Table 2. In vitro mucosal-to-serosal sugar flux

	Flux Glucose	Galactose	3-O-methylglucose	Fructose
Control	86.8 ± 7.3	41.1 ± 9.2	19.8 ± 2.2	13.4 ± 3.6
Glucose infused	248.0 ± 29.4	110.0 ± 15.3	39.4 ± 4.7	11.3± 2.3
Galactose infused	218.3 ± 20.8	94.2 ± 18.7		15.9 ± 3.9
Fructose infused	179.8 ± 34.0			32.9 ± 6.5
Control + cycloheximide	90.0 ± 11.5			
Glucose infused + cycloheximide	68.8 ± 16.9	33.7 ± 6.0	15.1 ± 5.3	
Fructose infused + cycloheximide				10.7 ± 3.6

Method and calculation as in table 1. n = 6–11. Cycloheximide was given intravenously in a dose of 1.5 mg/kg at the beginning of the i.v. sugar infusion; in the control: 4 h prior to the killing of the animal.

glucose infusion because there was no increase in the mucosal-to-serosal flux of sugars in the intestine of control animals infused with the same concentration and volume of mannitol. The protein synthesis inhibitor, cycloheximide, completely inhibited the enhancement of hexose transport following hyperglycemia, hypergalactosemia and hyperfructosemia, without any effect upon the basal sugar transport (table 2).

The stimulation of the mucosal-to-serosal flux of sugars was not necessarily accompanied by an intracellular accumulation, particularly in the gut of animals with moderate hyperglycemia. This agrees with the finding of Olsen and Rosenberg[7] that the intestine of rats which were maintained in a hyperglycemic state (degree not specified) for 5 days by intravenous glucose infusion did not display an increased tissue accumulation of 3-O-methylglucose.

These findings support the following hypothesis: the increase of the sugar level in the blood enhances the transepithelial sugar transport. It is most likely that the enhancement is the consequence of an induced synthesis of new transport carriers which would explain why the high blood sugar has to be maintained for several hours before it takes effect. It was also observed that the enhanced sugar transport is maintained for a few hours after the blood sugar returned to a normal level. The complete inhibition by cycloheximide of the increased sugar transport produced by hyperglycemia, hypergalactosemia and hyperfructosemia strongly indicates that the transport enhancement is connected with the synthesis of additional transport receptors (carriers).

The interesting finding of the present study is the existence of a substrate-specific adaptive transport system which is rather rapidly activated, presumably through the synthesis of new transport receptors (carriers). The system is not localized in the brush border but most probably in the basolateral membrane; hence its inhibition by phloretin but not by phlorizin.

The effect of cobra venom factor (CVF) activation of the complement cascade on leukocyte circadian variation in the rat[1]

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Summary. Administration of cobra venom factor (CVF) at different time periods over a 24-h-period produced a leukocytic response which varied according to the time of day the factor was given. The resulting leukocytic circadian rhythm was achieved by a marked variation and increase in the neutrophil population.

Serum complement consists of 9 separate protein macromolecules (C_1–C_9) and is sequentially activated by a series of enzymatic reactions in the presence of antigen-antibody complexes. An alternate pathway in the complement cascade can be activated by purified cobra venom factor (CVF) or the polysaccharide inulin[2]. Biological activities resulting from activation of the various components of the complement cascade include chemotaxis (C_{3a}, $C_{5a,6,7}$), opsonization (C_{3b}), anaphylaxis (C_{3a}, C_{5a}), and lysis and lesion formation at the cell membrane (C_8, C_9)[3]. Recently a new biological activity of complement has been described following activation by inulin and purified cobra venom factor of the alternate pathway[4]. An immediate neutropenia followed by a marked neutrophilia occurred. Accordingly, this response was studied to determine it might be time sensitive and display a circadian rhythm. This investigation determined the effects of CVF administered equally at different time periods over a 24-h-period on leukocyte response and rhythm.

