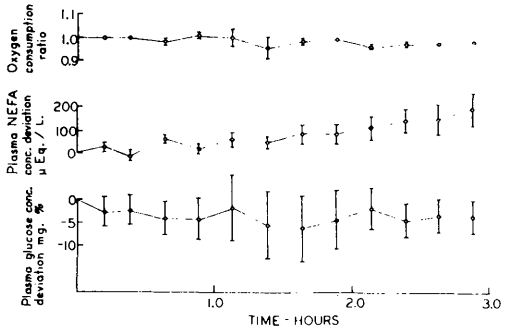

FIG. 1. Oxygen consumption ratio and deviation of plasma glucose and Nefa concentrations in four anesthetized dogs.

tration in four dogs was 91 mg/100 ml initially and remained within ± 5 mg/100 ml throughout 3 hours. Average Nefa concentration was 397 μeq/liter initially and exhibited a slow increase over the 3 hours. Regression analysis of deviations from the initial plasma Nefa concentration revealed a relationship: Nefa = −11.9 + 65.2(t) where t is time in hours, with standard error of estimate = 122.0. The slope was significant at $p < .01$; the intercept was found not to differ significantly from zero. The rate of oxygen consumption in these animals remained essentially constant. This variable is plotted in Fig. 1 as the ratio to the value at completion of the 1 hour stabilization period.

Response of normal animals to DNP. As may be seen in Fig. 2*a*, oxygen consumption rose abruptly, then remained relatively constant at about 2.25 times the pre-DNP level, an increase comparable to that of mild exercise. Plasma glucose concentration in these six animals began rising immediately following DNP administration, reached a peak 30 mg/100 ml above the basal level in 30 min, and then slowly fell to about 15 mg/100 ml above basal, where it then remained over the last hour. The increase in plasma Nefa concentration during the first 2 hours after DNP administration, Nefa = −25.8 + 65.0 (t), did not differ from that of the control group. During the third hour, however, the slope constant increased to 276.0 μeq/liter per hour, which differed significantly ($p < .01$) from both the previous 2-hour slope (65.0 μeq/liter per hour) and the slope of the control group (65.2 μeq/liter per hour).

Response of reserpinized animals to DNP. To investigate the possibility that the DNP induced hyperglycemia and elevated Nefa level resulted from the action of endogenous catecholamines, these experiments were repeated in five other animals pretreated with reserpine. Average oxygen consumption ratio of the reserpinized dogs was found by group comparison t test to be significantly greater than that of the nonreserpinized animals, $p < .05$, (Fig. 2*b*), however, the change in plasma glucose concentration of the reserpinized group was not detectably different from that of the normal group. The DNP induced change in Nefa concentration in reserpinized animals was found by analysis of variance to be significantly lower ($p < .05$) than that of the normal nonreserpinized group for the first 2 hours, although the rise during the third hour could not be shown

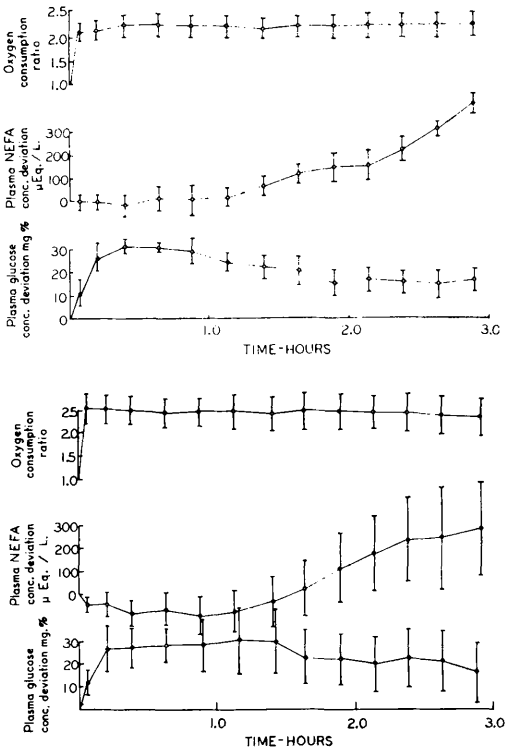

FIG. 2. Time course of oxygen consumption ratio and deviation in plasma glucose and Nefa concentrations from basal after DNP: (A, top). Six nonreserpinized dogs. (B, bottom). Six reserpinized dogs.

