three times at 3-day intervals, and antibody titrations in the subjects' serum were determined 2 days after the last RNA injection.

Controls. In order to detect the possible presence of antigen or of antibodies in preparations, the RNA was separated from admixtures. For this purpose the RNA was treated with warmed ribonuclease (10 μg/100 μg RNA) and then dialysed against a 0.15 M solution of NaCl for 5–6 h at 37 °C and then for 10–12 h at 4 °C for the complete removal of the degraded RNA. The residue remaining after dialysis was injected intravenously (dosage: 2 mg/kg body weight) into 5 new-born rabbits. The injections were repeated 3 times at 3-day intervals. Antibody titrations were determined 2 days after the last injection. Finally, antibody titrations were determined on the serum of 20 new-born rabbits that had received no RNA treatment.

Results. After injections of RNA obtained from the spleens of animals that had been immunized with *S. typhi* 'H' antigen, antibody titrations in the serum of new-born recipient subjects were found to be 1/30,000, 1/35,000, 1/25,000, 1/30,000 and 1/40,000. No antibodies were found, however, in the serum of the new-born control subjects or of the new-born animals injected with the residue obtained after destruction and removal of RNA. Therefore only the RNA molecule in its native state is the agent of the immunization transfer, while the small percentages of proteins, polysaccharides and DNA injected together with RNA had no influence. The last result is in accordance with the data obtained by FUKS et al.[14].

For the time being, the difficulty of obtaining an adequate supply of RNA from the spleens of immunized animals does not allow an all-round picture to be obtained of how the phenomenon occurs – when antibody production begins, how long it lasts, which are the minimum active dosages, etc. Even though the experiment has so far been performed with inadequate supplies of experimental material the results nevertheless seem clearly to suggest that the possibility does exist of obtaining an antibody synthesis in vivo by means of RNA.

Riassunto. L'iniezione ad animali normali di ARN estratto dalla milza di animali immunizzati con antigene 'H' di *S. typhi*, provoca la comparsa, nel siero degli animali riceventi, di anticorpi diretti contro tale antigene.

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Clinica Medica dell'Università di Pavia (Italy), September 30, 1965.

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The Tonically Acting Pulmonary Receptors Innervated by C Fibres

The investigation of the activity of single afferent vagal fibres has revealed pulmonary stretch receptors (ADRIAN[1]) which are innervated by A fibres. However, the majority of vagal afferent fibres, about 80%, including afferent fibres from the lungs, belong to C fibres (AGOSTONI et al.[2]). The function of these fibres up to now remained obscure.

In 1955 and 1957 PAINTAL[3,4] established the existence of vagal pulmonary afferent fibres excited by deflation of the lung and supposed that these fibres innervated pulmonary deflation receptors. By their conduction velocity these fibres belonged to B fibres. But in 1964 the author reported that most of them belonged to C fibres.

In 1965 COLERIDGE et al.[5] distinguished vagal pulmonary afferent fibres which also belonged to C fibres. It is very important to emphasize that according to the authors' findings these fibres show spontaneous activity which is not synchronized with the cycles of breathing. COLERIDGE et al.[5], contrary to the data obtained by PAINTAL[3,4], found that the deflation of the lungs did not excite these fibres but that they were excited by hyperinflation.

The observations of the influence exerted by some drugs evoking respiratory chemoreflexes on these fibres are also contradictory. According to PAINTAL[6], phenyl diguanide excited these fibres while veratrine did not. Quite the contrary is observed by COLERIDGE et al.[5]. In their experiments veratrine excited C fibres while phenyl diguanide did not.

The preparation of single C nerve fibres is very difficult because they are very thin and are often injured. Therefore we used the method of colliding impulses (DOUGLAS and RITCHIE[7]). This method has also another advantage: it permits us to establish the function of the greatest part of fibres of a definite group.

Method. The experiments were made on 25 cats weighing 2.0–4.0 kg anaesthetized with urethane and chloralose. The chest was widely opened and artificial respiration was carried out. The right cervical vagus was cut just below the nodose ganglion. A pair of stimulating platinum electrodes was placed close to the peripheral cut end of the nerve, and another pair of recording electrodes was placed about 50–90 mm lower. For stimulation of the nerve rectangular pulses of current of 0.1–0.5 msec duration were used, the stimuli used being supramaximal for A or C components. The vagus was stimulated during the inspiration or expiration. To exclude the impulses from other organs, the left and right vagi were cut over the diaphragm and the majority of cardiac branches of the right vagus were cut too. Thus only the excitation of pulmonary receptors may act on the compound action potential of vagus.

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Results. As seen from the picture (Figure 1, top record), during the inspiration the wave A of the compound action potential decreases. It means that impulses evoked by excitation of the stretch receptors during each inspiration and spreading orthodromically along the A fibres collide with impulses evoked by the artificial electrical stimulation of the vagus and spread antidromically along the same A fibres. On the contrary, the size of the wave C remains the same during inflation and deflation (Figure 2, top record). The wave C does not depend on the change of the volume of the lungs. It means that most of the spontaneous activity of C fibres indeed has not any connection with the cycles of respiration and acts tonically. This fact is of great interest in view of the following findings.