Materials and methods. 45-day-old male Holtzman rats weighing between 200 and 250 g were housed 5 per cage and allowed free access to food and water. The rats were maintained rigidly on a 12-h-light(7.00 a.m. to 7.00 p.m.)-

1 This study was supported by U. S. P. H. S. Grant No. HL 16769 and Grant No. AI 09169.

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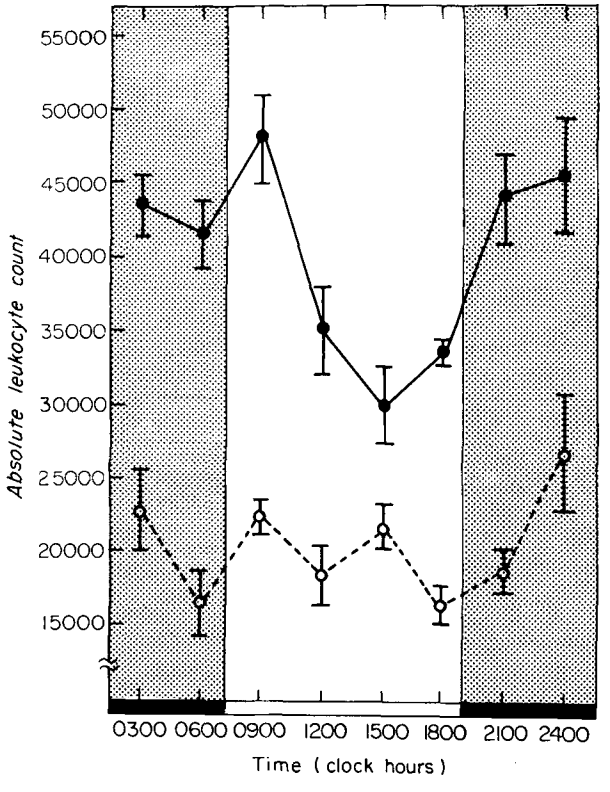
dark cycle and allowed 1 week to acclimate prior to experimentation.

Eight time periods at 3-h-intervals (03.00, 06.00, 09.00, 12.00, 15.00, 18.00, 21.00 and 24.00 clock hours) were investigated. Each hour consisted of 2 groups. The first (controls) received an injection of 0.5 ml of phosphate buffered saline (PBS). The second group (experimentals) received an injection of 0.15 ml of purified cobra venom factor (CVF) diluted in 0.35 ml of PBS (total volume of 0.5 ml). All injections were given via a jugular vein cutdown, and blood samples were taken 45 min later by cutting approximately 1 mm off the tip of each tail. The first drop of blood was discarded and the second was drawn up into a blood-diluting pipette. The pipette was then emptied into a vial containing 10 ml of isotonic diluent and 2 drops of lysing agent. The absolute leukocyte count was then obtained using a Coulter Counter. A third drop of blood was smeared on a glass slide. This was later stained by Wright's method and 100 cells were differentiated on each slide in order to determine the percentage of each cell type. Statistical analysis was performed using Student's t-test.

Means and standard errors of neutrophils and lymphocytes per mm³ at 3-h-intervals over a 24-h-period

Hour	Neutrophils Control	Neutrophils Experimental*	Lymphocytes Control	Lymphocytes Experimental
03.00	3362± 206**	28,893±1514	19,461±2563	12,754±1311
06.00	2212± 344	26,052±1958	14,480±1920	14,438± 909
09.00	1811± 476	27,527±1800	20,421± 915	18,621±2230
12.00	L1658± 747	21,734±1412	16,529±1174	L12,588±1848
15.00	2410± 566	16,187±3220	19,288±1817	13,007±1325
18.00	2829± 427	L13,828±2882	L13,701± 739	H19,639±2523
21.00	2916± 604	27,581±3484	15,763±1577	14,749±2957
24.00	H5387±1336	H29,787±3667	H21,245±2175	14,868±1150
Ave.	2823± 353	24,184±1254	17,608± 696	15,036± 702
Probability				
H vs. L	<0.05	<0.02	<0.01	<0.05
H vs. Ave.	>0.05	>0.05	>0.05	>0.05
L vs. Ave.	>0.05	<0.01	<0.01	>0.05

*CVF-treated rats. **Standard error of the mean (5 animals per group) H = High values; L = low values.



