conclusion can be drawn from this observation. Quantitative measurements reported in the Table showed a significant increase in LAP activity only in gastrocnemius of mice of the older group.

Theoretically, growth hormone, through its influence on connective tissue[7], might have been an activator of dystrophic degeneration and have induced an increase of LAP activity. However, treatment of the animals with this hormone produced no measurable effect on LAP, and did not stimulate body growth; apparently, the growth hormone preparation used here, though growth-promoting in rats[11], was ineffective in dystrophic mice.

Leucine aminopeptidase activity in muscles of dystrophic mice[a]

Groups	50–60 days old		100–125 days old	
	Untreated	Growth hormone		
Controls	3.2 ± 0.4	2.9 ± 0.3	5.3 ± 0.8	$p < 0.002$
Dystrophic	4.2 ± 0.5	3.8 ± 0.8	10.5 ± 1.1	

[a] Expressed in μg of naphthylamine produced per g of tissue after 2 h of incubation ± S.E.

The present quantitative results confirm previous histochemical reports on the presence of LAP activity in muscles of dystrophic mice[3,4]. They do not indicate an acceleration of the enzyme activity prior to connective tissue proliferation in dystrophic muscles but suggest that both changes occur simultaneously[12].

Résumé. L'activité de la leucine aminopeptidase (LAP) a été mesurée dans le muscle de la souris dystrophique. La LAP est peu active dans le muscle dystrophique de la souris jeune (50 à 60 jours). D'autre part, l'activité de l'enzyme est fortement augmentée dans le muscle de la souris plus agée (110 à 125 jours). Un traitement de 5 jours à l'hormone de croissance chez la souris jeune n'a pas modifié significativement l'activité de la LAP dans les muscles des souris dystrophiques ou témoins.

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Département de Pharmacologie, Faculté de Médecine, Université de Montréal (Canada), Octobre 3, 1963.

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[12] *Acknowledgments.* This research was supported by a grant from the Muscular Dystrophy Association of Canada.

Inhibitory Action of γ-Aminobutyric Acid (GABA) on *Ascaris* Muscle

Piperazine, the drug most commonly used for the treatment of roundworm infections, produces a flaccid paralysis of the somatic musculature of *Ascaris lumbricoides*. A recent investigation has shown that this effect cannot be accounted for by a block or curarization of excitatory nerve-muscle synapses, as was generally assumed. New experimental evidence suggests that piperazine behaves as a pharmacological analogue of an inhibitory neuromuscular transmitter[1].

The repetitive spike potentials responsible for the contraction of *Ascaris* muscle are not transmitted from motor nerve fibres by discrete bursts of synaptic activity. Instead, they are generated by pacemakers located in a specialized region of the muscle which we have called the *syncytium*[2,3]. This is a structure formed by the terminal arborizations of the *arms*, extensions or processes, sent by each muscle cell to the nerve cord (see Figure 1).

The functional properties of the muscle syncytium resemble those of mammalian visceral muscle. On the one hand, the muscle cell arms are electrically interconnected at this level, i.e. current injected into any muscle cell flows along its arm into the syncytium where it spreads electrotonically to the surrounding arms across low impedance pathways[2]. On the other hand, the syncytial surface membrane possesses autorhythmic properties, generating spike potentials which are conducted within the syncytium itself as well as away from it along the arms, to the contractile region of the muscle cell or *spindle* (see Figure 2).

The frequency of the rhythmic spike potentials is modulated by excitatory and inhibitory synapses established between the nerve cord fibres and the muscle syncytium. The chemical transmitter liberated by the former, believed to be acetylcholine or a related choline ester, exerts a depolarizing effect on the syncytial membrane increasing the frequency of the spike activity[3]. The chemical transmitter, of unknown nature, released at the inhibitory synapses causes a hyperpolarization of the syncytium and a decrease in the frequency of the spikes, which disappear when the potential difference across the membrane increases above 40 mV.