It is well known that cutting of the vagi leads to rare and deep breathing. HERING[8] and BREUER[9], nearly 100 years ago, showed that cutting of the vagi led to such an effect not only during natural breathing but also when the movements of the lung were artificially excluded. This observation was firmly forgotten. Meanwhile, on the basis of this observation, HERING[8] and BREUER[9] made a very significant conclusion; namely, that besides pulmonary nerve endings which were excited during inspiration there must be nerve endings constantly exciting the respiratory centre. As established later, the former – stretch receptors – are innervated by A afferent vagal fibres. Our experiments permit us to assume that the latter – receptors exerting a tonic influence on the respiratory centre – are innervated by C fibres.

Further, our experiments showed that the receptors innervated by C fibres were excited both by veratrine and phenyl diguanide. As shown in Figure 1 (middle record), injection into the right atrium of 1 ml saline containing 25–150 μg of veratrine decreases the amplitude of A wave. This means that according to the generally adopted view veratrine excites the pulmonary stretch receptors. Simultaneously the amplitude of C wave decreases too (Figure 2, middle record). It means that veratrine excites not only the pulmonary stretch receptors but also the pulmonary receptors innervated by C fibres.

Injection of 1 ml saline containing 100–450 μg phenyl diguanide into the right atrium also decreases the wave C.

The increase of activity of receptors acting tonically on the respiratory centre can obviously increase the excitability of the latter and thus facilitate the reactions of the respiratory centre to other stimuli. These data are very important for the analysis of the mechanism of chemoreflexes evoked by these drugs which was not quite clear up to now.

As shown in our previous article (SERGEEVA and FRANKSTEIN[10]) the reflectory change of respiration during local pneumonia and lung oedema probably also depends on the increase of activity of receptors innervated by C fibres.

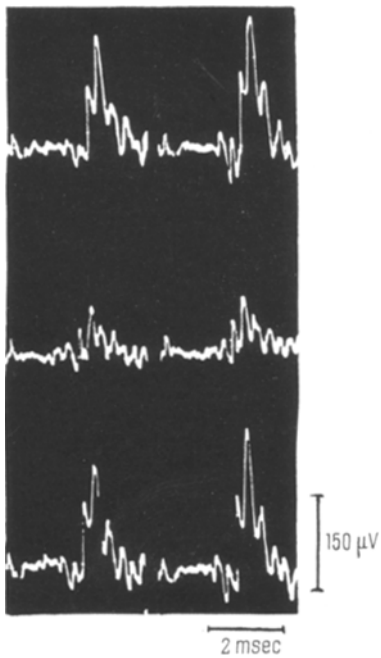

Fig. 1. Records of the A elevation of the action potential in a cat's chest vagus. Top records: before injection of veratrine the amplitude of A elevation during inflation (left) is smaller than that of A elevation during deflation (right). Middle records: after injection of 50 μg (15 μg/kg) veratrine into the right atrium the amplitude of A elevation decreases during inflation (left) and deflation (right). Bottom records: 2 min after injection the amplitude of A elevation is normalized. The distance between the stimulating and recording electrodes was 82 mm. Each record was made by superposing 5 sweeps.

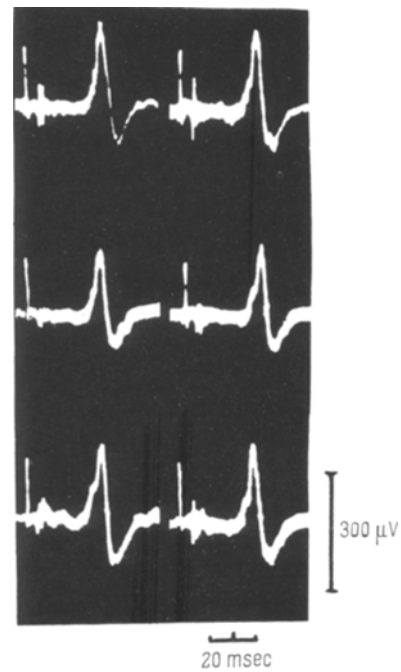

Fig. 2. Records of the C elevation of the action potential in a cat's chest vagus. Top records: before injection of veratrine the amplitude of C elevation during inflation (left) is equal to that of C elevation during deflation (right). Middle records: after injection of 50 μg (15 μg/kg) veratrine into the right atrium the amplitude of C elevation decreases during inflation (left) and deflation (right). Bottom records: 2 min after injection the amplitude of C elevation is normalized. The distance between the stimulating and recording electrodes was 70 mm. Each record was made by superposing 3 sweeps.

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Выводы. Помимо легочных рецепторов растяжения, иннервируемых волокнами группы А, существуют легочные рецепторы, иннервируемые волокнами группы С, оказывающие постоянное тоническое влияние на дыхательный центр. Некоторые химические вещества (вератрин, фенилдигуанид) увеличивают активность этих рецепторов. Такое же действие на них оказывают воспаление и отек легких. Это следует учесть в анализе дыхательных хемо – и патологических рефлексов.

