

During the first 2 weeks of sampling and recordings, the insulin-resistant dog showed a very uniform recurrence of the MMC at regular intervals ( $90 \pm 3$  min). Exogenous insulin was followed by a postprandial pattern of less than 3 h vs. 6 h in the control and feeding disrupted the MMC for only 0.5 to 3 h. The plasma IRI level before ( $30.3 \mu\text{U} \cdot \text{ml}^{-1}$ ) and after ( $\Sigma\text{IRI } 8,190$ ) feeding was higher than in the control dog ( $10.9 \pm 8.1 \mu\text{U} \cdot \text{ml}^{-1}$  and  $\Sigma\text{IRI } 1,042 \pm 456$  SEM). A decrease in the initial insulin resistance of the dog might be suspected since the last

week of recording its natural bulimy slightly decreased and the  $\Sigma$  IRI value fell to 870. Despite a reduced food intake (300 vs. 400 g), the duration of the postprandial pattern was increased (Figure 2) and in the last week of time the MMC also recurred more irregularly during the interdigestive periods, resembling that of the control dog.

**Discussion.** A direct action of insulin on intestinal smooth muscle in vitro was described as early as 1932<sup>8</sup>. A low level of insulin may have a permissive role in vivo in the recurrence of the basic MMC pattern, the postprandial hyperinsulinemia being responsible for the disruption of the MMC. An imbalance between hyperinsulinemia and insulin peripheral resistance seems to exist in both genetically obese rats and the bulimic dog. A relatively greater increase in plasma IRI level than in intestinal insulin resistance would explain the irregular MMC pattern in obese rats during day-time. On the other hand, a higher insulin resistance as shown by the diminished response to exogenous insulin may explain the brevity of the disruption of the MMC pattern after feeding in the bulimic dog as well as the regularity of this pattern during the interdigestive periods.

Insulin may not be the only hormone regulating gastrointestinal electrical activity. Other intestinal hormones, such as enteroglucagon, motilin and gastric inhibitory polypeptide may be involved: recent observations of hyperplasia of gut endocrine cells in genetically obese mice suggest that changes in these hormones may also be significant in the Zucker rat<sup>9</sup>. However, the present results give further support to a major role of insulin in the control of gut electrical spiking activity.

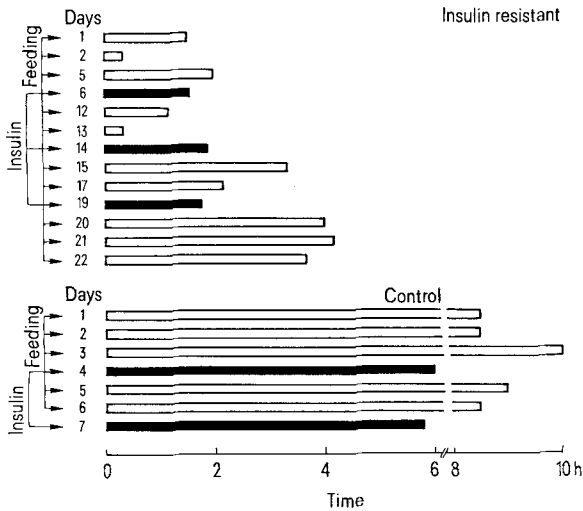


Fig. 2. Duration of the postprandial pattern of electrical activity after a daily meal (400 g) or i. v. injection of insulin (3 IU/kg) in an insulin-resistant dog recorded over 22 days compared with a control dog over 7 days.

<sup>8</sup> I. PAVEL and S. M. MILCOU, C. r. Soc. Biol., Paris 109, 776 (1932).  
<sup>9</sup> J. M. POLAK, A. G. E. PEARSE, L. GRIMELIUS, S. R. BLOOM and V. MARKS, J. Endocr. 67, 67 P (1975).

Ultradian and Other Rhythms in Human Respiration Rate

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**Summary.** Twelve male subjects had their respiration rate (RR) measured at 3 min intervals for 6 h. Although there were substantial individual differences, most subjects demonstrated a  $90 \pm 15$  min rhythm of RR, with several subjects also showing  $60 \pm 10$  min and  $30 \pm 3$  min rhythms.