The addition of piperazine (to a concentration of $10^{-3} M$) to the saline bathing *Ascaris* preparations causes hyperpolarization of the muscle cells and suppression of the spike activity. Electrophoretic application of this compound to different regions of the muscle cells has shown that the piperazine-receptors are located in the muscle syncytium and are probably identical with the postsynaptic receptors of the inhibitory nerve-muscle junctions. The activation of such receptors results in an increased conductance of the syncytial membrane to Cl^- ions, which, moving into the cell along the existing con-

[1] J. del Castillo, W. C. de Mello, and T. Morales, submitted to Brit. J. Pharmacol. (1963).

[2] J. T. de Bell, J. del Castillo, and V. Sanchez, J. cell. comp. Physiol. *62*, 159 (1963).

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centration gradients, bring about an increased negativity of the cytoplasm.

Experiments are now being performed to see whether these inhibitory receptors are activated by compounds other than piperazine and the natural inhibitory transmitter; particularly by those known to inhibit in low concentrations invertebrate nerve and muscle cells. One of the chemicals already tested, γ-aminobutyric acid (GABA), has been found to exert a powerful hyperpolarizing action on *Ascaris* muscle cells.

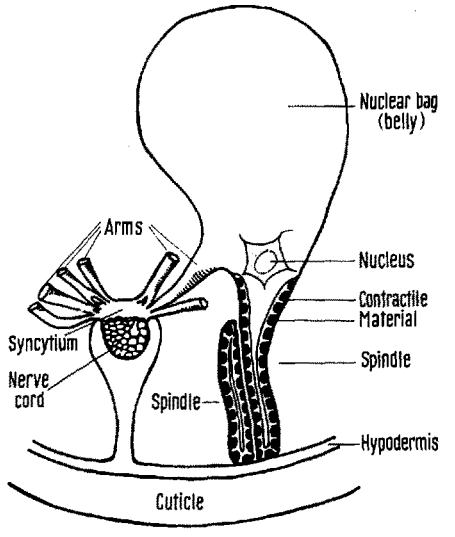

Fig. 1. Cross section of a somatic muscle cell and the nerve cord in *Ascaris lumbricoides*. Three different parts can be distinguished in the former: a voluminous nuclear bag, or *belly*; a long and flattened *spindle* parallel to the axis of the worm, which contains the contractile material; and a process, the muscle cell *arm*, which extends from the belly to the nerve cord. The terminal arborizations of the arms form a structure, the muscle *syncytium*, which extends across the channel of hypodermal tissue enclosing the nerve cord. Excitatory and inhibitory synapses are established between the nerve cord fibres and the muscle syncytium. Part of other six cell arms are also shown in this drawing, as well as the spindle of another muscle cell sectioned some distance away from the corresponding nuclear bag.

Curve A in Figure 3 represents the change in muscle membrane potential recorded with intracellular microelectrodes as a function of the concentration of GABA in the surrounding solution. Each point is the average of 120 measurements (20 cells in each of six preparations) expressed as the difference from the average initial value in the absence of GABA in the solution.

As can be seen, a low concentration of GABA ($10^{-7}M$) exerts a small but significant ($P < 0.0002$) *depolarizing* action on the muscle cells. However, an increment of about 15 mV in the average membrane potential occurs when the concentration of GABA is increased from about 10^{-6} to $10^{-5}M$. A further increase in concentration is not accompanied by a corresponding increment in the membrane potential which, instead, tends to decrease slightly.

In order to compare the hyperpolarizing actions of GABA and piperazine, the curve B, based on data from a previous paper[1], is included in Figure 3. This curve shows the membrane potential as a function of piperazine concentration. Each point is the average of 100 measurements (20 cells in each of five different preparations).

The maximal levels of membrane potential induced by both GABA and piperazine are nearly equal. The initial resting potential in curve A, in the absence of GABA, was of 30.5 mV (S.E. $\pm$ 0.34) which was increased to an average of 46.3 mV (S.E. $\pm$ 0.88) by a $10^{-5}M$ concentration of GABA. The initial membrane potential of B, in the absence of piperazine, was of 30.7 mV (S.E. $\pm$ 0.37). The highest point in this curve, corresponding to a piperazine concentration of $2.15 \times 10^{-3}M$, was of 45.2 mV (S.E. $\pm$ 0.62).

