deuterated cells. The striking effect of partial deuteration upon the circadian rhythm in *Euglena* has previously been reported (*5*), and it will be interesting to explore this behavior in fully deuterated organisms.

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Autonomic Mediation of the Effect of Raised Arterial Glucose upon Free Fatty Acids

Abstract. *The intravenous infusion of 600 milligrams of glucose over 30 minutes caused a 17 percent fall in the concentration of free fatty acids in arterial blood of subjects who had fasted overnight. This response to glucose was abolished in subjects treated previously with ganglionic or adrenergic blocking agents. Small amounts of insulin were secreted in response to these glucose infusions, but in insulin-dependent diabetics incapable of altering plasma insulin to any great extent, the effect of glucose upon free fatty acids could be obtained provided the subjects were primed with long-acting insulin before the infusions were begun. The response of free fatty acids to doses of glucose which elevated the concentration of glucose in arterial blood by only 3 mg percent and the blockade of this response by autonomic and adrenergic blocking agents suggest that centers in the central nervous system exist which are capable of responding to elevations of arterial glucose by inhibiting the sympathetic tone partially responsible for sustaining lipolysis in fasting.*

The role of the sympathetic nervous system in sustaining lipolysis during fasting has been amply documented (*1*, *2*). Tonic activity of the sympathetic fibers supplying the adipose tissue cells directly (*1*) and centrally mediated release of epinephrine and norepinephrine from the adrenal medulla (*3*) have been established as potential pathways for this activity. The stimuli important for initiating and sustaining this tonic sympathetic activity have received little attention. Dunér (*3*) demonstrated that raising the concentration of glucose in the carotid circulation or directly in the hypothalamus caused a prompt decrease in adrenal medullary secretion in cats. Dunér did not examine the rate of lipolysis in his experiments but rather concluded that the mechanisms under study were primarily concerned with regulating the concentration of glucose in the blood. In this report we present evidence that small increases in the concentration of glucose in the arterial blood of man inhibit lipolysis by decreasing central sympathetic tone (*4*).

Experimental subjects were studied after an overnight fast, in bed, in a quiet room. An intravenous needle for infusion was placed in a forearm vein and an indwelling needle for drawing blood samples was placed in a brachial artery. After a 30- to 45-minute rest period, two or three control samples were withdrawn at 15-minute intervals. Thereafter, solutions were delivered intravenously at a constant rate with a motor-driven syringe pump, and samples of arterial blood were collected periodically. Plasma was analyzed in duplicate for glucose (glucose oxidase) and free fatty acids (*5*).

To study the mechanism of action of glucose in decreasing lipolysis, the concentration of glucose in blood from fasting subjects was raised with small intravenous infusions of glucose. Doses of glucose were sought which would be just large enough to produce measurable changes in the free fatty acids but small enough to avoid major or prolonged alteration of arterial glucose concentration. The dose of glucose which would regularly produce a measurable decline in the free fatty acids in normal subjects proved to be 500 to 600 mg. This amount of glucose, delivered either as a single injection or at a rate of 20 mg/min for 30 minutes, was accompanied by a 20 percent decrease in free fatty acids; after the infusion, the concentration of these acids increased above the control values. To avoid the unpredictable surge of arterial glucose during the first circulation after a single injection, the short infusion technique was adopted for use in further studies. The pattern of response to infusion with 20 mg of glucose per minute for 30 minutes in eight normal subjects is illustrated in Fig. 1. The mean decrease in free fatty acids was −17 percent from the control values. At the same time, arterial glucose was raised by 2 to 3 mg percent. Increasing the rate of infusion to 60 mg of glucose per minute for 30 minutes caused an average decline of 25 percent in the concentration of free fatty acids, and after the infusion there was a more pronounced rise in the concentration (Table 1). Arterial glucose was raised 4 to 7 mg percent by this infusion rate.

