Au contraire, le 5-bromo-4-chloro-3-indolyl-β-D-galactopyranoside[3] est un substrat chromogénique: incolore en solution, il donne, après action d'une galactosidase, un produit de réaction insoluble et coloré en bleu turquoise. Ce substrat a été utilisé par PEARSON et al.[4] pour localiser la galactosidase dans les tissus.

Un immunsérum de lapin anti-suc digestif d'*Helix pomatia* est obtenu par la méthode suivante: la première semaine, deux injections de suc dilué au 1/20 (environ 10 mg d'azote/ml) avec adjuvant de Freund, puis, pendant trois semaines, une injection intramusculaire de la même quantité du suc, alternativement dans la cuisse droite et dans la cuisse gauche, quinze jours de repos puis reprise des injections intra-musculaires alternées pendant deux semaines.

La technique d'immunoélectrophorèse utilisée est celle de SCHEIDEGGER[5]. Une quinzaine de lignes de précipitations apparaissent avec le suc total (Figure 2a). Pour la caractérisation de l'enzyme, une solution du substrat chromogénique (0,00016 *M* dans le tampon acétate 0,1 *M* pH 5,3, 0,001 *M* en chlorhydrate de spermidine) est déposée dans la rigole des lames d'immunoélectrophorèse lavées préalablement durant deux jours après la réaction immunologique. La réaction enzymatique s'effectue en chambre humide pendant 6 h à 37°C: deux arcs sont ainsi colorés spécifiquement en bleu turquoise, l'un dans la région des γ-globulines, l'autre au niveau des α_2-globulines (Figure 2c). Par leur migration électrophorétique, ces deux lignes correspondent donc aux deux principales zones d'activité enzymatique mises en évidence sur cellogel.

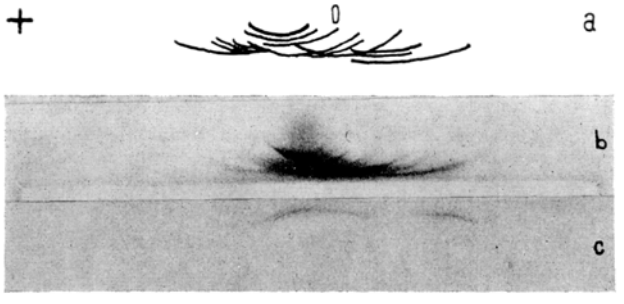

Fig. 2. Immunoélectrophorèse du suc digestif d'*Helix pomatia*. a, b, Schéma et photographie des lignes de précipitation révélées par l'amidoschwartz. c, Réaction spécifique des arcs de précipitation possédant une activité hydrolytique sur le 5-bromo-4-chloro-3-indolyl-β-D-galactoside.

On peut en conclure qu'il existe au moins deux β-galactosidases dans le suc digestif d'*Helix pomatia*, toutes les deux antigéniques, différant par leur mobilité électrophorétique.

L'activité hydrolytique du suc sur l'ONPG passe par un maximum à pH 5,3: des essais de dosage effectués à d'autres pH sur les zones séparées sur cellogel n'ont pas permis de différencier les galactosidases par leur pH optimum.

Un travail de purification de ces enzymes est en cours et permettra, sans doute, de mieux caractériser ces différents enzymes[6].

Summary. Digestive juice of *Helix pomatia* was studied by immunoelectrophoresis: after incubation with a chromogenic substrate, 5-bromo-4-chloroindol-3-yl-β-D-galactopyranoside, two β-galactosidases were revealed.

R. GOT, A. MARNAY et P. JARRIGE

Laboratoire de Biochimie, Faculté de Médecine, Paris (France), le 12 avril 1965.

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Effect of Mephenesin on Skeletal Muscle Myofibrils

Mephenesin, 3-(2′-methylphenoxy)propane-1,2-diol, has been classified as a skeletal muscle relaxant or depressant[1]. It acts primarily on the spinal cord through inhibitions of polysynaptic pathways and more specifically on the internuncial cells[2]. In the past, the pharmacological research with this drug has been directed toward its primary target, the spinal cord[3,4]. Except for some studies regarding the action potential[5], the pharmacodynamic effect of mephenesin on muscle has received little attention.

In some early work, BATE-SMITH and BENDALL[6] found that mephenesin administration resulted in a marked delay in the onset of rigor mortis in muscle. More recently, SINK et al.[7] showed that the sarcomere length in the muscle myofibril was highly correlated with the length of this delay in the onset of rigor. From these researches, it should follow that the administration of mephenesin, by delaying the onset of rigor mortis, should produce sarcomeres that are longer than those normally observed in the post-rigor muscle myofibril. Consequently, the pur-

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pose of these experiments was to test the hypothesis that the pharmacodynamic effect of mephenesin on muscle is the longer sarcomere in the myofibril.

Experimental procedure. Four experimental animals (*Sus domesticus*), averaging approximately 75 kg and with similar genetic and nutritional backgrounds, were used in these studies. Two animals were intraperitoneally given doses of mephenesin (E. R. Squibb & Sons, New York) prepared in 25% ethanol to a concentration of 100 mg/ml. Two different doses, at levels of 750 and 1350 mg/kg of body weight, were administered. 20 min after the injection of the drug, the animals were sacrificed.