This close similarity between the effects of GABA and piperazine is not surprising, since other experiments (to be published elsewhere) have revealed that the immediate effect of GABA on the syncytial membrane is also an increase of its conductance to Cl^- ions. Therefore, one may assume that the 46 mV level corresponds to the chloride potential.

As mentioned above, the spike activity of *Ascaris* muscle is suppressed when the average resting potential increases above 40 mV, although occasionally spikes have been seen in cells with higher membrane potentials. This level is indicated by the shaded stripe in Figure 3. It can be seen that the concentrations of GABA and piperazine

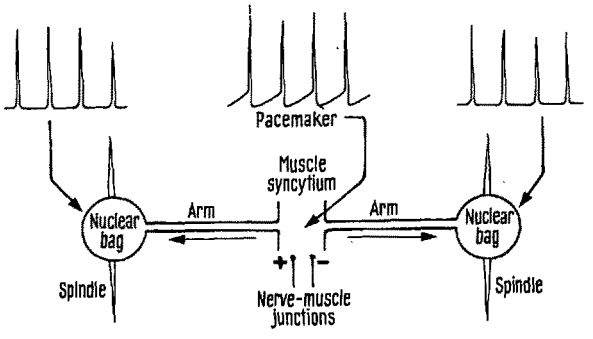

Fig. 2. Functional organization of the neuromuscular system of *Ascaris*. The muscle pacemakers at the syncytium generate spike potentials which are conducted along the cell arms to the nuclear bags and spindles. Only one muscle cell has been drawn at each side although approximately 85 arms join the muscle syncytium per mm of length. The frequency of the spike potentials produced by the muscle syncytium are modulated by excitatory (+) and inhibitory (–) nerve-muscle junctions.

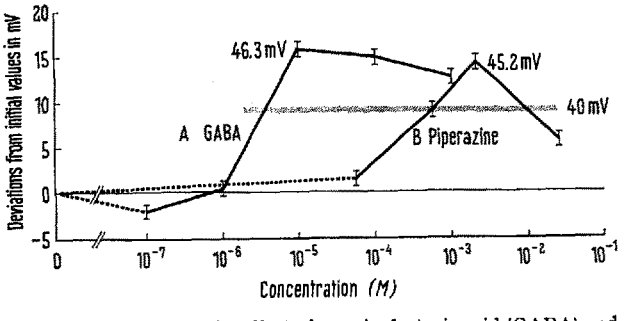

Fig. 3. Graph showing the effect of γ-aminobutyric acid (GABA) and piperazine on the membrane potential of *Ascaris* muscle cells. Ordinates represent average membrane potential in mV, expressed as the difference from the initial values in the absence of drugs. Abscissae: concentration of the drugs in moles. Each point in curve *A* (GABA) is the average membrane potential of 120 cells (six preparations), while the points in curve *A* (piperazine) are the average of *100* measurements (five preparations). The lines drawn through the points are equal to 2 × the S.E. of the mean.

needed to produce such a hyperpolarization differ by two orders of magnitude.

The decrease in membrane potential observed when the piperazine concentration increases above $4 \times 10^{-3} M$ appears to be due to a non-specific influence of this compound over the entire surface of the muscle cells. The effects of comparable concentrations of GABA have not been investigated, although the shape of curve A suggests that they would exert a similar depolarizing action.

The marked hyperpolarizing action of GABA on *Ascaris* muscle leads one to think that this compound might serve as a basis for the development of new anthelmintics[4].

Zusammenfassung. Die somatische Muskulatur von *Ascaris lumbricoides* wird durch γ-Aminobuttersäure in Konzentrationen von 10^{-6}–$10^{-5} M$ völlig gehemmt. Eine hnliche Hemmung wird durch Piperazin ($10^{-3} M$) erzielt. Beide Substanzen steigern die Cl-Permeabilität der Muskelmembran.

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Department of Pharmacology, School of Medicine, and Laboratory of Perinatal Physiology, N.I.N.D.B., U.S. Public Health Service, San Juan (Puerto Rico), October 28, 1963.

[4] This work was supported by U.S. Public Health Service, Research Grant No. 02021–05, from the National Institute of Neurological Diseases and Blindness.

