The cytoplasm is also labelled after a short pulse, when this is followed by a chase with cold precursor (Figure 4). We have not noticed a decrease of nuclear label in this case; however, the chase periods tried were not longer than $1\,^1/_2$ h.

The incorporation of H^3-uridine follows the same pattern described for H^3-cytidine, and the extraction with ribonuclease yields negative radioautographs (Figures 5, 6).

Discussion. Our experiments with H^3-thymidine prove that, even if a cytoplasmic store of DNA exists, an exogenous precursor can also be utilized.

The results obtained with H^3-cytidine and H^3-uridine give evidence of uptake into RNA and show a pattern of incorporation similar to that seen in tissue cultures[7]. The cytoplasmic label probably corresponds to ribosomic RNA, as is suggested by its time of appearance in relation to the nucleolar label[7], and by the higher probability of retaining heavy ribosomic RNA after the fixation and the embedding procedure. In Echinoderms and Amphibia, the incorporation of labelled uridine during cleavage is low and it is not recovered from the heavy ribosomal fraction[8–11].

Furthermore, labelled uridine in our preparations is extracted by ribonuclease, while it is partially incorporated into the DNA of cleaving Amphibia eggs[12]; a finding also reported for the egg of the marine snail *Ilyanassa*[13].

The effect of actinomycin D also points to a difference between mouse ova, their development being arrested[14], and Amphibia or Echinoderm eggs, which are not affected prior to the blastula stage[15,16].

In conclusion the pattern of nucleic acid metabolism of mouse morulae differs from that known for cleaving Echinoderm and Amphibia eggs; this coincides with their difference in genetic control[17].

Résumé. L'incorporation de précurseurs marqués des acides nucléiques dans les morulae de souris, suit un cours différent de celui qui est connu pour les œufs en segmentation d'Echinodermes et d'Amphibiens; ceci pourrait se rapporter à leur contrôle génétique différent.

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Escuela de Medicina, Universidad Católica, Santiago (Chile), February 26, 1965.

[7] R. P. Perry, Nat. Cancer Inst. Monogr. *14*, 73 (1964).
[8] M. Nemer, Proc. Nat. Acad. Sci. USA *50*, 230 (1963).
[9] F. H. Wilt, Devl. Biol. *9*, 299 (1964).
[10] J. Brachet, J. cell. comp. Physiol. *60*, 1 (1962).
[11] D. D. Brown and J. D. Caston, Devl. Biol. *5*, 435 (1962).
[12] N. Bieliavzky and R. Tencer, Exp. Cell Res. *21*, 279 (1960).
[13] J. R. Collier, Exp. Cell Res. *32*, 442 (1963).
[14] S. Silagi, Exp. Cell Res. *32*, 149 (1963).
[15] J. Brachet, H. Denis, and F. de Vitry, Devl. Biol. *9*, 398 (1964).
[16] P. R. Gross, L. I. Malkin, and W. A. Moyer, Proc. Nat. Acad. Sci. USA *51*, 407 (1964).
[17] This work was supported by P.H.S. research grant HD.00579. It was presented at the 6th Regional Meeting of Intern. Union Physiol. Scien. (November 1964).

Muscle Spindles in the Rat Diaphragm

Antidromic activity (ADA), which under certain conditions occurs in motor nerves and ventral roots, was first demonstrated by Masland and Wigton[1]. Their finding has been confirmed and subjected to further study by other authors (Feng and Li[2]; Eccles et al.[3]; Riker et al.[4]; Werner[5]; Blaber and Bowman[6]). Under appropriate conditions, ADA has been demonstrated also in the phrenic nerve-diaphragm preparation (Van der Meer and Meeter[7]; Barstad[8]; Randić and Straughan[9]). As the recordings in the latter experiments have been made from the phrenic nerve trunk, activity in afferent fibres within this nerve presents a potential source of error. Recording from the cervical ventral roots of the rat being technically difficult, Randić and Straughan[9] circumvented the intricacy by sensory denervation of the phrenic nerve three weeks in advance of their experiments. For a similar purpose, van der Meer and Meeter[7] employed longitudinal splitting of the nerve.

