that melanin microgranules gradually disperse within dendritic epidermal melanophores of teleost fishes as a response to dark backgrounds and reaggregate in the center of the cell, giving the animal a pale appearance in white-reflecting aquariums. Such light-induced reactions are regarded by many naturalists (*16*) as being valuable for survival by contributing to the animal's concealment from predators.

Direct or reflected illumination as opposed to dim light, however, evokes responses opposite from the above in the choroid melanophores of elasmobranchs, inducing a dispersal of light-shielding melanin over the specular tapetum in bright light and, in darkness, retreat of the pigment into the mother-cells. The tapetal surface is thus exposed in dimly illuminated environments. Gilbert (*4*) quotes and agrees with Nicol, who pointed out that visual acuity is likely to be improved on a black, absorbing surface, and sensitivity increased by a specular tapetum. It is conceivable that a shark, adapting itself for improved vision in moderately lighted waters by partially shading its tapetum, may also profit to some degree through diminution of its conspicuous eye-shine, but that at deeper and darker levels the increased tapetal exposure, augmenting the predator's visual perceptiveness, should more than compensate for the enhancement of the eye-shine and its incidental warning signals.

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Regulation of Cockroach Fat-Body Metabolism by the Corpus Cardiacum in vitro

Abstract. *Incubation in vitro of young, adult, male* Leucophaea maderae *fat body with extracts of corpora cardiaca or intact corpora cardiaca results in stimulation of oxygen consumption but reduction in carbon dioxide evolved from carbohydrate. The carbohydrate is preferentially used for trehalose synthesis, and the endogenous metabolism of the fat body appears to be supported by increased lipid utilization. A hormone from the corpus cardiacum is most likely responsible for these effects and may act at two points, at least, in the glycolytic pathway.*

The corpus cardiacum is an important component of the neuroendocrine axis that regulates the growth and development of insects. It is located between, and connected to, the brain (source of the brain hormone) and the corpus allatum (source of the juvenile hormone). In addition to the proposed role of the corpora cardiaca as storage centers or modifiers of the brain hormone during the molting cycle, these structures have also been implicated as regulators of physiological processes vital to the existence of the organism. For example, the corpus cardiacum stimulates the muscular contraction of the insect heart, hindgut, and malphigian tubules (*1*); it depresses the spontaneous activity of the central nervous system (*2*); and it blocks inhibitory centers of the nervous system (*3*). The release of the humoral substance or substances from the corpus cardiacum may be a consequence of normal feeding (*4*) or may be induced by enforced hyperactivity or electrical stimulation (*5*). The chemical nature of the factor or factors responsible for the various actions of this gland is not known with certainty, although there have been some attempts to separate the components of the gland by differential centrifugation and solvent partitioning (*6*).

The injection of corpus cardiacum extracts into cockroaches (*Periplaneta americana* and *Blaberus discoidalis*) results in the mobilization of fat-body glycogen and a consequent increase in blood trehalose (*7*). These alterations in substrate concentration may be due to an increase in the concentration of active phosphorylase (*8*). We have shown with techniques in vitro that the corpus cardiacum stimulates the release of trehalose from the fat body of *Leucophaea maderae*, with a concomitant decrease in fat-body glycogen (*9*). In the course of experiments designed to perfect a quantitative bioassay for this "hyperglycemic hormone," we observed the release of trehalose from the C^{14}-labeled glycogen from the fat body and noted new effects of the corpus cardiacum on fat-body metabolism that have bearing on the mechanism of action of the hormone.