Circadian rhythm of total leukocytes at 3-h-intervals for control (○—○) and CVF-treated (●—●) rats. Each point represents the mean value of 5 animals with vertical bars representing the standard error of the mean. Nonshaded and shaded areas represent the light-dark cycle in which the animals were maintained.

Results. For control animals, a peak absolute leukocyte count of 24,950 ± 4041 (per mm³) occurred at 24.00 h and a low of 16,704 ± 1139 was seen at 18.00 h (figure); these were significantly different from each other ($p < 0.05$). The low point was also significantly different ($p < 0.01$) from the overall 24-h-mean (20,624 ± 866).

In CVF-treated rats, a high total leukocyte count of 48,137 ± 2960 occurred at 09.00 h while a low of 29,990 ± 2772 occurred at 15.00 h (figure), which were significantly different from each other ($p < 0.01$). The high and low points were significantly different ($p < 0.05$ and $p < 0.01$, respectively) from the overall 24-h-mean (40,576 ± 1323).

When the leukocytes were differentiated (table), a peak neutrophil count of 5387 ± 1336 for control animals was seen at 24.00 h and low of 1658 ± 747 was found at 12.00 h; these were significantly different from each other ($p < 0.05$). No significant difference was observed between the high and low points and the overall 24-h-mean (2823 ± 353).

For CVF-treated rats, a peak neutrophil count of 29,787 ± 3667 occurred at 24,00 h while a low of 13,823 ± 2882 occurred at 18.00 h, which were significantly different from each other ($p < 0.02$). The low point was significantly different ($p < 0.01$) from the overall 24-h-mean (24,184 ± 1254).

The peak lymphocyte count of 21,245 ± 2175 in control animals was observed at 24.00 h and low of 13,701 ± 739 occurred at 18.00 h; these were significantly different from each other ($p < 0.01$). The low point was significantly different ($p < 0.01$) from the overall 24-h-mean (17,608 ± 696).

In rats treated with CVF, the peak lymphocytic count of 19,639 ± 2523 occurred at 18.00 h and a low of 12,588 ± 1848 was observed at 12.00 h, which were significantly different from each other ($p < 0.05$). The high and low points were found not to be significantly different from the overall 24-h-mean (15,036 ± 702).

The increase between low and high points for total leukocytes, represents a change of 61% for both control and CVF-treated rats. For neutrophils, the increase was 225% for control and 115% for CVF-treated animals. The increase between low and high points for lymphocytes was 55% for control and 56% for rats treated with CVF.

Discussion. The data demonstrate the existence of a daily cycle in the numbers of total leukocytes, neutrophils, and lymphocytes in the rat, a finding similar to that previously reported[5]. Closer evaluation of the absolute leukocyte rhythm in the present investigation demonstrates the variation to be the result primarily of the lymphocytic population. When CVF is administered, the leukocytic response which follows also exhibits circadian rhythmicity, although at considerably higher levels. This CVF-induced rhythm is achieved mainly by neutrophil variation.

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Examination of the differences between control and CVF-treated animals at each 3-h-period demonstrates that the total leukocytic and neutrophilic response to CVF is dependent upon the time of day the factor is given. Total leukocytic and neutrophilic response is greater during the early morning (06.00–09.00 h) than during the afternoon (12.00–18.00 h). The lymphocytes on the other hand, showed little difference in variation and increase following CVF administration.