Studying ADA in the rat phrenic nerve-diaphragm preparation, the present authors occasionally recorded series of spike potentials which had the main characteristics of the afferent firing from muscle spindles. Records from these experiments are shown in Figure 1.

Afferent firing in the phrenic nerve has been recorded in the rabbit by Cardin[10,11] and Cuénod[12] and in the cat by Yasargil[13,14]. According to Hinsey et al.[15] approximately 10% of myelinated fibres in the cat phrenic nerve are sensory. Landau et al.[16] found 35–45% afferents in the phrenic nerve of the dog.

Histologically several authors have demonstrated the occurrence of muscle spindles in the diaphragms of different mammalian species, including man (Dogiel[17];

[1] R. L. Masland and R. S. Wigton, J. Neurophysiol. *3*, 269 (1940).
[2] T. P. Feng and T. H. Li, Chin. J. Physiol. *16*, 37 (1941).
[3] J. C. Eccles, B. Katz, and S. W. Kuffler, J. Neurophysiol. *5*, 211 (1942).
[4] N. F. Riker jr., G. Werner, J. Roberts, and A. Kuperman, Ann. N.Y. Acad. Sci. *81*, 328 (1959).
[5] G. Werner, J. Neurophysiol. *23*, 453 (1960).
[6] L. C. Blaber and W. C. Bowman, Int. J. Neuropharmacol. *2*, 1 (1963).
[7] C. van der Meer and E. Meeter, Acta physiol. pharmac. néerl. *4*, 454 (1956).
[8] J. A. B. Barstad, Exper. *18*, 579 (1962).
[9] M. Randić and D. W. Straughan, J. Physiol. *173*, 130 (1964).
[10] A. Cardin, Archo. Sci. biol. Bologna *25*, 51 (1939).
[11] A. Cardin, Archo. Sci. biol. Bologna *30*, 9 (1944).
[12] M. Cuénod, Helv. physiol. pharmac. Acta *19*, 360 (1961).
[13] G. M. Yasargil, Helv. physiol. pharmac. Acta *19*, 360 (1961).
[14] G. M. Yasargil, Helv. physiol. pharmac. Acta *20*, 39 (1962).
[15] J. C. Hinsey, K. Hare, and R. A. Phillips, Proc. Soc. exp. Biol. Med. *41*, 411 (1939).
[16] B. R. Landau, K. Akert, and T. S. Toberts, J. comp. Neurol. *119*, 1 (1962).
[17] A. Dogiel, Arch. mikrosk. Anat. *59*, 1 (1902).

TIMOFEJEW[18]; GREGOR[19]; MASUMOTO[20]; WINCKLER and DELALOYE[21]). MASUMOTO's paper[20] also included the demonstration of a muscle spindle from the rat diaphragm. Apart from this, the literature seems to contain scanty direct evidence regarding the occurrence and distribution of muscle spindles in this muscle. To procure some additional knowledge about the interference of firing from muscle spindles with experiments on ADA, and moreover to explore the possible applicability of the rat diaphragm as a single-spindle preparation, the present authors undertook a limited histological examination of this muscle.

Adult albino rats weighing 200–300 g were used, without regard to sex. The diaphragm was taken out immediately after the animal had been killed, fixed in formol or a formol acetic acid ethanol mixture (ROMEIS[22]; PALMGREN[23]) and embedded in paraffin. Different staining procedures were tried. The best results were obtained with Hematoxyline-Eosine (ROMEIS[22]), the silver staining method of PALMGREN[23] and with methylene blue (BOYD[24]).

Figure 2 shows a transverse section through the equatorial region of a spindle. By thorough microscopic inspection of serial sections of 10 and 25 μ thickness as well as of teased muscle preparations, we regularly found three spindles in each hemidiaphragm. One of them was almost constantly found in the pars lumbalis. The two muscle spindles usually found in the pars costalis – or one of them rarely in the pars sternalis – were almost invariably localized to the costal side of the nerve insertion. Our results do not justify any conclusions with regard to the finer structure and composition of the spindles.

