the entrance of the inferior laryngeal or recurrent nerve (R); these have been depicted in our Figure and labelled aA. A few mm below the branching of the recurrent nerve, three or more thin twigs are noted to diverge from the main vagal trunk. These can be traced in proximity to the atria, ventricles and pulmonary artery, although their actual ending cannot be identified owing to their abundant anastomosing in the cardiac plexus. Therefore, they have loosely been labelled as 'cardiac' vagal fibers (C). Shortly below the 'cardiac' vagal branches, several twigs depart from the main trunk directed toward the pulmonary hilus. As for the so-called 'cardiac' fibers, their actual ending cannot be determined exactly, although some of them appear to originate from the bronchial tree[4], their section being associated with the known respiratory changes due to the interruption of the Hering-Breuer reflex. We have loosely labelled these fibers as 'pulmonary' (P). Caudad of the 'pulmonary plexus' and near to the diaphragm, the main vagal trunk itself bifurcates into two divisions which enter the abdomen after uniting with similar divisions of the right vagus nerve.

The effect of intrathoracic vagus sectioning at three different levels was studied (I) on the amplitude of the pressor response to carotid occlusion, in animals with the carotid sinuses intact, or (II) on the basal level of arterial pressure, in animals with previous removal of the carotid sinus receptive regions. Both tests gave concordant results. Left vagus interruption just above its terminal supradiaphragmatic division (section 3 in our Figure) was always without effect, while a slightly more rostral section (2 in our Figure), above the 'pulmonary' twigs, constantly induced either an increased pressor response to carotid occlusion or an enduring augmentation of basal arterial pressure. Further pressor effects were, however, induced by subsequent section of the cervical vagus. Intrathoracic vagal severing just above the cardiac twigs, well below the entrance of the inferior laryngeal nerve and the aortic arch (section 1), elicited pressor reactions which were often larger than those produced by section 2 ('pulmonary' fibers), but then no further pressor change could be induced by subsequently cutting the left cervical vagus. Any substantial contribution of accessory aortic fibers to the circulatory effects of intrathoracic or cervical vagus sectioning was finally ruled out by showing that vagal severing above the pulmonary hilus had conspicuous effects, unmodified by later cutting of the cervical trunk, also after the aortic arch had been surgically denervated by stripping and all vagus branches from its entrance into the thorax to a level just above the pulmonary hilus had been resected.

To sum up, those afferent fibers in the left cervical vagus that have been shown to exert a tonic inhibitory influence upon the circulation[2], appear to originate from intrathoracic receptive areas, which are likely to include atria, ventricles, pulmonary vessels, and, possibly, the bronchial tree. More precise identification of the receptive fields is prevented by the distribution of these vagal endings in diffuse plexuses. Accessory aortic fibers, claimed by NONIDEZ[3] to join the left vagus at the level of the recurrent nerve, would be responsible for quite an unsubstantial part, if any, of the influence.

Riassunto. Le fibre afferenti presenti nel vago cervicale, e dotate di tonica attività inibitrice sui fenomeni circolatori, originano da aree recettrici intratoraciche, non provengono in misura importante dall'arco aortico, ma si dipartono da ampie zone della regione cardio-polmonare.

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Istituto di Patologia Medica, Università di Siena (Italy), October 31, 1961.

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Day-Night Periodicity in Pentobarbital Response of Mice and the Influence of Socio-Psychological Conditions[1]

The pharmacological effectiveness of barbiturates in man and animals is known to be subject to many varied influences. Psychological factors in barbiturate responsiveness of man and of Rhesus monkeys have been cited by SHAGASS[2] and by CHEN[3] respectively. Physiological factors affecting degree of barbiturate response have been studied to a greater degree. Important influences observed in rodents include state of water balance[4,5], environmental and body temperature[5–8], adrenocortical hormone secretion[9], blood level of insulin or epinephrine[10], and circulatory state[11] in addition to basic attributes of age, sex, and strain. With such information in mind we have anticipated that one or more physiological factors displaying circadian periodicity might affect barbiturate response significantly. Various workers no doubt have considered time of day as a variable to be controlled in tests of barbiturate sleeping time prolongation commonly employed in characterizing new central nervous system drugs. Observations reported here demonstrate the importance not only of circadian physiological periodicity, but also of socio-psychological conditions in the response of mice to a standard anesthetic dose of pentobarbital sodium.