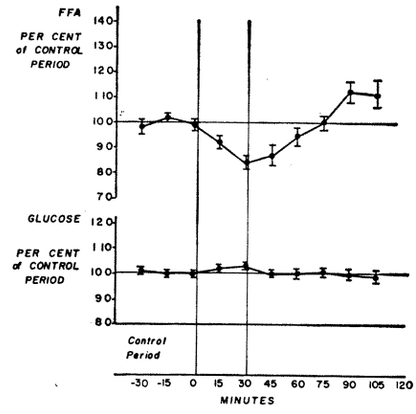

Fig. 1. The response to intravenous infusion of glucose at a rate of 20 mg per minute for 30 minutes (total dose, 600 mg) in eight normal fasting subjects. The vertical lines show two standard errors. Arterial samples were analyzed in duplicate for free fatty acids (*FFA*) and glucose.

Utilizing these small glucose infusions as stimuli, we examined the effect of treating the subjects with various autonomic blocking agents prior to and during the infusions. In normal fasting subjects under the conditions of our experiments, short-acting drugs such as hexamathonium (ganglionic blockade) or the adrenergic blocking agent, *n*-isopropylmethoxamine (*6*), produced a 30 to 35 percent fall in free fatty acids. Infusion of glucose at a rate of 60 mg/min failed to produce a further fall in free fatty acids at the height of blockade with either agent. To avoid the problem of low concentrations of free fatty acids at the time of challenge, prolonged ganglionic blockade was induced in three subjects with pentolinium. Subcutaneous injections of 5 mg were given every 4 hours during the 16 hours before study, and 10 mg was injected 30 minutes before the control period. The subjects were kept in bed through both treatment and experimental periods. The adequacy of blockade was easily demonstrated in all three subjects by

the presence of profound orthostatic hypotension after the final blood sample was obtained. With prolonged blockade, the concentrations of free fatty acids and glucose were in the normal range after overnight fast (Table 1). Infusion of glucose at the supra-threshold rate of 60 mg/min failed to induce significant changes in the free fatty acids in these subjects, despite a 6-percent rise in arterial glucose during the infusion.

From these data two points seem to be established. First, glucose in small doses (2 to 7 calories), which only slightly alter the concentration of glucose in arterial blood, can induce a decline in arterial free fatty acids which approaches that produced by acute autonomic blockade. Secondly, the effect of glucose upon free fatty acids could no longer be elicited after ganglionic or adrenergic blockade.

The concentration of insulin in samples of plasma from several of the experiments was assayed by Berson's technique. Plasma insulin was raised near the end of the infusion period by a factor of 1 to 3 in many (but not all) subjects, particularly after the larger infusions (60 mg/min). When the insulin was elevated during an infusion, it always fell promptly to fasting values after the infusion was stopped. Since the rate of lipolysis is known to be very sensitive to insulin (*7*), the experiments were performed on completely insulin-dependent, pancreatic, and juvenile diabetics to assess the importance of changes in endogenous insulin in mediating the response under study.

The infusion of glucose at rates of 20, 60, or 80 mg/min was not accompanied by fall in the concentration of free fatty acids in insulin-dependent diabetics if they were tested without being primed with insulin before the experiment (Table 1). However, if 40 percent of the daily dose of long-acting insulin [such as Isophane (NPH) or Protamine Zinc insulin (PZI), U.S. Pharmacopeia] was injected 1 hour prior to study, glucose at 20 mg/min evoked a significant decline in free fatty acids in two of three subjects tested (Table 1). In these patients the concentration of free fatty acids rose promptly after the infusion of glucose to values observed prior to the infusions, in spite of continued downward adjustment of the arterial glucose concentration under the influence of long-acting exogenous insulin. This biphasic response in the absence of any possible acute change in available insulin suggests that glucose acts through means exclusive of insulin secretion. The necessity for priming with depot insulin, however, suggests that insulin has a permissive role.

Table 1. The concentration (μeq/liter) of free fatty acids before, during, and after the intravenous infusion of glucose in groups of fasted subjects.