TABLE I. Basal (Pre-DNP) Plasma Nutrient Concentrations. (Mean ± SE)

	Normal	Depleted	Probability level (%)
Glucose	86.0 ± 4.5	112.2 ± 18.2	< 5
Nefa	439 ± 88	562 ± 63	>10

to differ from that of either the normal or the control animals. The mean basal pre-DNP) glucose and Nefa concentrations were found by group comparison *t* test to be significantly higher in reserpinized than in normal animals at the probability levels indicated in Table I. There was no difference in basal oxygen consumption.

Discussion. The action of dinitrophenol appears to be primarily an uncoupling of the oxidation and phosphorylation processes (7). Thus, although nutrients are oxidized, the transfer of this energy of oxidation to the formation of high-energy phosphate occurs with reduced efficiency. Consequently an increased rate of nutrient metabolism is necessary to meet basal energy requirements of the tissues. That the plasma pool of glucose is increased rather than depleted under such conditions is of particular interest. One possible interpretation is that this hyperglycemia represents the response of a system capable of sensing the increased metabolic rate and evoking a release of stored glucose. Were this the case it would not be surprising that a hyperglycemic steady state should result since this and the consequent hyperinsulinemia would facilitate glucose uptake to meet cellular energy requirements.

The proposal of such a regulatory system is not without precedent. The existence of an unidentified "metabolic rate sensor" has been proposed to account for the hypernea of exercise (8). It is interesting to speculate that the hyperglycemic response reported here might be of similar origin.

Although the action of dinitrophenol on intermediary metabolism is basically different from that of exercise, both forces lead to an increased cellular requirement for nutrients. Furthermore, many investigators report a significant transient hyperglycemia during exercise (9,10), suggesting that both forces result in qualitatively similar responses. It has been demonstrated that DNP hyperglycemia is not the result of general asphyxia or hypercapnia (11); however, further study is necessary to eliminate the possibility of a direct effect of DNP on glycogenolysis.

Reserpine, when given intraperitoneally on each of 2 days preceding experimental use, has been found to deplete nerve endings of cardiac muscle, adrenal medulla, and adipose tissue to less than 10% of their normal catecholamine content (12–14). It does not, however, block the recovery of blood glucose concentration following induction of insulin hypoglycemia (15). The absence of any difference in plasma glucose response of normal and catecholamine depleted animals suggests that catecholamine release, either via the sympathetic nervous system or as transported in plasma, is unimportant in the hyperglycemic response to DNP. However, the higher basal glucose concentrations in the reserpinized group appear to be in conflict with what would be expected in the absence of catecholamine and suggest the introduction of another effect of reserpine. The data of other investigators, using reserpinized animals in metabolic studies, reveal similar differences, particularly in fasting blood glucose concentration (15–17). The occurrence of a reserpine induced release of pituitary corticotropin resulting in extended glucocorticoid responses, as well as the classical acute catecholamine responses, has been reported and confirmed in recent literature (18,19). A corticoid induced increase in gluconeogenesis might explain the elevated basal glucose and even mask the effects of catecholamine depletion on the plasma nutrient response to DNP.

The initial drop in plasma Nefa concentration detected in reserpinized animals suggests either a greater dependence of this group on plasma Nefa for nutrient material or a decreased rate of release from adipose tissue.

Summary. The response of plasma glucose and Nefa concentrations to DNP has been investigated. An overshoot in the transient response of plasma glucose concentration to a DNP-induced step increase in metabolic rate was apparent, with roughly 2 hours re-

quired for attainment of the steady state. We have failed, however, to detect any impairment of this response by prereserpinization. The Nefa concentration was found to increase continuously in response to DNP at a rate greater than that found in anesthetized control animals. The reserpinized animals differed only by an initial decrease in plasma Nefa concentration following DNP.

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Transaminase Inhibition by Decaborane* (32776)

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The use of the boron hydrides, pentaborane and decaborane, in recent years as high energy propellants has stimulated interest in the mechanism(s) by which this class of compounds expresses its toxic effects.

Boron hydrides are known to cause marked derangements in the biochemical and functional relationships of the central nervous system (1). While there is indirect evidence that many of the effects may be due to inhibition of aromatic amino acid decarboxylases (2), direct evidence has been obtained that 5HTP decarboxylase (EC 4.1.1.26) is inhibited by decaborane ($B_{01}H_{14}$) (3); these are all pyridoxal-requiring enzymes. For this reason, we chose to examine a number of pyridoxal enzymes, as well as several non-pyridoxal-requiring enzymes, to test the hypothesis that the toxic manifestations of boron hydrides are primarily due to their inhibition of pyridoxal enzymes.

We are reporting our initial results showing the effects of decaborane ($B_{10}H_{14}$) upon glutamic-oxalacetic transaminase (aspartate aminotransferase, EC 2.6:1.1) activity in the

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