The experimental insects were male adults 4 to 7 weeks old; they were raised at 26°C, 85-percent relative humidity, on dog biscuits and water. Experimental conditions in vitro were designed to minimize effects on other organs that could respond to the hormone and secondarily affect the fat body. Hormone extracts were prepared by homogenizing corpora cardiaca in buffered Ringer solution (0.005*M* tris, *p*H 7.5), heating in a water bath at 100°C for 7 minutes, and using the supernatant after centrifugation of the boiled homogenate at 3000*g* for 5 minutes (*7*). The fat bodies from 8 to 12 decapitated cockroaches were dissected out under cold buffered Ringer solution, gently separated into small pieces, and randomly distributed in four to six reaction flasks. Incubation at 30°C in an oscillating water bath was followed by removing and rinsing the tissues, and homogenizing them in a mixture of chloroform and methanol (2:1) to extract the lipid. The "lipid-free dry weight" or lean dry weight was determined gravimetrically and was a convenient and reproducible representation of the tissue mass. Glycogen was extracted from the lipid-free material with trichloroacetic acid and subsequently precipitated with ethanol. Preparation of sodium palmitate and assay of the lipid, glycogen, and CO_2 fractions by liquid-scintillation spectrometry was as previously described (*10*), except that in the present work the glycogen was

suspended in 4 percent Cab-O-Sil (Packard) prior to counting.

The $C^{14}O_2$ resulting from the oxidation of uniformly labeled glucose-C^{14} by fat body in the presence of corpus cardiacum was only about 70 percent that of the controls (Table 1). In addition, the tissue incubated in the presence of hormone yielded a glycogen fraction in which the net radioactivity was about 15 percent that of the control; the radioactivity of the lipid fraction derived from the experimental tissue was also lower. The results of the glycogen assay are in general agreement with the hypothesis of hormonal activation of phosphorylase and consequent increased rate of glycogen degradation. This degradation would particularly apply to the radioactive glucose molecules recently incorporated into the distal ends of the glycogen molecule. However, the decreased rate of $C^{14}O_2$ release in the presence of hormone was unexpected because of the stimulation in vitro of oxygen consumption (QO_2) in fat body from female *Leucophaea* by intact corpora cardiaca (*11*). We have confirmed this increase in QO_2 with male fat body, using hormone extract as well as intact glands (Table 2). When the tracer and manometric experiments were combined, we demonstrated consistently the simultaneous decrease in $C^{14}O_2$ from labeled glucose in the presence of hormone and a concomitant increase in QO_2. The observations may be due to the action of corpus cardiacum hormone in stimulating the metabolic rate while causing fat-body utilization of noncarbohydrate substrates. If so, one should discern a decrease in respiratory quotient of the fat-body tissue in the presence of hormone. There is a tendency for a decrease in respiratory quotient when the tissue is incubated in the presence of hormone, indicating a lesser rate of carbohydrate catabolism and a shift toward increased utilization of lipid or protein (Table 2).

The increase in the active form of phosphorylase by the action of the corpus cardiacum in *Periplaneta* (*8*) and *Leucophaea* (*9*) cannot by itself account for the foregoing results. The consequence of increased phosphorylase activity would be a rise in the concentration of phosphorylated sugars, possibly leading to a higher rate of glycolysis, trehalose release, or resynthesis of glycogen. None of these would directly cause a decrease in either the respiratory quotient or the oxidation of labeled glucose to $C^{14}O_2$. These data indicated that the hormone must act on the fat body by decreasing the amount of carbohydrate available for glycolysis, while increasing the quantity available for other tissues in the form of newly synthesized trehalose. In examining the possible compensation by other substrates for decreased carbohydrate oxidation, one may expect an increase in the oxidation of acetate-1-C^{14} to $C^{14}O_2$ by the fat body in the presence of hormone. That this is so is shown by the summary in Table 2. Under these conditions the hormone stimulates production of $C^{14}O_2$ from labeled acetate at a rate about 30 percent greater than that of the controls. Concentrations of from 0.02 to 2.0 pairs of corpora cardiaca (as extract) per milliliter of incubation medium showed similar effects, as did experiments with fat body and corpora cardiaca from female *Leucophaea* (*9*). Thus, acetate is consumed at a greater rate by the fat body in the presence of hormone, and glucose is catabolized at a lesser rate.

