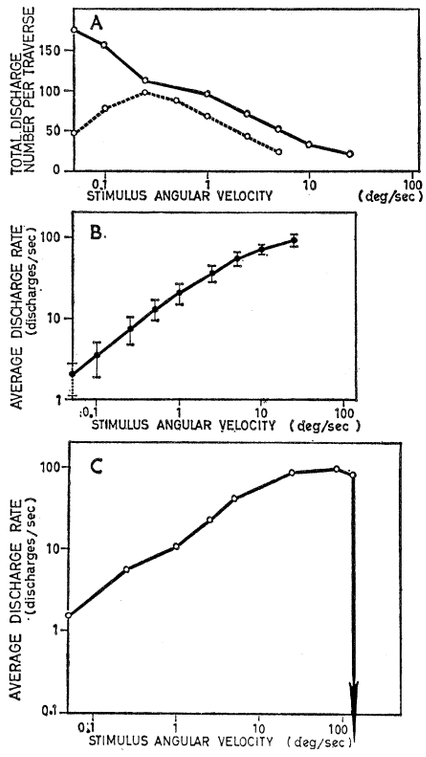

Fig. 2. (*A*) Total discharges per traverse for two units tested with a 1.2°-stimulus. The variability in response patterns demonstrated by these two neurons was found in many of the neurons tested. (*B*) Dependence of the means of the average discharge rates of 25 units on the velocity of a 1.2°-stimulus. Bars represent standard deviation of the collective values. (*C*) Dependence of average discharge rate of a single neuron on the velocity of a 0.7°-stimulus.

of dark followed by 60 seconds of light) in order to eliminate the effects of neural and photochemical adaptation (*4*). The neural responses were displayed on a cathode-ray oscilloscope and photographed together with a synchronized display of the stimulus movement which was monitored by a potentiometer.

Figure 1 shows the recordings of a single neuron responding to two of the nine angular velocities. We examined two qualities of the neural response: (i) the total discharge number per stimulus traverse and (ii) the average discharge rate per stimulus traverse.

The total discharge number per traverse showed no simple relation to the angular velocity of the stimulus. The angular velocity which elicited the maximum number of discharges per traverse varied for different class 2 neurons. Sixteen of the 25 units gave a maximal discharge number at the two slowest angular velocities possible with our perimeter (0.05° and 0.1° per second). The responses of two of the 25 units are illustrated in Fig. 2*A*.

The discharge rate (a measure of neural activation probably of more physiological significance than the total discharge number) was not constant as the stimulus moved through the ERF (see Fig. 1). The average discharge rate per stimulus traverse showed a strict dependence on the angular velocity of the stimulus in all 25 neurons that were tested (Fig. 2*B*). The relation may be expressed by the power function

$$R = k_0 \cdot v^{0.7}$$

where R is the average discharge rate per second, k_0 is a constant, and v is the angular velocity of the stimulus in degrees per second. With a 1.2°-black spot and a black–white luminance ratio of 1 : 34, the value of k_0 is 20 discharges per degree. This power function is valid for values of v from at least 0.05° to 10° per second. In examining the relation of the discharge rates in the peripheral thirds of the ERF or in the central third of the ERF to the angular velocity of the stimulus, similar power functions were found, differing only in the value of k_0. It appears, therefore, that the class 2 neurons perform the same operation at different degrees of excitability in different parts of the ERF.

The stage that carried the stimulus was moved by hand in order to observe the neural response to angular velocities higher than 24° per second. Figure 2*C* illustrates that the class 2 operation breaks down rapidly at angular velocities above 100° per second, and, at 140° per second, this neuron responded with only one discharge per traverse.

Class 2 ganglion cells code a number of characteristics of a visual stimulus, such as size, shape, contrast, and angular velocity. We used a motor-driven perimeter, varied the angular velocity of the stimulus over a series of fixed values, and kept the other visual parameters constant. The relation of the average discharge rate of ganglion cells to the angular velocity of the stimulus suggests the possibility of further quantitative studies in order to analyze the functional properties of the neural network which determines the class 2 operation.