The time course of rigor mortis was approximated on an excised strip (1 · 1 · 6 cm) of parallel muscle fibers, with the use of a rigorometer[8] maintained at 37°, 100% relative humidity, nitrogen atmosphere, and 50 g weights applied at 2 min intervals. Onset of rigor mortis is defined as the time at which the muscle begins to lose its elasticity. After all the elasticity disappears, rigor mortis is complete[9].

24 h post-mortem samples of the Gluteus medius, Rectus femoris, Longissimus dorsi, Trapezius thoracis, Serratus ventralis, the outer and inner portions of the Biceps femoris, and the light and dark portions of the emitendinosus muscles were used in determining the sarcomere length of the myofibril. The repeating unit of structure in the myofibril is the sarcomere and is defined as the region bounded by two adjacent Z-lines[10].

Approximately 400 mg of the excised muscle tissue sample were homogenized in 10 ml of cold 0.08 *M* potassium chloride[11] in a Lourdes tissue homogenizer for 1 min. An unfixed preparation of this homogenate was examined under an A. O. Spencer Phasestar microscope equipped with an ocular scale calibrated with a stage micrometer. Since muscle tissue has been shown to be heterogeneous with respect to the length of the individual sarcomeres[12,13], measurements of the sarcomere length of 25 randomly selected, isolated myofibrils were made for each unfixed preparation. The mean and standard error, for each muscle sample, were calculated in the usual manner[14].

Results and discussion. Following the administration of mephenesin, both animals showed flaccid paralysis and a loss of the righting postural reflex within 10 min. Muscular paralysis was of the ascending type. The posterior limbs and the lower half of the body were affected first with the anterior limbs and the neck being affected last. Respiration appeared normal. Salivation in both animals was observed as well as slight nausea and vomiting. During paralysis, the animals remained motionless and were completely limp.

The onset of rigor mortis was noticeably delayed in the two treated experimental animals. The onset of rigor occurred in approximately 1 h in the controls, 6 h in the 750 mg/kg treated and 8 h in the 1350 mg/kg treated animals.

From the data presented in the Table, the effect of mephenesin dose on the sarcomere length of the muscle myofibril can be observed. Considering each muscle individually, some small differences were noted between those from the 750 mg/kg treated and the 1350 mg/kg treated animals. With the exception of the Biceps femoris muscle, the use of the larger dose resulted in a slightly longer mean sarcomere length. As a group, there happened to exist no difference between the mean sarcomere length (2.65 μ) of the muscles from the animals administered mephenesin.

It is quite obvious, after comparing the mean sarcomere length of the mephenesin treated animals (2.65 μ) with that measured in the controls (2.27 μ), that the original hypothesis has been supported.

Another very interesting observation, worthy of comment, is that mephenesin produces a more uniformly contracted state in the myofibril. The variation in the mean sarcomere length of the muscles from the control animals was $\pm$ 0.28 μ, whereas the variation was only $\pm$ 0.15 μ in the mephenesin treated animals.

Thus, it has been quantitatively shown that mephenesin affects the structure of skeletal muscle by preventing the sarcomere from contracting normally. In mediating muscle contraction, the heterogeneity in sarcomere length, both within and between muscles, is greatly reduced[15].

Effect of mephenesin dose on the sarcomere length of the muscle myofibril (mean $\pm$ standard error)

Muscle	Sarcomere length in the myofibril (in μ)		
	Controls μ	750 mg/kg μ	1350 mg/kg μ
Trapezius thoracis	2.70 $\pm$ 0.24	2.74 $\pm$ 0.18	2.76 $\pm$ 0.14
Serratus ventralis	2.55 $\pm$ 0.27	2.72 $\pm$ 0.14	2.72 $\pm$ 0.14
Semitendinosus			
Dark portion	2.77 $\pm$ 0.24	2.74 $\pm$ 0.09	2.75 $\pm$ 0.15
Light portion	2.35 $\pm$ 0.33	2.59 $\pm$ 0.14	2.62 $\pm$ 0.17
Rectus femoris	2.25 $\pm$ 0.30	2.67 $\pm$ 0.12	2.71 $\pm$ 0.18
Biceps femoris			
Outer portion	2.09 $\pm$ 0.23	2.69 $\pm$ 0.12	2.65 $\pm$ 0.14
Inner portion	1.98 $\pm$ 0.31	2.76 $\pm$ 0.11	2.54 $\pm$ 0.16
Longissimus dorsi	1.94 $\pm$ 0.33	2.50 $\pm$ 0.19	2.52 $\pm$ 0.17
Gluteus medius	1.81 $\pm$ 0.24	2.49 $\pm$ 0.23	2.62 $\pm$ 0.13
All muscles	2.27 $\pm$ 0.28	2.65 $\pm$ 0.15	2.65 $\pm$ 0.15

Résumé. Ces expériences ont montré quantitativement que la méphénésine affecte la structure des muscles du squelette en empêchant le sarcomère de se contracter normalement. Par l'affaiblissement de la contraction du muscle, l'hétérogénéité de la longueur des sarcomères se trouve fortement réduite.

J. D. SINK

Department of Biochemistry, University of Wisconsin, Madison (USA), June 4, 1965.

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