Infusion rate (mg glucose/min)	Control period			Infusion period		Postinfusion period		
	−30 min	−15 min	0	15 min	30 min	45 min	60 min	75 min
Normal subjects								
60	400	387	426	374	310	426	490	568
60	480	517	541	467	406	554	615	652
60	935	972	972	800	664	775	947	1021
Normal subjects with permanent ganglionic blockade (pentolinium)								
60		473	495	528	517	572	649	
60	642	692	704	642	667	679	692	
60	549	627	616	594	582	632	650	
Diabetics, no insulin for 24 hours								
20		476	451	488	500	512	488	
60		817	793	830	817	891	915	
80		854	854	840	938	1022	1190	
Diabetics, primed with Isophane or Protamine Zinc insulin								
20		313	313	286	258	313	354	394
20		687	674	612	562	512	524	687
20		317	317	288	288	306	311	

Under prolonged ganglionic blockade with pentolinium, the concentration of insulin in plasma doubled in one patient and did not change significantly in two of the three patients during infusion of glucose at a rate of 60 mg/min. In the patients under adrenergic blockade, plasma insulin rose normally during glucose infusion. These data raise the possibility that insulin secretion in response to these small doses of glucose is centrally mediated in man. The fact that the concentration of free fatty acids failed to decrease further after glucose infusion in the individuals with adrenergic blockade, in spite of insulin being released, again suggests that the insulin response is not the primary event in reducing free fatty acids under these experimental conditions. However, the finding that insulin secretion was apparently blocked at the same time that the concentration of free fatty acids failed to decline with glucose infusion in patients under ganglionic blockade suggests caution in absolutely excluding insulin secretion as the mediator of this response in normal subjects. Whether insulin secretion is the primary event or a parallel event, however, the participation of the central nervous system in these responses of free fatty acids to glucose would seem to be established.

Several lines of evidence point to glucose-sensitive regulatory centers in the central nervous system. These centers have been looked upon as important primarily for the regulation of plasma glucose itself. The effect of lowered concentrations of glucose in arterial blood perfusing the brain and spinal cord in discharging the sympathetic nervous system is well established (*8*). The effect of raising the concentration of glucose in the carotid circulation upon adrenal medullary secretion has already been mentioned (*3*). In addition, Zunz and LaBarre (*9*) and Sakata *et al.* (*10*) have reported experiments which suggest that selective elevation of cerebral blood glucose causes a lowering of the extracerebral blood glucose, provided the integrity of the nervous system is maintained. Anand *et al.* (*11*) have recently provided more direct evidence for glucose receptors in the hypothalamus. In support of the glucostatic theory of appetite regulation, they showed that raising the concentration of arterial glucose increased the directly recorded activity of the medial hypothalamic "satiety centers" and decreased the activity of the lateral "feeding" centers. In addition, it has long been appreciated that lesions in various parts of the brain cause alteration of glucose regulation (*12*). Since the central nervous system is completely dependent upon the availability of glucose for its normal metabolism, the participation of the central nervous system in regulating plasma glucose is not surprising. In fasting, however, there are two major circulating substrates for cellular metabolism, glucose and free fatty acids. Our experiments suggest that there exists a center (or centers) acting through the autonomic nervous system which alters the balance

of these substrates in response to small changes in arterial glucose. If verified by more direct evidence, the possible relations of such a center(s) to appetite regulation and normal weight maintenance will be of great interest.

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Streptomycinoid Antibiotics: Synergism by Puromycin

Abstract. *Puromycin synergizes the lethal action of streptomycin and related antibiotics. This is interpreted to mean that puromycin action uncovers a sensitive site (or sites) on the 30S ribosome. The streptomycinoid antibiotics can then associate more readily with the ribosome and inhibit further synthesis of valid protein.*

The antibiotics streptomycin, kanamycin, neomycin, paromomycin, gentamycin, viomycin, and hygromycin B all strongly inhibit polypeptide synthesis in cell-free bacterial extracts (*1*), and all possess certain common features in their chemical structures (*2*). Mutants of microorganisms that are resistant to one of these "streptomycinoid" antibiotics frequently show resistance to others in the group (*3*). These observations and those of other investigators suggest a similarity in the mode of action of these antibiotics (*4*). In this report we provide evidence that all members of this group are bactericidal against *Escherichia coli* (following a brief lag) and that this action is antagonized by chloramphenicol and synergized by puromycin, although both of these antibiotics inhibit protein synthesis. Interpretation of this evidence indicates that the streptomycinoids are closely similar in their lethal mode of action.