McCall et al.[4] reported alterations in neutrophil kinetics in the rabbit after CVF activation of the complement cascade. Following intravenous injection of 0.6 to 1.0 ml of purified cobra venom factor, a profound neutropenia occurred within 60–120 sec followed by a marked neutrophilia. In this same study, CVF was injected into C_6-deficient rabbits which again caused an initial neutropenia followed by a neutrophilia. To further characterize the active factor, fresh plasma was treated with CVF and filtered through a 20,000 MW filter. The filtrate was then injected into the rabbit ear vein and the same neutrophilic response obtained. These results suggested that the neutrophilic changes depended on the elaboration of a factor of low molecular weight ($<20{,}000$) which may be derived from either C_3 or C_5[4].

Because the present investigation was concerned with only a 45-min-response, a neutropenia was not observed. However, a 10fold increase between the 24-h-mean control and experimental neutrophil levels was evident, confirming the neutrophilia following CVF activation of the complement cascade[4].

It is not known whether complement regulates hourly variations in neutrophil kinetics. However, this study has demonstrated that the extent of the neutrophilia following CVF complement activation in the rat is a time-dependent phenomena which varies according to the time of day the factor is given and has a circadian rhythm.

Electrically excitable neurosecretory cell bodies in the periphery of the stick insect, Carausius morosus

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Summary. Intracellular recordings have been made from the cell bodies of both neurosecretory and non-neurosecretory multipolar neurons in the periphery of Carausius morosus. The neurosecretory neurons have cell bodies which are electrically excitable and produce overshooting action potentials, whilst the cell bodies of the non-neurosecretory neurons are electrically inexcitable.

There is a sparsity of electrophysiological data about the basic membrane properties underlying neuroendocrine integration and regulation in insects. A suitable preparation for such a study is found in the stick insect, in which multipolar neurons showing the ultrastructural characteristics of being neurosecretory have been described[2,3]. These multipolar neurosecretory neurons, the link nerve neurons (LNNs), lie with their cell bodies on or near the link nerve and have processes passing superficially along a number of major peripheral nerves[2,3]. Each of the processes propagates action potentials towards their terminals where presumably release of neurosecretory material is triggered[3].

As a preliminary to investigating the control mechanisms of these cells, intracellular recordings were made to determine the properties of the membrane of the cell body. Previous intracellular recordings from nerve cell bodies of insects have been confined to monopolar neurons in the central nervous system. The majority of these cell bodies are electrically inexcitable[4], although a specific group of dorsally situated cell bodies are electrically excitable[5,6].

In this study we provide the first account of intracellular recordings from multipolar neurons in insects and present evidence for the presence of overshooting action potentials recorded from the cell bodies of LNNs.

Adult stick insects were dissected mid-dorsally, pinned out on 'Sylgard' (Dow Corning Corporation) and the gut removed. The preparation was flooded with a modified version of Wood's saline[7] (composition: KCl, 18 mM; $MgCl_2$, 50 mM; $CaCl_2$, 7.5 mM; NaH_2PO_4 6 mM; $NaHCO_3$, 9 mM; glucose 185 mM; made up to 1000 ml H_2O). A window was cut through the cuticle and tissue underlying the LNNs of an abdominal segment, and the preparation viewed under a compound microscope by interference contrast using transmitted light. In this way the insertion of microelectrodes was under visual control, providing no doubt as to the intracellular nature of the recordings and their origin in the soma. Glass micro electrodes of between 40 and 60 MΩ resistance filled with 3 M KCl were used for intracellular recording, and current injection was provided through the same electrode using a bridge circuit (Mentor N-950 Intracellular probe system).

Insertion of a microelectrode into a neurosecretory cell body revealed negative resting potentials of 30–62 mV (mean 46 mV, $n = 50$). The total membrane resistance of the cells was found by applying hyperpolarizing current pulses and varied from 40 to 80 MΩ (mean 58 MΩ, $n = 10$). Total time constant was found to be 30–50 ms (mean 43 ms, $n = 10$). Assuming the cell to be a sphere of 40 μm diameter and the time constant to be the time taken for the membrane to reach 63% of its final value[8] (10 ms), the specific membrane resistance can be calcu-

1 We are grateful to the Science Research Council for financial support and to Drs Benjamin, Swindale and Slade for allowing us a preview of their manuscript in press.

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