Acetate is not a storage substrate but is an intermediate in the catabolism of either lipid or carbohydrate and readily enters the Krebs cycle. Because we demonstrated that the increased QO_2 is not a result of carbohydrate degradation, our attention turned to the lipid that is present in large quantities in the fat body of this cockroach. In the presence of hormone extract, the con- all cases, intact glands used as the source of hormone produced metabolic effects indistinguishable from those of the corpus cardiacum extracts. Moreversion of palmitate-1-C^{14} to $C^{14}O_2$ by the fat body is about 35 percent greater than that of the controls (Table 2). In

Table 1. Effect of the corpus cardiacum on the incorporation and catabolism of glucose-C^{14} by the fat body in vitro. The incubation medium contained 3 ml of buffered Ringer solution in the first four experiments or unbuffered Ringer solution in the next two experiments, at *p*H 7.5; 1.5 percent trehalose; 2×10^6 count/min uniformly labeled glucose-C^{14}. Control vessels contained either no extract or muscle (*m*) extract. Experimental vessels contained hormone extract equivalent to one pair of corpora cardiaca per milliliter of incubation medium or intact (*i*) glands. The reaction took place at 30°C for 60 or 90 minutes in an oscillating water bath. The release of $C^{14}O_2$ was linear for at least 3 hours. The results are presented as counts per minute per hour of incubation per milligram of tissue (lean dry weight, LDW). All counts were corrected for quenching and efficiency. S.E.D. is the standard error of the difference between control and experimental.

Carbon dioxide		Glycogen		Lipid	
Control	Exptl.	Control	Exptl.	Control	Exptl.
		Experiment 1			
407	353	306	49	46	34
		Experiment 2			
408	323	83	32	126	79
364 (*m*)	293 (*i*)	56 (*m*)	27 (*i*)	121 (*m*)	90 (*i*)
		Experiment 3			
563	359 (*i*)				
499					
		Experiment 4			
635	321	263	29	422	157
	340 (*i*)		30 (*i*)		175 (*i*)
		Experiment 5			
355	200	209	15		
		Experiment 6			
221	178	133	26	101	57
Mean 431	296	175	30	163	99
S.E.D. = ±35		S.E.D. = ±39		S.E.D. = ±45	

Table 2. Summary of effect of the corpus cardiacum on key metabolic factors of the fat body in vitro. In the experiment on oxygen consumption (QO_2) and respiratory quotient (RQ), conventional manometric methods were used; oscillating 14-ml Warburg flasks at 30°C contained 3 ml of buffered Ringer solution, *p*H 7.5, and, with one exception, 1.5 percent trehalose. Controls contained either no extract or muscle extract (one determination), whereas the test preparations contained one pair of corpora cardiaca per milliliter of solution, as extract or as intact glands (two determinations). Oxygen consumption is expressed as microliters per hour per 100 mg (LDW) of tissue. In the experiments on oxidation, the incubation medium contained 3 ml of buffered Ringer solution and 2×10^6 count/min of sodium acetate-1-C^{14} or sodium palmitate-1-C^{14}. In the acetate experiment, one determination each was made with extract at the following concentrations: 0.01, 0.02, 0.1, and 2.0 glands per milliliter. The results are given as the number of counts per minute of labeled CO_2 released per hour of incubation per milligram (LDW) of tissue. S.E.D. is the standard error of the difference between the means of the control and the experimental series. The numbers in parentheses indicate the numbers of determinations.

QO_2	RQ	Oxidation (CO_2 released)	
		Acetate	Palmitate
	Control		
129 (9)	0.944 (3)	3170 (6)	863 (5)
	Experimental		
165 (9)	.836 (3)	4065 (7)	1138 (6)
	S.E.D.		
±5.9	±0.05	±165	±115

over, control extracts of muscle or testis were without effect.