The general features of the lethal action of the streptomycinoids are illustrated in Fig. 1. These data were obtained using gentamycin, but quite similar curves have been obtained with streptomycin and the other streptomycinoids (Table 1). With gentamycin alone (Fig. 1, curve *C*) a characteristic lag is followed by exponential killing of the microorganisms. There is also a final period during which the survivors die more slowly. In the presence of either chloramphenicol or puromycin alone viability is constant or rises slightly during the first 2 hours (not shown). The antagonism of the lethal effect by chloramphenicol (curve *A*) and the synergism by puromycin (curve *D*) present a striking contrast. After the first 20 minutes puromycin antagonizes further killing by gentamycin. This late antagonism by puromycin can be enhanced by incubating the bacteria with puromycin for 60 minutes before adding gentamycin (curve *B*). Chloramphenicol does not give a synergistic effect at any concentration.

Although the best evidence for the mode of action of a streptomycinoid antibiotic has been obtained for streptomycin itself, we propose that the model presented below for streptomycin action is valid for the entire group. Streptomycin inhibits bacterial protein synthesis in growing cultures (*5*) as well as in extracts, but, since many other biological effects of streptomycin have been described, there has been some question whether the inhibition of protein synthesis per se is, in fact, its lethal action (*4*). The existence of the lag and of the antagonism by chloramphenicol have even been interpreted to mean that protein synthesis is actually required for expression of the lethal action of streptomycin. The puromycin synergism contradicts this idea and clearly calls for an interpretation based on events at the ribosome, which is the common site of action of all three antibiotics. The ribosome may be thought of as operating in a cycle (Fig. 2): the free ribosome associates with a molecule of messenger RNA; a polypeptide

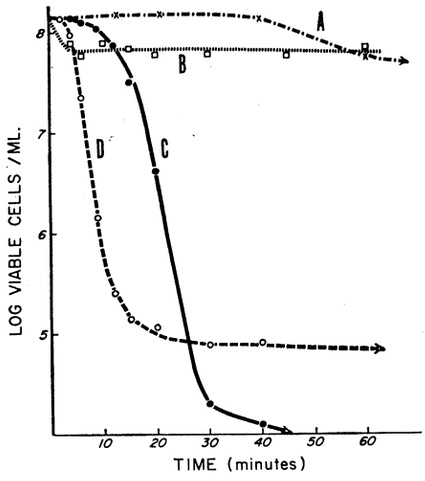

Fig. 1. A phenylalanine-requiring mutant of *Escherichia coli* strain 15 was grown exponentially at 37°C in a tris-buffered minimal medium (*17*) containing glucose and phenylalanine (generation time, 50 minutes). Antibiotics (*18*) were added as follows: *A*, gentamycin and chloramphenicol at 0 minutes; *B*, puromycin, 60 minutes before the addition of gentamycin, and gentamycin at 0 minutes; *C*, gentamycin at 0 minutes; *D*, gentamycin and puromycin at 0 minutes. Concentrations: gentamycin, 10 μg/ml; puromycin, 500 μg/ml; and chloramphenicol, 200 μg/ml. Serial dilutions of the samples were made in ice cold saline and were plated on Difco nutrient agar.

chain is initiated, grows, and is released; and messenger RNA is also ultimately released (*6*). Streptomycin (or other streptomycinoids) may be postulated to associate irreversibly with the ribosome, thereby eliminating that ribosome as a further site of protein synthesis. It is the irreversibility of this association that is lethal. In addition it must be postulated that this irreversible association occurs only when the ribosome is in a particular, sensitive state, which we take to be the free state (*7*). These postulates lead to the following interpretation of streptomycin action.

When streptomycin is added to an exponentially growing culture, only a few of its 10^4 ribosomes are in the sensitive state. These ribosomes are quickly inactivated, but the cell remains viable until a sufficient portion of its ribosomes have cycled around to the sensitive state and have been inactivated in turn. The inactivated ribosomes can still associate with messenger RNA (*7, 8*), but evidently cannot catalyze the normal synthesis of protein; as a result the cell can no longer form a colony on nutrient agar and is by definition nonviable. Chloramphenicol apparently holds a large fraction of the ribosomes in insensitive states by a mechanism