The phenomenon of REM cycling throughout sleep has produced the proposal<sup>1</sup> that there is a basic rest activity cycle (BRAC) which is a fundamental 90 min rhythm in sleep and wakefulness. Others<sup>2,3</sup> are less specific about such a rhythm and consider that there may be desynchronized ultradian rhythms (URs) in many body functions, with a duration of about 80–120 min. Whilst there are many equivocal findings with the presence of URs in wakefulness, perhaps a more definite UR is with orality<sup>4,5</sup> and gastric motility<sup>6</sup>. The equivocality may be due to such factors as; 1. insensitive measures, 2. URs do not exist in many body functions, 3. URs are vulnerable to intervening variables, e.g. light and darkness<sup>3</sup>, and circadian rhythm modulation. Since the putative BRAC is associated with changes in levels of activity and wakefulness, measures of wakefulness may be particularly sensitive. There is some evidence<sup>7</sup> of a UR in waking

EEG activity. It has been proposed<sup>8</sup> that respiration is a very sensitive measure of levels of wakefulness, with the proximity of the bulbo-pontine respiratory pacemaker

<sup>1</sup> N. KLEITMAN, *Sleep and Wakefulness*, (University Press, Chicago 1963).  
<sup>2</sup> G. G. GLOBUS, E. C. PHOEBUS, J. HUMPHRIES, R. BOYD and R. SHARP, *Aerospace Med.* 44, 882 (1973).  
<sup>3</sup> D. F. KRIPKE, *Advances in Sleep Research* (Spectrum, New York 1974), p. 305.  
<sup>4</sup> T. WADA, *Arch. Psychol. Monogr.* 8, 1 (1922).  
<sup>5</sup> I. OSWALD, J. MERRINGTON and S. LEWIS, *Nature, Lond.* 225, 959 (1970).  
<sup>6</sup> J. F. HIATT and D. F. KRIPKE, *Psychosomatic Med.* 37, 320 (1975).  
<sup>7</sup> D. F. KRIPKE, *Psychosomatic Med.* 34, 221 (1972).  
<sup>8</sup> K. BÜLOW, *Acta physiol. scand.* 59, suppl. 209 (1963).

to the reticular formation being the key factor. The effect of changes in the level of relaxation are known<sup>9</sup> to influence respiration. In a study<sup>10</sup> unrelated to URs, 1–2 h oscillations of oxygen consumption in resting man have been reported. The present study investigated the possibility of a UR in respiration rate (RR).

**Method.** 12 male subjects, 18–25 years, non-smokers and medically fit took part. RR was measured by a nasal thermistor connected to a strain gauge coupler and paper output polygraph. Subjects were measured individually and were confined to a sound-proofed room controlled at 20°C, for 6 h between the time limits of 1200–2000 h. All time cues were removed and subjects were unaware of the phenomenon under investigation. Throughout each run subjects remained comfortably seated at a desk, and were allowed to read books of their choice and to write. To avoid interference with actual measurement and with arousal, subjects were not allowed to eat or drink during the run. They were instructed to ensure that they ate and drank sufficiently beforehand. Each paper record was divided into 3 min epochs, totalling 120 epochs, and the RR was assessed for each epoch. The digital data were fourier analyzed with a program of low resolution in the range 0.1–0.008 Hz (10–120 min period). A Hewlett Packard Fourier Analyser, model HP 5451A was used for this purpose. The low resolution was more able to cope with frequency modulations (FMs). The most dominant frequencies were assessed for significance by the following criteria. Epoch data for each subject were randomized 100 times and fourier analyzed each time. These 100 analyses were

compiled and used for comparison with the original data. Thus the energy value for any dominant frequency from the original analysis could be compared with the other 100 values for that particular frequency. If an original value was never exceeded by the 100 values then it was considered to be significant at a  $p \leq 0.001$  level, if it was exceeded between 1 and 5 times it was considered to be significant at a  $p \leq 0.05$  level. The frequency values were converted to periods in a later part of the analysis.

**Results.** From the Table of the five most dominant periods it can be seen that 7 subjects demonstrated a significant period within a UR range of  $90 \pm 15$  min, with 3 subjects showing highly significant ( $p \leq 0.001$ ) levels. With the averaging of the original fourier analyses for the group of subjects it can be seen from Figure 1 that there are at least 2 other period ranges of prominence. One apparently at  $30 \pm 5$  min and the other at  $60 \pm 10$  min. The Table shows that 7 subjects have significant periods within  $30 \pm 5$  min and 4 subjects with a significant  $60 \pm 10$  min period. There are clear individual differences in the distribution of these significant periods across the subjects, ranging from subject 3 with no significant periods at all, to subject 5 with all the 3 main periods plus one at 42 min.