[5] On leave of absence from the Instituto de Biofísica, Universidade do Brazil, Rio de Janeiro (Brazil).

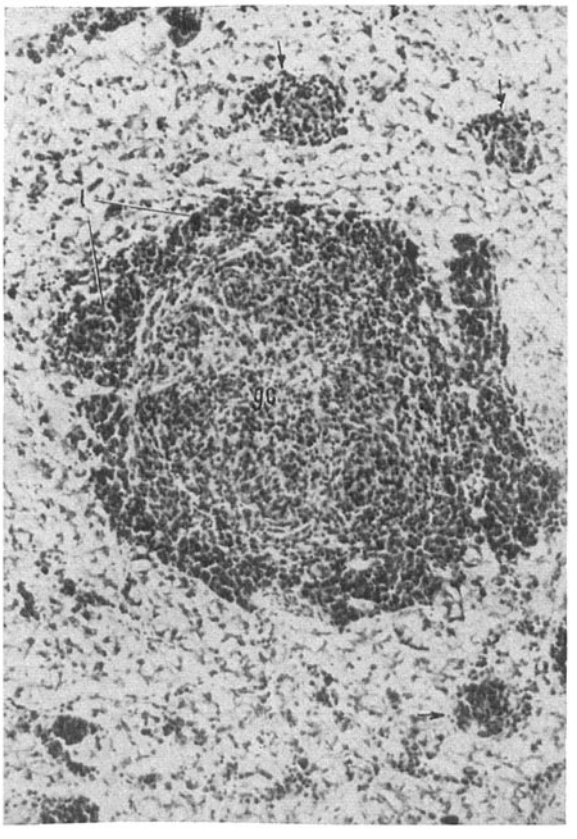
Benzpyrene-Induced Tumours in the Clawed Toad, *Xenopus laevis*

Although a variety of spontaneous neoplasms have been discovered in a large number of anuran species[1], attempts at the chemical induction of tumours in these amphibians have been relatively unsuccessful[2]. Recently, however, it has been shown that the implantation of methylcholanthrene crystals into the South African clawed toad, *Xenopus laevis*[2], provokes the development of lymphoid tumours similar to those occurring spontaneously in this species[1]; moreover, these tumours are readily transplantable into other *Xenopus*[3] or into the urodele species *Triturus cristatus*[4].

In view of the suggestion of LEONE[5] that, uniquely in the amphibia, sarcomas develop after methylcholanthrene treatment and carcinomas after benzpyrene application, experiments have been performed in an attempt to induce neoplasia in an anuran species using benzpyrene. Single doses (1.5 mg) of benzpyrene crystals (Roche) were placed in the abdominal cavity of 13 adult *Xenopus laevis laevis* through small cuts in the abdominal wall near the liver, and each wound was closed with a single stitch (Experiment I). Secondly, single small doses of benzpyrene crystals mixed with egg albumen were placed in the dorsal lymph sac or abdominal cavity of 20 immature animals of the same species (Experiment II).

The results of the first experiment are summarized in Table I, which shows that 11 of the 13 animals developed lymphosarcomas between 86 and 288 days after treatment. In 7 of the 11 positive cases the induced tumours affected a multiplicity of visceral organs, notably the liver and kidneys, but in four cases the tumours were less widespread when the animals were killed. The development of lesions in the abdominal wall muscle or skin of five animals is a reflection of the difficulty of introducing the carcinogen without leaving some crystals in the wound area.

The benzpyrene-induced tumours appeared to be histologically very similar to those lymphoid tumours appearing spontaneously in *Xenopus* or induced with methylcholanthrene crystals. A very large nodule some 2 cm in diameter, found in the intestinal mesentery of Case 10, consisted of groups of lymphoid cells separated by connective tissue. Under the abdominal skin of the same ani-



Transfer series case 4. The liver parenchyma lacks the large groups of pigment granules normally found in this organ, but contains a large lymphoid tumour consisting of peripheral small lymphocytes (l) and a 'germinal centre' (gc). Smaller tumour nodules such as those arrowed were spread throughout the liver. H. & E. × 200.

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