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19 July 1965

Growth Hormone: Important Role in Muscular Exercise in Adults

Abstract. *Measurement by radio-immunoassay of growth hormone in the plasma of adults showed consistently low concentrations in subjects in bed in the morning. Exercise caused marked increases except when the subjects ingested carbohydrate during the exercise.*

Pituitary growth hormone is secreted in the adult in response to muscular exercise. Small increases in its concentration in plasma were found in two subjects after they had walked 8 km (*1*), and concentrations were high in three subjects after they had played squash for 2 to 3 hours (*2*). Injection of growth hormone raises the concentration of nonesterified fatty acids in the plasma (*3*), and these constitute the principal fuel during exercise (*4*). This report, based on measurement of growth hormone in plasma by radio-immunoassay (*5, 6*), suggests an important role for the hormone in mobilizing fuel for muscular exercise. As a corollary it shows that studies of growth hormone in plasma are of little value unless the subject's rate of expenditure of energy is known.

After an overnight fast, plasma samples from normal adults sometimes show high values for growth hormone, although most have values below the sensitivity of present assays [1 to 2 ng(μmg)/ml] (*6, 7*). In our laboratory ten adult males sampled in bed before rising all had concentrations below 1 ng/ml, whereas 9 of 23 subjects, who traveled to the laboratory and rested for 1 hour before sampling, yielded 17 elevated values (range, 2 to 53 ng/ml) from a total of 44 samples from the 23. Eight of the nine individuals showing high concentrations were later sam-

pled before rising; none had a value greater than 2 ng/ml. We therefore conclude that in the absence of exercise an overnight fast does not stimulate secretion of growth hormone in normal adults.

Measurements of concentrations of growth hormone, blood sugar, and nonesterified fatty acids in plasma, and of respiratory quotients during a 12.8-km walk at 6.4 km/hr on a treadmill, in six subjects after an overnight fast, are shown in Fig. 1. Exercise at this rate expends energy approximately five times faster than remaining at rest. Association of a steadily rising concentration of fatty acids in plasma, a falling respiratory quotient implying increasing combustion of fat, and a marked increase in growth hormone in plasma (with peak concentrations ranging from 17 to 53 ng/ml) during the walk is clear. The pattern of secretion of growth hormone in three subjects showed a peak at 1 hour, and in all but one subject there were decreases during the 2nd hour. In all subjects the concentration of fatty acids in plasma continued to rise throughout the walk.

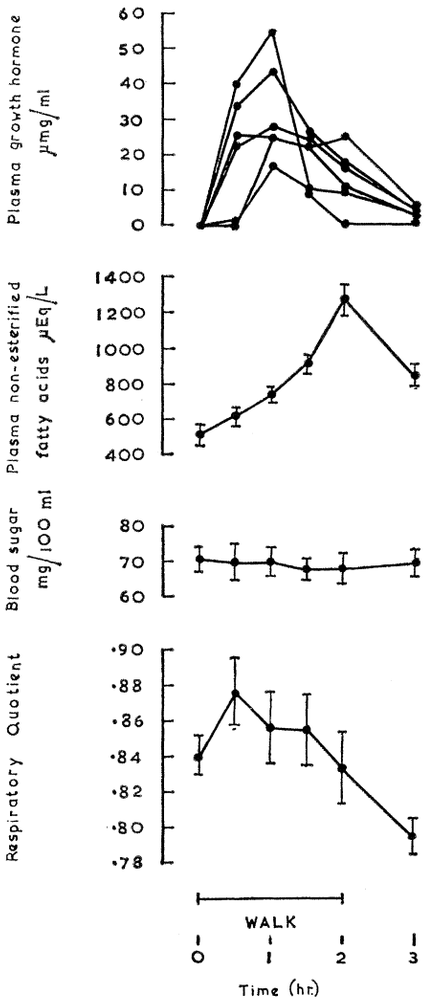

Fig. 1. Growth hormone (*12*) and nonesterified fatty acids in plasma, venous blood sugar, and respiratory quotient during and after a 12.8-km walk at 6.4 km/hr in six normal adults aged 19 to 37. Figures for the nonesterified fatty acids, sugar, and respiratory quotient are means plus or minus standard error of the mean.

Concentrations of growth hormone in six male physicians whose plasma was sampled at hourly intervals during ward duty throughout a normal working day were all less than 3 ng/ml —many times lower than the peak values found in our walking subjects. This difference emphasizes the important effect of the intensity of exercise on secretion of the hormone.