If our findings, which comprise slightly more than 20 reliable observations of spindles and a few less reliable ones, give a correct picture of the occurrence and distribution of these end organs in the rat diaphragm, this muscle seems to lend itself as a suitable basis for preparing a mammalian single-spindle preparation, when care is taken not to overdo its dimensions. This conclusion is also supported by our electrophysiological experiments, in which the amplitude and frequency of the afferent spikes sometimes indicated discharges from one, sometimes from two end organs.

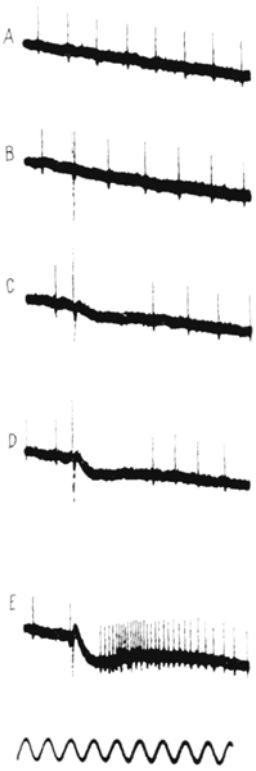

Fig. 1. Nerve recordings from an isolated phrenic nerve-diaphragm preparation. The nerve was placed on five platinum electrodes in paraffin oil. One pair of the electrodes close to the cut end of the nerve was used for recording, a second pair, distally placed, for nerve stimulation (with square waves of 50 μ sec duration). The fifth electrode was placed between the two pairs and connected to earth. The nerve was crushed between the recording electrodes. The muscle was fixed to a perspex holder and immersed in Tyrode solution which was equilibrated with a mixture of oxygen and carbon dioxide (5%) at 37°C. Slight stretch was applied to the muscle until regular afferent firing was seen (A). The records B–E show the effect of increasing stimulus intensity. The stage at which the pause in afferent firing occurred (C), coincided with the first sign of muscle contraction (watched through a stereomicroscope). Calibration sine wave: frequency 50/sec; peak to peak 50 μV.

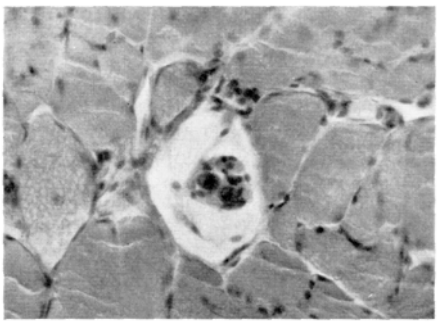

Fig. 2. Part of transverse section from rat diaphragm. Hematoxyline-eosine. × 200. A muscle spindle, cut through the lymph space, lies in the centre surrounded by extrafusal muscle fibres.

Zusammenfassung. Elektrophysiologische Registrierungen afferenter Impulse des Nervus phrenicus aus dem isolierten Rattenzwerchfell wurden durch eine histologische Untersuchung des Zwerchfelles unterbaut. Das Vorkommen von durchschnittlich 3 Muskelspindeln in jeder Zwerchfellhälfte wurde nachgewiesen.

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[18] D. A. TIMOFEJEW, Arch. mikrosk. Anat. *59*, 629 (1902).
[19] A. GREGOR, Arch. Anat. Physiol. *113, 1904*, 113.
[20] K. MASUMOTO, Mitt. med. Fak. K. jap. Univ. *10*, 1015 (1934).
[21] G. WINCKLER and B. DELALOYE, Acta anat. *29*, 114 (1957).
[22] B. ROMEIS, *Mikroskopische Technik* (15. Aufl. R. Oldenburg, München 1948).
[23] A. PALMGREN, Acta zool. Stockh. *29*, 377 (1948).
[24] J. A. BOYD, J. Physiol. *141*, 10P (1958).