The Figure illustrates the variation in duration of response to pentobarbital with time of day detected in several strains of mice. Curves obtained from mice of inbred albino or non-albino, and non-inbred albino strains are similar. To detect such circadian patterns considerable care must be used to maintain constancy of conditions during and prior to the experimental observations. Male mice of several strains were housed in isolation or in groups with food and water available *ad libitum*. Room

[1] This work was initiated with the aid of a grant from the University of Oklahoma Faculty Research Committee and was later supported by grant B-2250, National Institutes of Health, U.S. Public Health Service.

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temperature was maintained constant. Automatically-controlled artificial illumination provided alternating 12 h periods of light (08:00 to 20:00) and darkness (20:00 to 08:00) which corresponded approximately to the natural daily light-dark sequence. Duration of anesthesia after the customary 60 mg/kg intraperitoneal dose of pentobarbital sodium was greater during the light period than during the dark period. The exact peak and trough of the 24 h response curves appear to be variable about the mid-points of the light and dark periods, respectively, between different groups of mice, or even between repeated determinations in a single group.

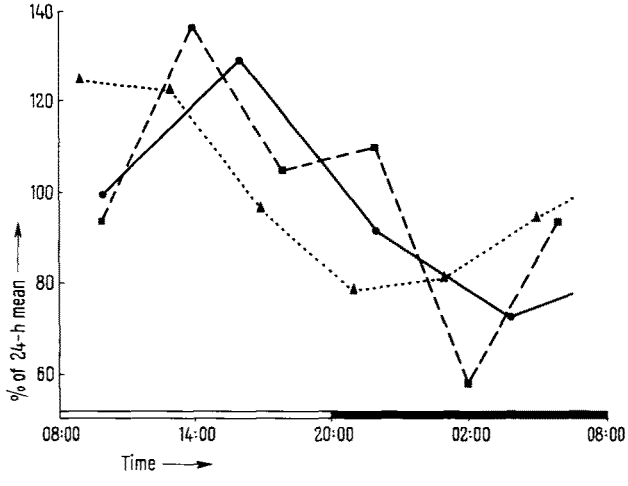
A primary role of the light-darkness cycle as environmental synchronizer of the factor(s) responsible for this circadian variability in pharmacological response may be anticipated from the findings of many investigations of 24 h biological rhythms. It was predicted that changing mice from a light-dark sequence to conditions of continuous light would disrupt or suppress the circadian pattern. This expectation is confirmed by the data of Table I. Seventy-five male C-57Bl mice were divided among eight sub-groups according to time of day, light schedule, and housing status. The mice exposed to constant light for four to six weeks prior to the experiments did not show a significant difference in duration of response to pentobarbital between 14:00 and 02:00. In contrast, the mice under day-night conditions showed a highly significant difference ($p < 0.001$). Experiments I and II are replications at an interval of two weeks using the same mice with crossing-over of individuals between the two observation times. The results of the two replications are quite obviously parallel.

Tab. I
Anesthesia duration—time of day, light and housing conditions

Conditions		Experiment I 14:00	Experiment I 02:00	Experiment II 14:00	Experiment II 02:00
Light–darkness	grouped	109.6	62.0	80.9	44.1
	isolated	80.0	61.4	67.2	44.0
Constant light	grouped	85.6	93.5	67.8	59.7
	isolated	75.1	78.1	58.8	59.0

Tab. II. Anesthesia duration—time of day, temperature conditions

Room temperature	10:00	16:00	22:00	04:00
26°	90.6	122.5	82.9	66.1
36° C	58.0	57.0	60.0	41.6