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Effect of Light and Dark Pulses on the Emergence Rhythm of *Drosophila pseudoobscura*

On the basis of studies on the petal movement of *Kalanchoe* flowers[1], it was suggested that light periods induce an optimal rhythm if the 'off-rhythm' (rhythm which is initiated by the transition from light to darkness) falls together with the 'on-rhythm' (rhythm which is initiated by the transition from darkness to light) in such a way that an off-maximum occurs at the same time as an on-maximum. This is schematically diagrammed in Figure 1. The superposition of an on- and off-rhythm would explain the fact that at certain day-lengths and/or night-lengths the petal movement reaches high amplitudes. It could further be a basis of time measurement in photoperiodism.

We tested this hypothesis in the case of the emergence rhythm of *Drosophila pseudoobscura*. *D. pseudoobscura* has the advantage of being well studied in regard to its emergence rhythm[2], and in contrast to *Kalanchoe* can be kept under continuous darkness (DD) during the whole development. At the time of emergence the cultures are exposed to single steps (LL-DD, DD-LL), which already results in a rhythmic pattern of emergence, or to single pulses (light period pulse LP, dark period pulse DP). Since a pulse contains both a light-on as well as a light-off signal, the question arises whether the observed results of pulse experiments are explainable in terms of the results of single step experiments.

A stock of *D. pseudoobscura* was kindly supplied by C. S. PITTENDRIGH and reared in the usual way. At 20 °C under LL or DD conditions the flies start to emerge after about 3 weeks and continue to emerge in a random fashion. If, however, the DD-cultures are transferred to LL (300 lux fluorescence tube light), a periodic emergence is induced and peaks occur at 1, 20, 50, 75, and 100 h after transfer until the rhythm fades away and again emergence becomes random (Figure 2). A periodic emergence is also achieved if LL-cultures are transferred to DD. In this case peaks occur 12, 39, 64, 89, and 111 h after transfer. Synchronization is sharper and longer maintained (Figure 2 below).

If both steps are combined in a single DP- or LP-pulse, the emergence distribution depends on the length of the pulse and the kind of pulse. Examples are given for 12 and 18 h DP and LP (Figure 3). Further results are shown in Figure 4, in which the DP was varied from 1 h up to 33 h and the LP from 3 h up to 39 h. Only the emergence distribution between 50 and 75 h after the start of the DP and between 40 and 65 h after the start

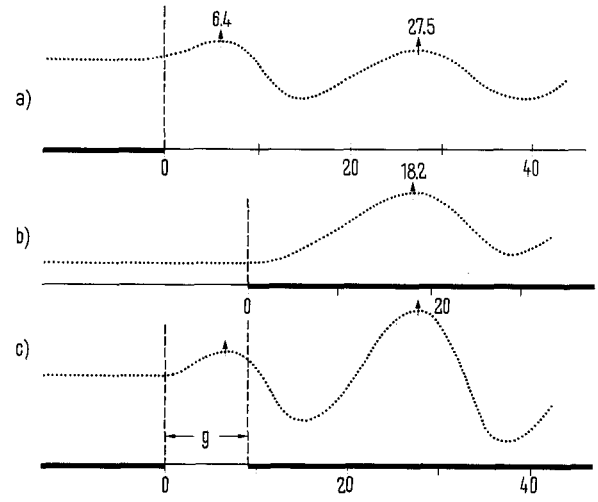

Fig. 1. Superposition of an on- and off-rhythm in the petal movement of *Kalanchoe blossfeldiana*. (a) Initiation of an on-rhythm by a single dark-light step; (b) initiation of an off-rhythm by a single light-dark step; (c) superposition of an on- and off-rhythm by a dark-light step followed by a light-dark step 9 h later. In this case the first maximum of the off-rhythm falls together with the second maximum of the on-rhythm (27.5-18.2 ≃ 9 h).

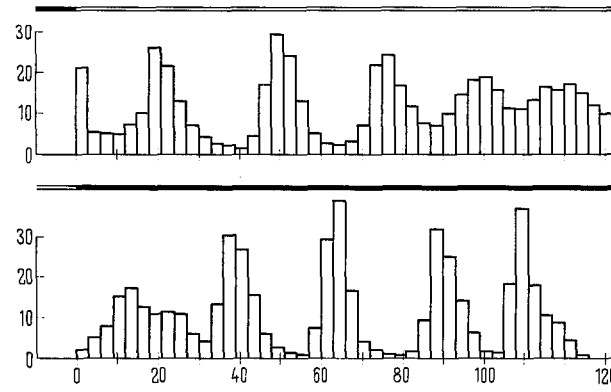

Fig. 2. Emergence rhythm in *Drosophila pseudoobscura* after transfer from continuous darkness to continuous light (above) and after transfer from continuous light to continuous darkness (below). Transfer at zero h. Abscissa: h after transfer. Ordinate: % of emerged flies (100% = sum of all flies between 2 minima).

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