We postulate that the hormone from the corpus cardiacum acts at perhaps two points in the pathway of carbohydrate metabolism. First, it increases the rate of glycogen degradation and, second, it diverts the mobilized hexose residues from the glycolytic pathway and into the blood as trehalose. As a consequence, the endogenous metabolism of the fat body shifts toward increased utilization of lipid. The increased metabolic rate reflected in an increased QO_2 should provide greater amounts of adenosine triphosphate for regeneration of the uridine triphosphate utilized in the endergonic process of trehalose synthesis. Thus, the lower rate of conversion of glucose to lipid in fat body treated with hormone (Table 1) may be due to inhibition of the conversion of phosphorylated hexoses to acetate and to increased utilization of lipid.

Where may the hormone act in addition to increasing the amount of active phosphorylase? We believe that the most probable activities are (i) inhibition of glycolysis at phosphofructokinase and (ii) activation of trehalose-6-phosphate synthetase. The regulation of carbohydrate oxidation by phosphofructokinase should be considered, since it may be a rate-limiting step in mammals (*12*) as well as in insects (*13*). This enzyme in rat heart appears to be easily inhibited by a number of metabolites (*12*) and is activated by serotonin and adenosine-3′,5′-monophosphate in *Fasciola* (*14*). The regulation of trehalose synthesis by trehalose-6-phosphate synthetase may occur by interconversion between active and inactive forms of the enzyme, such as is known to occur for phosphorylase and for glycogen synthetase (*15*). Hormonal control of either enzyme could cause the shifts in metabolic pattern which the data indicate.

Trehalose itself inhibits the trehalose-6-phosphate synthetase, but an increased concentration of glucose-6-phosphate relieves this inhibition (*16*). The corpus cardiacum hormone must upset this homeostatic state in favor of a rate of synthesis of trehalose greater than that expected from simple changes in chemical equilibria. This supposition is supported by experiments in vivo (*8*) and in vitro (*9*) that show that trehalose is synthesized by the fat body and released into the blood at a much greater rate in the presence of corpus cardiacum hormone. Whether this hormone is a product of the corpora cardiaca or is synthesized elsewhere and stored in the corpora cardiaca is still a matter of conjecture. However, it has been firmly established that the corpus cardiacum of *L. maderae* does possess the morphological characteristics of an endocrine gland (*17*).

In situations of stress (*18*) or hyperactivity, relatively large amounts of trehalose are made available to other tissues such as muscle. Under these conditions the central nervous system may regulate the release of hormone from the corpus cardiacum, and thereby cause an increased rate of trehalose synthesis in the fat body and release into the blood. This view is in accord with proposals by others (*1, 7, 8*).

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Glycerol Metabolism in the Human Liver: Inhibition by Ethanol

Abstract. *Glycerol is metabolized predominantly in the liver, the first step presumably being phosphorylation to α-glycerophosphate. When ethanol is present in the blood the rate of glycerol uptake by the splanchnic organs is reduced to about one-third of the control value. At the same time glycerophosphate accumulates in the liver. Hepatic blood flow and oxygen consumption are not influenced by the combined infusion of glycerol and ethanol. The phenomenon may be connected with the increased concentration of the reduced form of diphosphopyridine nucleotide present in the liver during ethanol metabolism.*

When ethanol is oxidized in the normal human liver, more than half the oxygen consumption of this organ is required for the transformation of ethanol to acetate, which is released into the venous blood (*1*). Major changes in the metabolic processes of the liver must therefore take place during alcoholemia, especially an inhibition of the tricarboxylic acid cycle. However, other changes are also observed, for example, in the metabolism of galactose (*2*) and fructose (*3*).

Lundsgaard (*4*) recorded the concentration of glycerol in the blood after oral ingestion of glycerol by human subjects. He observed a marked reduction in the slope of the curve when ethanol was given together with glycerol. This observation could be explained by the increased release of glycerol from peripheral tissues or by the decreased rate of its removal from the circulation by the liver and possibly other organs.

We have now studied the metabolism of glycerol in livers of patients who were considered metabolically normal. Glycerol was administered as a continuous infusion for about 50 minutes (first period). Three samples of arterial blood and hepatic venous blood were collected during the last half of the period, when the concentration had be-