**Discussion.** The criteria for whether a period is significant or not were objective but arbitrary. If the top 3 dominant periods are taken regardless of significance values, then 10 subjects have a period in the  $90 \pm 15$  min range, 5 in the  $30 \pm 5$  min range and 8 in the  $60 \pm 10$  min range. With 30, 60 and 90 being multiples of each other it is likely that least one is a harmonic. But any harmonic analysis is difficult to determine because 1. ranking of the dominance periods within subjects shows no consistent trend, 2. fourier analysis, like most frequency analysis techniques assumes that a waveform is linear, but with the present data FM was present, and even though a low resolution analysis was employed FM would have reduced the efficiency of the analysis. With only 6 h of data collection possible the presence of any periods longer than 120 min would become increasingly noisy owing to fewer complete periods for analysis. Interestingly, subjects 4 and 12 each show 2 significant periods within  $90 \pm 15$  min. From the raw data of subject 4 in Figure 2 it can be seen from the troughs marked ○ there is about a 78 min periodicity, and from the troughs marked ● there is about a 96 min period. These periods appear to be independent, suggesting that there may be more than one UR factor influencing RR. Although gastric activity was

Top five most dominant periodicities with significance levels, for all subjects

Subject No.	Length (min) of dominant periods				
	1	2	3	4	5
1	84 <sup>a</sup>	39 <sup>a</sup>	69	27	33
2	27 <sup>a</sup>	78 <sup>a</sup>	69	33	93
3	54	24	111	36	45
4	78 <sup>a</sup>	48 <sup>a</sup>	96 <sup>a</sup>	30	46
5	90 <sup>b</sup>	63 <sup>a</sup>	42 <sup>a</sup>	57 <sup>a</sup>	33 <sup>a</sup>
6	60 <sup>a</sup>	90	42	15	36
7	35 <sup>b</sup>	90 <sup>a</sup>	114	45	54
8	90 <sup>b</sup>	57 <sup>a</sup>	45 <sup>a</sup>	39	21
9	27 <sup>a</sup>	78	21	63	42
10	27 <sup>a</sup>	54	93	39	63
11	65 <sup>b</sup>	27 <sup>b</sup>	38 <sup>a</sup>	19 <sup>a</sup>	24 <sup>a</sup>
12	102 <sup>b</sup>	77 <sup>b</sup>	43 <sup>a</sup>	31 <sup>a</sup>	25 <sup>a</sup>

<sup>a</sup>Significant at 0.05 level. <sup>b</sup>Significant at 0.001 level.

<sup>9</sup> J. MEAD, *J. appl. Physiol.* 15, 325 (1960).  
<sup>10</sup> D. BAILEY, D. HARRY, R. E. JOHNSON and I. KUPPRATZ, *J. appl. Physiol.* 34, 467 (1973).

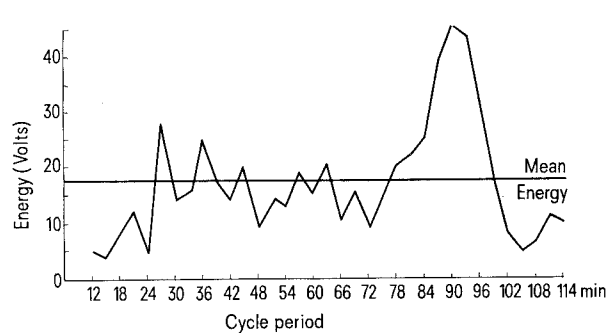


Fig. 1. Average power spectrum for all subjects ( $n = 12$ ).

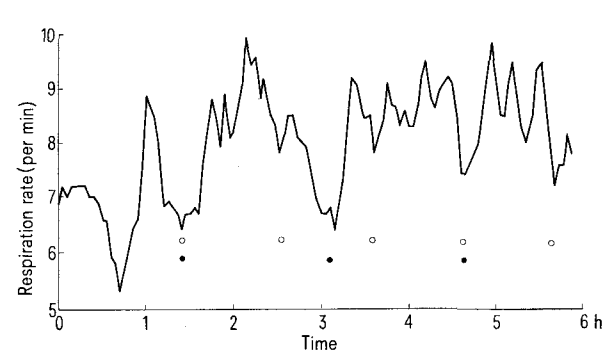


Fig. 2. Subject 4 respiration rate changes over a 6 h period.

not measured, it is possible that any gastric or oral UR<sup>4-6</sup> may have some influence on RR outside the central nervous system (CNS).

The rationale behind the present study was oriented towards CNS arousal factors in RR control and not necessarily towards changes in metabolic rate per se. But if there is a resting UR of daytime metabolic rate, which may be reflected in the changes in oxygen consumption reported earlier<sup>10</sup>, then these small changes in oxygen re-

quirements may be made at the expense of tidal volume and not necessarily in RR<sup>11</sup>. Thus a further study might assess changes in respiration flow rate<sup>12</sup>.

<sup>11</sup> F. J. CLARK and C. VON EULER, J. Physiol., Lond. 222, 267 (1972).

<sup>12</sup> Acknowledgment. We would like to thank Dr RAY MEDDIS of this department for his helpful advice and assistance in the data analysis.