The data of Table II suggest at least a partial basis for the circadian variation in anesthetic duration. Male Swiss albino mice were divided among four observation times and two levels of ambient temperature during experimental observations ($N = 17$ for each group). The higher ambient temperature prevented hypothermia which occurs during barbiturate anesthesia at the lower, customary room temperature. As expected, the higher temperature thus produced an overall shortening of duration. Those mice observed at 26° C manifested the usual pattern of response variability between the light and dark periods, while those tested at 36° C showed a considerable levelling of the pattern due mainly to a disproportionate reduction in duration at the usual peak time. These data suggest that a body temperature factor must in part be responsible for the differences observed with time of day. A lesser resistance to hypothermia during the light period than during the dark period may occur and may cause the longer pentobarbital duration in the day at usual room temperature. This would be in accordance with the known occurrence of a circadian body temperature rhythm and with knowledge of the role of hypothermia in duration of barbiturate response in rodents[5–8].

An influence of socio-psychological conditions on barbiturate response is evident in data of Table I if the values for grouped and isolated mice on light-dark conditions are compared. Although mice under both conditions of housing showed almost identical values at the trough (dark) time, the grouped subjects consistently gave significantly greater durations ($p < 0.01$) at the peak (light) time than did the isolated mice, i.e., the amplitude of their pentobarbital response rhythm was greater. Experimental evidence to provide an explanation of this finding has not yet been obtained. However, we suggest it may be related to a difference in amplitude of the secretory rhythm of the adrenal cortex between isolated and grouped mice in view of the observation that isolated mice have significantly lighter adrenals than grouped mice[12], and that adrenalectomized mice show a decreased amplitude of the body temperature rhythm[13]. These observations seem to exemplify the interaction of a 'socio-ecological' environmental factor (aggregation) with a 'physico-chemical' environmental factor (light) as suggested by HALBERG et al. in their analysis of the participation of the nervous system in the control of 24 h periodic functions of the body[13]. Additionally, the data reported here demonstrate a circadian periodicity of responsiveness to a 'therapeutic' level of drug action, rather than to the toxicologic level of action which has been noted previously in several interesting reports[14–17].

Résumé. L'étude de la réaction de la souris à des doses anesthésiques de pentobarbital montre une périodicité

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nycthémérale. La réaction est forte pendant le jour, faible pendant la nuit. Chez les souris soumises à des conditions qui empêchent l'hypothermie ordinaire de se produire pendant l'anesthésie, l'amplitude de périodicité diminue. La périodicité disparaît le plus souvent lorsqu'on éclaire les souris sans interruption. L'amplitude de la périodicité diminue lorsque les souris sont parquées individuellement plutôt qu'en groupes. Les mécanismes physiologiques répondant à ces observations sont suggérés.

W. M. Davis

University of Oklahoma College of Pharmacy, Norman (Oklahoma USA), February 22, 1962.

PRO LABORATORIO

A Pump for Use in Measuring Arteriovenous Differences in Concentration[1]

Accurate discrimination of arteriovenous differences – for instance of pH, oxygen saturation or concentration of labelled substances – calls for knowledge of the time relation and the blood volume between the arterial and venous blood samples. Important factors are the blood volume of, and rate of flow in, the organ from which the samples are drawn, and the sampling technique. The blood volume and flow must be determined or estimated for the individual organ and on each occasion of measurement, whereas the sampling method can be standardized.

The simplest way of standardizing the sampling procedure is to withdraw the blood at the same rate simultaneously from the respective sites of measurement. This can be effected by means of a pump that can draw blood through two channels at the same speed irrespective of pressure fluctuations in, or pressure differences between, the vessels from which the blood is withdrawn. To avoid variations in blood volume during continuous measurement, the blood should be returned at the same rate as it is withdrawn. Of the numerous pumps hitherto available for extracorporeal circulation and for perfusion of organs[2], none has been found that meets these requirements.

