

medium consisted of Eagle's basal medium (Institut Pasteur, Paris) supplemented with 20% fetal calf serum (GIBCO). In addition, experimental medium contained 10% of one of the following extracts: a) brain-mesodermal extract prepared from brain including the surrounding covering membranes as used in previous studies<sup>1,12</sup>; b) brain extract prepared from the brain tissue after removal of the surrounding membranes; c) mesodermal extract prepared from the covering membranes. All these extracts were prepared from 8-day-old chick embryos at a concentration of 20% in Tyrode solution and centrifuged 1 h at 105,000 *g*. The experimental medium was added to the cells after 48 h incubation and renewed every 2 days.

**Results.** The development of 5-day-old chick embryo brain cells under minimal nutritional conditions and the stimulatory effect of the total brain extract (brain-mesodermal extract) on the differentiation of these cells have previously been described in detail<sup>1</sup>. After 2 weeks cultivation, bipolar and multipolar neurons had developed and were dispersed upon a monolayer of flat polygonal astroblasts (Figure 1). Under the effect of total brain extract, the size of the cell body was increased; the nerve fibres were thicker, longer, possessed many ramifications and formed bundles as compared to the control cultures (Figure 2). The brain extract, prepared without the covering membranes, was observed to have no significant effect on the young neuroblasts from these 5-day-old chick embryos. However, under the effect of the mesodermal extract large multipolar neurons developed rich in Nissl bodies and large fibre bundles were observed after 2 weeks in culture (Figure 3).

The relative amount of large multipolar neurons were obtained by analysis of the cultures and by a visual quantification. Detailed measurements of length and number of nerve fibres was not made due to the often tortuously ramified fibres and the difficulty in estimating accurately the number of fibres in nerve bundles. In control cultures, an average amount of 3 to 5 large multipolar neurons for a total of 20 neurons were seen, while in cultures treated with mesodermal extract an average of 10 to 12 large neurons had developed.

It has previously been demonstrated that total brain extract stimulates the differentiation of nerve cells from 7-day-old chick embryo with the same intensity as it influences the cells from the 5-day-embryo<sup>1</sup>. The dif-

ferentiation of these nerve cells is also stimulated by brain extract prepared without the covering membranes. Compared to cultures without the added extract, more large multipolar neurons develop as well as neurons with ramified fibres after 2 weeks cultivation (Figure 4). Thick bundles of nerve fibres appeared in several areas of the culture (Figure 5). In contrast, the mesodermal extract has a very low influence on these brain cells.

**Discussion.** These results showed that the young neuroblasts from 5-day-old chick embryo, which are still morphologically undifferentiated respond differently to the various extracts studied. While brain-mesodermal extract and mesodermal extract influenced the maturation of these neuroblasts, the brain extract had no effect. Therefore, at this stage of the embryonic development, the maturation and the differentiation of the neuroblasts seem to be influenced only by the mesodermal covering membranes. Later on, when the neuroblasts became pyriform and have already started the differentiation process (7-day-old chick embryo), they are mainly stimulated by factors produced by the brain-cells and respond only slightly to mesodermal influence.

It can be concluded that the surrounding mesenchyme of the brain stimulates the differentiation of the morphological undifferentiated neuroblasts. Later on the maturation of the neuroblasts is influenced by factors produced by the brain cells themselves.

**Summary.** Extracts prepared from the mesodermal tissue surrounding the brain stimulate the differentiation of morphologically undifferentiated neuroblasts, while the differentiation of more mature neuroblasts is influenced by brain extracts.

Y. CAM, M. SENSENBRENNER<sup>13</sup>  
and P. MANDEL<sup>14</sup>

*Centre de Neurochimie du C.N.R.S., 11, rue Humann,  
F-67085 Strasbourg Cedex (France), 23 May 1975.*

<sup>12</sup> P. ATHIAS, M. SENSENBRENNER and P. MANDEL, *Differentiation* 2, 99 (1974).

<sup>13</sup> Maître de Recherche au C.N.R.S., France.