In a second experiment (Fig. 2), one subject, a 29-year-old male, performed the same 2-hour walk at 6.4 km/hr (i) in the fasting state, (ii) taking glucose orally in four doses, each of 25 g, immediately before and at ½-hour intervals during the walk, and (iii) taking protein (casein) in similar quantities and at similar times. After ingestion of glucose there was no increase in blood sugar, but the availability of exogenous glucose completely abolished the increase in fatty acids and the decrease in respiratory quotient, with no increase in concentration of growth hormone in plasma. After ingestion of casein there was a more than twofold increase in amino nitrogen in plasma over the fasting concentration throughout the walk, without increase in urinary nitrogen during this 2-hour period. Concentrations of fatty acids were lower than in the fasting state. The increase in fatty acids associated with fasting was similarly partially suppressed by administration of glutamine, leucine, or analine (*8*). The concentration of growth hormone in our experiment after ingestion of casein (iii), however, was higher than after fasting. This increase in concentration of growth hormone during a walk taken during ingestion of protein was confirmed in a second subject, who also showed suppression of secretion of

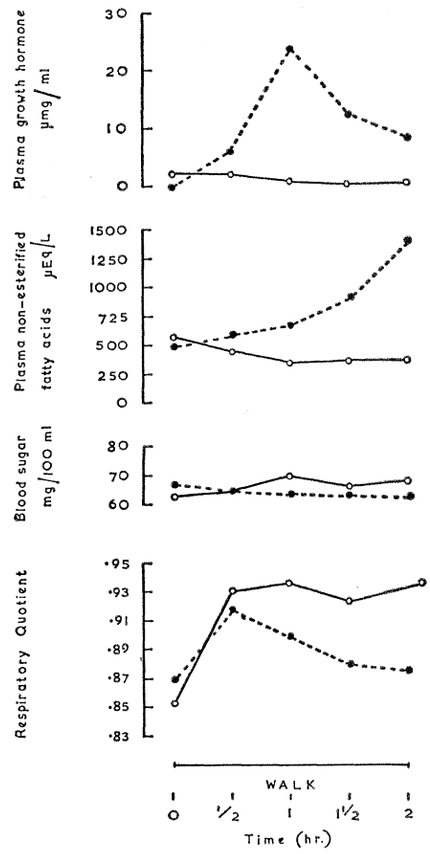

Fig. 2. Measurements during 12.8-km walks by a 29-year-old male in fasting state, ---●---, and while ingesting glucose, —O—; each point is the mean of values from duplicate experiments.

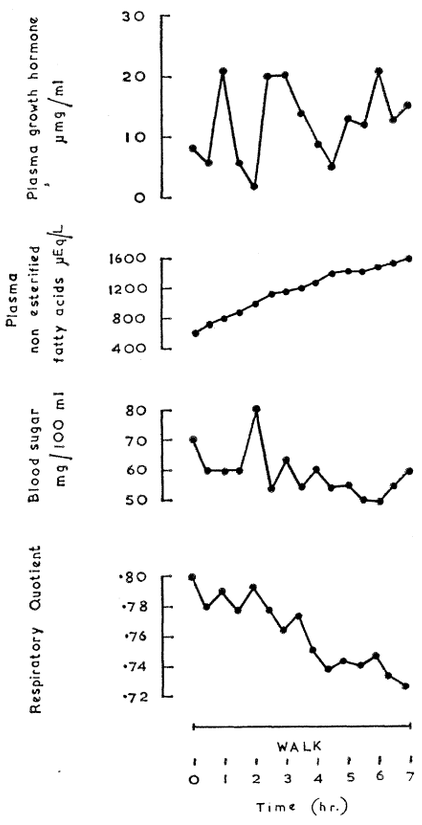

Fig. 3. Measurements during 45-km walk by 20-year-old male in the fasting state.

growth hormone in duplicate walks while ingesting glucose; he also showed similarly increased concentrations of fatty acids and growth hormone during a walk after fat had been introduced into the third part of the duodenum by nasogastric tube, as during a walk when starving. This finding agrees with the current concept that ingested fat is utilized only after its uptake by adipose tissue and subsequent release as fatty acids.