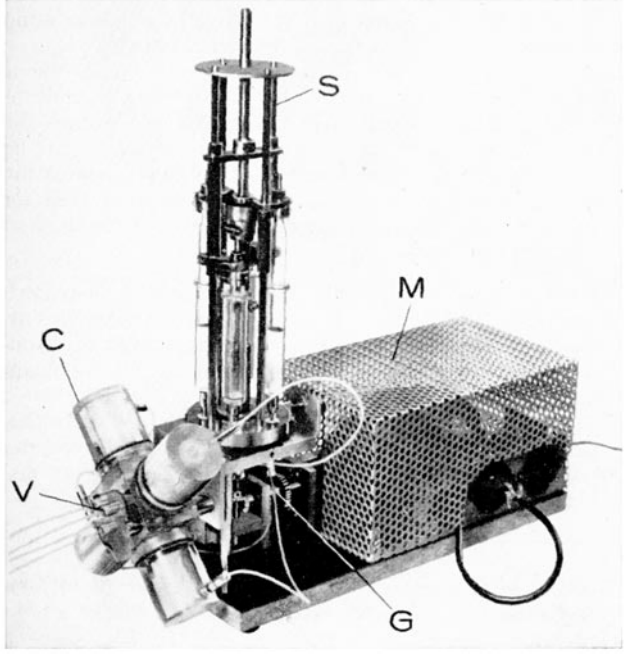

Fig. 1. General view of the pump. *M* motor, *S* stand for the syringes, *G* gear transmission system, *C* Perspex cylinders, *V* valve.

The pump described in this paper was designed to overcome the prevailing deficiencies.

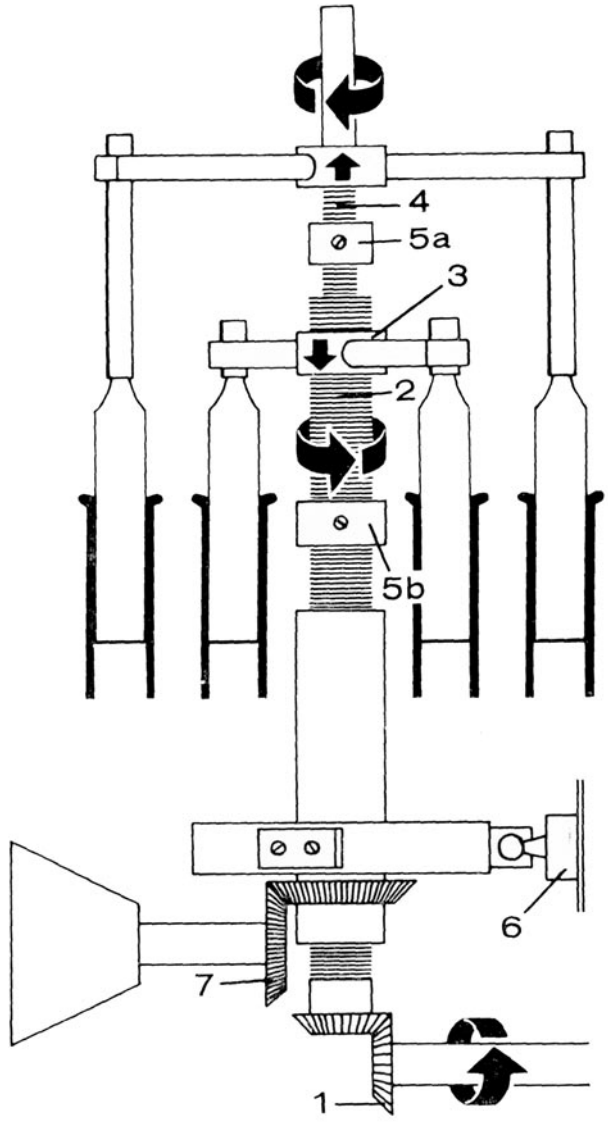

Fig. 2. Schematic view of the pump's mechanical construction. 1 gear, 2 rotating tube, 3 threaded block, 4 rod that screws into tube 2, 5 adjustable stop nuts, 6 switch for reversing the motordirection, 7 mitre gear.

[1] A grant from 'Reservationsanslaget' of the Karolinska Institutet is gratefully acknowledged.

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