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## Muscular Respiratory Receptors in Self-Regulation of Normal Breathing in Man

Vagal blockade in a normal man evokes no change in the pattern of respiration. Thus the lung receptors do not take part in self-regulation of normal breathing in man<sup>1</sup>. As a consequence the respiratory muscle receptors were brought into focus of interest<sup>2,3</sup>. But to establish the role of the afferents from the respiratory muscles in self-regulation of breathing one must investigate the effect of posterior rhizotomy of the respiratory muscles. Unfortunately the results of such investigations in animals, as well as in man, are contradictory<sup>4-6</sup>. This probably depends on the difficulties of the operation which may involve a damage of the ventral roots of the

spinal nerves, as is supported by the following example. Although the diaphragm has a very scanty supply of spindle muscles<sup>2,3</sup>, even paralysis of the diaphragm

<sup>1</sup> A. GUZ, M. I. M. NOBLE, J. H. EISELE and D. TRENCHARD, in *Breathing*. Hering-Breuer Centenary Symposium (J. and A. Churchill, London 1970), p. 17.

<sup>2</sup> V. CRITCHLOW and C. EULER, *J. Physiol. Lond.* 168, 820 (1963).

<sup>3</sup> T. A. SEARS, *J. Physiol., Lond.* 174, 295 (1964).

<sup>4</sup> H. C. COOMBS, *Ber. ges. Physiol. Pharmac.* 55, 342 (1930).

<sup>5</sup> P. W. NATHAN and T. A. SEARS, *J. Neurol. Neurosurg. Psychiat.* 23, 10 (1960).

<sup>6</sup> G. STELLA, *J. Physiol., Lond.* 93, 10 (1938).



Fig. 1. Respiratory discharges of the chest muscles recorded during relaxation are replaced by a tonic activity as soon as the dog stands up and begins to walk. Calibration, 100  $\mu$ V and 1.5 sec.

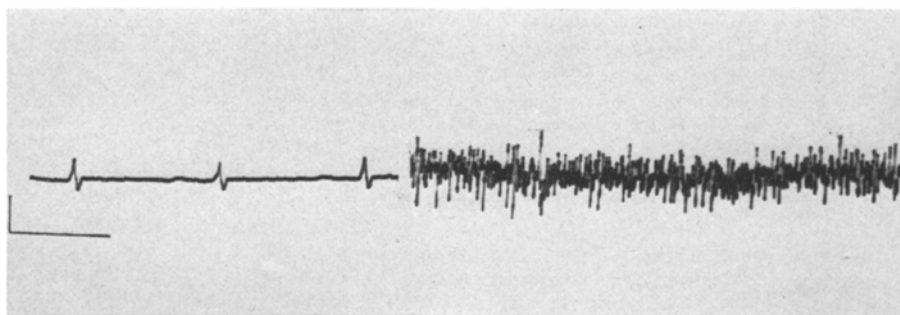


Fig. 2. Records taken with surface electrodes from the chest muscles in man. During relaxation there is no electrical activity from the chest muscles (except the ECG). Upon leaning forward, a tonic electrical activity appears. Calibration, 150  $\mu$ V and 0.5 sec.

(paradoxal movements) was described as a result of posterior cervical rhizotomy<sup>5</sup>.

In order to avoid surgical interference we used another method. The first experiments were performed on 3 adult dogs. Silver electrodes were implanted in the interchondrial portion of the internal intercostal muscles and in the external intercostal muscles (IV, V, VI and VII intercostal spaces).

The human subjects were 7 adults, 3 males and 4 females. The skin over the muscles to be studied was thoroughly cleansed with acetone. Cup-shaped silver electrodes approximately 1 cm in diameter were filled with electrode jelly and placed on the skin about 1 inch apart. A small piece of elasticized adhesive tape held the unit in place. Electrode pairs were placed over the intercostal spaces as follows: parasternally in the second and midaxillary in the tenth. The investigation was made with electromyograph.

The electrical activity in the intercostals of the dog during relaxation is very variable. Usually, phasic inspiratory or expiratory discharges, and sometimes low impulses between them, are recorded. But as soon as the animal stands up and begins to walk a high tonic electrical activity appears, and sometimes it is difficult to detect respiratory variations in the electromyogram against a background of postural activity (Figure 1).