## A Paradoxical Effect of 2,4-Dinitrophenol in Stimulating the Rooting of Hypocotyl Cuttings of *Phaseolus mungo*<sup>1</sup>

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**Summary.** 2,4-dinitrophenol enhanced root formation on dark-grown hypocotyl cuttings of *Phaseolus mungo*. This effect is probably related to uncoupling of oxidative phosphorylation and not through IAA-metabolism as is evident from studies with respiratory inhibitors (Cd<sup>2+</sup>) and non-phenolic uncouplers of oxidative phosphorylation (arsenate).

While examining the effect of some phenolic compounds on hypocotyl cuttings of *Phaseolus mungo*, increased root formation was observed with 2,4-dinitrophenol (DNP). Work was therefore undertaken to investigate this unusual phenomenon of increased rooting with an uncoupler of oxidative phosphorylation<sup>2,3</sup>.

Healthy, uniform seeds of *Phaseolus mungo* were germinated in sterilized Petri-dishes (15 cm diam.) lined with cotton pads, maintained at 28 ± 2°C in the dark. Uniform seedlings were made into cuttings with 3.5 cm hypocotyl and 6.5 cm epicotyl by excising the cotyledons, the upper epicotylar and lower hypocotylar portions. These were planted in holes on tin-foils stretched over specimen tubes (3 × 7.5 cm), each containing 20 ml of the requisite test solution. Cultures were maintained in the dark and the test solutions were changed after every 24 h. Observations on the number of cuttings rooted and the number of roots were recorded after 7 days. The experiment was repeated 3 times with similar trends of results.

The results, together with the treatments presented in the Table, show that all cuttings rooted in water, as well as in 1 µg/ml IAA. 1% sucrose alone or with IAA, enhanced rooting, showing thereby that the optimal production of adventitious roots was limited by the level of endogenous nutrition and that a proper balance between auxin and nutrition was necessary for the process<sup>4-7</sup>.

DNP alone, with IAA (1 µg/ml), sucrose (1%) or IAA + sucrose enhanced rooting (Table). Auxin activity of the substituted phenols is lost when the position *para* to hydroxyl group is substituted by strong electron attracting groups<sup>8,9</sup>. However, the present investigation reveals that in the case of DNP, a *para* substitution results in an active molecule. It was decided to investigate why a *para* substituted phenol which acts as an uncoupler of oxidative phosphorylation also should enhance rooting.

Phenolic compounds are known to enhance certain auxin-caused responses<sup>10-12</sup>. DNP might, therefore, exert its influence via interaction with some hormonal mecha-

<sup>1</sup> The research has been financed by a grant from United States Department of Agriculture.

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<sup>4</sup> K. GURUMURTI and K. K. NANDA, Phytochemistry 13, 1089 (1974).

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<sup>6</sup> K. K. NANDA and M. K. JAIN, New Phytol. 70, 949 (1971).

<sup>7</sup> K. K. NANDA and M. K. JAIN, New Phytol. 71, 825 (1972).

<sup>8</sup> D. B. HARPER and R. L. WAIN, Ann. appl. Biol. 64, 395 (1969).

<sup>9</sup> R. L. WAIN and D. B. HARPER, Nature, Lond. 23, 1155 (1967).

<sup>10</sup> J. P. NITSCH and C. NITSCH, Ann. Physiol. Veg. 4, 211 (1962).

<sup>11</sup> R. L. WAIN and H. F. TAYLOR, Nature, Lond. 207, 167 (1965).

<sup>12</sup> C. E. HESS, Plant Physiol. 40, Suppl. 45 (1965).

Effect of DNP, DNP + Cd<sup>2+</sup> and arsenate alone and in combination with IAA, sucrose and IAA + sucrose, on the number of dark-grown *Phaseolus mungo* hypocotyl cuttings with excized apex and cotyledons that rooted out of 10 and the number of roots produced per rooted cutting (Figures within parentheses)

Treatment	Combination with			
	Water	IAA (1 µg/ml)	Sucrose (1%)	IAA (1 µg/ml) + sucrose (1%)
Water	10 (4.0 ± 0.3)	10 (4.3 ± 0.1)	10 (6.0 ± 0.4)	9 (7.0 ± 0.3)
DNP (5.0 µg/ml)	9 (9.3 ± 0.9)	10 (11.7 ± 0.9)	10 (10.3 ± 0.9)	10 (19 ± 1.0)
DNP (5.0 µg/ml) + Cd <sup>2+</sup> (2 × 10 <sup>-6</sup> M)	10 (4.8 ± 0.4)	5 (4.1 ± 0.4)	×	9 (3.1 ± 0.2)
Arsenate (10 <sup>-6</sup> M)	9 (4.4 ± 0.2)	10 (6.0 ± 0.2)	10 (8.2 ± 0.7)	10 (11.5 ± 1.0)

±, Standard error. ×, Cuttings decayed.