Three subjects walked 30, 37, and 45 km, respectively, at 6.4 km/hr while fasting; Fig. 3 shows the data for one of them. There was a slow decrease in concentration of blood sugar, and a more marked and steady increase in nonesterified fatty acids in plasma throughout the walk; data on respiratory quotient show progressive increase in the proportion of fuel derived from fat, which reached nearly 100 percent toward the end of the walk. If, as seems probable, the increase in nonesterified fatty acids resulted principally from the secretion of growth hormone, it is perhaps surprising that the hormone concentrations indicate a series of intermittent bursts of secretion rather than a steady increase with time. The same general pattern was found in the other two long walks. We suggest that growth hormone mobilizes fat by a series of "triggering" actions. Rabinowitz, Klassen, and Zierler (*9*) showed that nonesterified fatty acids in plasma were still increasing 35 minutes after a close arterial infusion of growth hormone was terminated.

Walks for ½, 1, 2, and 5 hours at 6.4 km/hr were performed by one subject, a 37-year-old male, in the fasting state. The recovery period was followed in detail for the two shortest walks. Concentration of growth hormone was less than 1 ng/ml immediately after the ½-hour walk, but rose to 5.7 ng/ml 45 minutes later and fell to less than 1 ng/ml during the next hour, showing that the concentration may be high even some time after a comparatively short period of moderate exercise. In the 1-, 2-, and 5-hour walks, peak concentrations of 22, 24, and 18 ng/ml were recorded after 1 hour. After the 1-hour walk the concentration fell exponentially to less than 1 ng, with a half-time of 22½ minutes, a rate of decrease identical with that which follows a single intravenous injection of the hormone (*10*). This suggests that secretion stopped at the end of this walk. In the two longest walks the rate of decrease was slower, suggesting that, if exercise is continued during the 2nd hour, secretion is reduced but not abolished. However, the pattern of concentration of growth hormone during the 2nd hour was little different whether the walk continued or not, whereas the concentration of nonesterified fatty acids always increased until the end of the walk and fell immediately thereafter. We suggest that some other factor must antagonize the nonesterified fatty acid releasing action of the growth hormone present during the recovery phase. It seems probable that secretion of insulin may increase at this time; the period is characterized by increase in ketone bodies, which have been shown to increase secretion of insulin (*11*).

Most of our experiments have been with men, but two women showed similar changes during a 12.8-km walk. Our subjects ranged in age from 19 to 54, but no age difference in response by growth hormone appeared.

These studies show that, during muscular exercise by normal human adults, unless exogenous carbohydrate is made available, the needs for fuel are increasingly met by mobilization of depot fat, and that secretion of growth hormone appears largely responsible for initiating and maintaining this process. The magnitude of the effect of exercise on concentrations of growth hormone is so great that precise control of energy expenditure is a prerequisite for any studies of the concentrations of this hormone in plasma.

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16 August 1965

Lysosomal and Free Acid Phosphatase in Salivary Glands of Chironomus tentans

Abstract. *In cells of salivary glands of last-instar larvae of* Chironomus tentans, *acid phosphatase activity is bound to (probable) lysosomes and a few other cell organelles. At the end of the pupal molt the salivary gland breaks down. While acid phosphatase in areas of nondegenerated cells is still restricted to the structures mentioned, in degenerated areas the enzyme is freely distributed in the cytoplasm.*

In vertebrates, many hydrolytic enzymes such as acid phosphatase are localized in cytoplasmic organelles termed lysosomes (*1*). It is assumed that in the process of cell breakdown the enzymes are released from the lysosomes and distributed throughout the cell. Biochemical and electron-microscopic data have been interpreted to support this idea (*1, 2*). This interpretation has been questioned, however, as possibly being based on artifacts resulting from the experimental procedures (*3*). In insects many tissues break down during the developmental transition from the larval to the adult stage in the course of metamorphosis. Lysosomes have never been convincingly shown to occur in insects, although their existence has been reported (*4*). In our studies of the relations between changes in gene-activity (puffing) patterns and enzyme patterns related to the breakdown of salivary-gland cells in *Chironomus tentans* (*5*), we not only found organelles resembling vertebrate lysosomes, but obtained clear evidence of the existence of free acid