There is usually no recordable electrical activity from the chest muscles during normal breathing in adult human subjects when surface electrodes are used. In some subjects a weak inspiratory activity was recorded.

But when the subject leans forward from the standing position and puts the hands on the floor a high tonic activity is recorded in the respiratory muscles (Figure 2).

Thus, in animals, a pronounced proprioceptive reflex from the chest muscles can be elicited very easily: as soon as the animal stands up the chest muscles become tonic. It is the natural phenomenon. In man, in the process of evolution the proprioceptive reflexes from the chest muscles have become weak. A pronounced tonic reflex from the chest muscles can be elicited only when a man takes the position of an animal, by leaning the trunk forward with his hands on the floor.

Consequently the proprioceptive reflexes from the chest muscles in man are much weaker than in animals. Thus, in man not only the lung volume reflexes mediated by the vagus but the reflex from the respiratory muscles probably do not influence normal breathing.

*Summary.* In man, in the process of evolution the proprioceptive reflexes from the chest muscle during apnea become weak. Probably the impulses from the respiratory stretch receptors do not take part in self-regulation of eupnea.

S. I. FRANKSTEIN, L. N. SERGEEVA  
Z. N. SERGEEVA and E. S. IVANOVA

*Institute of Normal and Pathological Physiology of the Academy of Medical Sciences, Baltijskaya 8, Moskwa (USSR), 25 February 1975.*

## Irrigation et sécrétion gastriques après ligature ou fistule pyloriques chez le rat, sous l'influence d'huile d'olive intra-duodénale

### Gastric Irrigation and Secretion in the Ligatured or Fistuled Pylorus Rat upon Influence of Intra-Duodenal Olive Oil

Parmi les nombreuses méthodes d'étude de la sécrétion gastrique chez le Rat, la ligature pylorique ou estomac de SHAY<sup>1</sup> a été largement utilisée, car, malgré certains inconvénients<sup>2</sup>, cette technique permet d'expérimenter très rapidement sur un grand nombre d'animaux<sup>3</sup>. Cette préparation est réputée pour favoriser la sécrétion acide<sup>4</sup> et paraît résistante à la gastrine<sup>5</sup>, sauf pour de fortes doses<sup>6</sup>. D'après BRODIE<sup>2</sup>, l'hyperacidité formée dans un estomac dont le pylore est ligaturé, ne serait pas due strictement à une libération de gastrine mais à une stimulation des récepteurs de l'antré; par contre, ISHII<sup>7</sup> admet plutôt l'intervention de la salive pour induire l'augmentation sécrétoire et insiste sur la complexité des mécanismes hormonaux contrôlant la sécrétion de l'estomac chez le Rat au pylore ligaturé.

Pour pallier les inconvénients d'une stase du suc dans l'estomac, certains auteurs<sup>8,9</sup> ont proposé de placer une fistule pylorique trans-duodénale; à part cette modifi-

<sup>1</sup> H. SHAY, D. C. SUN et M. GRUENSTEIN, *Gastroenterology* 26, 906 (1954).

<sup>2</sup> D. A. BRODIE, *Am. J. digest. Dis.* 71, 231 (1966).

<sup>3</sup> D. AURES et J. H. THOMPSON, *Eur. J. Pharmac.* 18, 323 (1972).

<sup>4</sup> D. A. BRODIE, R. W. MARSHALL et O. M. MORENO, *Am. J. Physiol.* 202, 812 (1962).

<sup>5</sup> M. D. KAYE, *Am. J. digest. Dis.* 16, 9 (1971).

<sup>6</sup> K. SEWING et M. ALBINUS, *J. Pharm. Pharmac.* 21, 58 (1969).

<sup>7</sup> Y. ISHII, *Jap. J. Pharmac.* 19, 125 (1969).

<sup>8</sup> S. BONFILS, G. ROSSI et A. LAMBLING, *Revue fr. Etud. clin. biol.* 3, 977 (1958).

<sup>9</sup> S. C. SKORYNA et D. R. WEBSTER, *Surg. Forum* 10, 193 (1959).