In addition to the above mentioned properties those newly synthesized acid-soluble proteins were discovered to display elution characteristics from Amberlite IRC-50 resin which resembled histones, and when labelled with [^{3}H]-arginine or [^{3}H]-lysine to have high concentrations of those amino acids. These findings are in agreement with previous studies which employed different embryos and developmental stages[12].

Both enucleated eggs and ovarian oocytes are also actively synthesizing histones (Table II). Previous studies on HeLa cells[13,14] emphasized the temporal relationship between DNA synthesis and histone synthesis. In those studies the synthesis of histone was linked to DNA synthesis. The findings described in this report indicate, however, that the amphibian oocyte, dormant in the synthesis of DNA[15,16], synthesized substantial amounts of histones. Indeed, histone synthesis also proceeds in the absence of functional nucleus. The apparent lack of a coordination between DNA and histone synthesis in oocytes may reflect the storage in the oocyte cytoplasm of histones which will be employed during the early cleavage stages when DNA synthesis and nuclear division proceed at exceptionally rapid rates. At 18 °C the number of cells doubles approximately once every 2 h[17].

Zusammenfassung. Proteine, die während verschiedener Entwicklungsstadien der Amphibien-Embryogenese auftreten, wurden isoliert und näher charakterisiert, wobei ein wesentlicher Anteil der mit ^{3}H-Leucin markierten Proteine aus Histonen besteht. In Eiern, deren Nukleus entfernt wurde, sowie in Ovarien-Oocyten, bei denen keine DNA-Synthese stattfindet, wurden jedoch Histone synthetisiert.

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[12] E. W. Byrd jr. and H. E. Kusinsky, Biochemistry *12*, 246 (1973).
[13] E. Robbins and T. W. Borun, Proc. natn. Acad. Sci., USA *57*, 409 (1967).
[14] D. Gallwitz and G. Mueller, J. biol. Chem. *244*, 5947 (1969).
[15] M. Izawa, V. G. Allfrey and A. E. Mirsky, Proc. natn. Acad. Sci. USA *50*, 811 (1963).
[16] J. B. Gurdon, Proc. natn. Acad. Sci. USA *58*, 545 (1967).
[17] This investigation was supported in part by N.S.F. Grant No. GB-36973.

Differentiation of Cultured Muscle in the Presence of α-Bungarotoxin

There is evidence that the acetylcholine receptor is present in cultured skeletal muscle at a very early stage of development, even in some mononuclear cells and often before the appearance of organized contractile elements[1,2]. It has been postulated that the cholinoreceptor plays a role in the early events of myogenesis. This possible function of the acetylcholine receptor has been investigated by culturing myoblasts in D-tubocurarine: no effect on development was seen during the first 48 h in vitro[1]. Although D-tubocurarine has a high affinity for the cholinoreceptor, its action is reversible and the receptor-antagonist complex is, therefore, in a

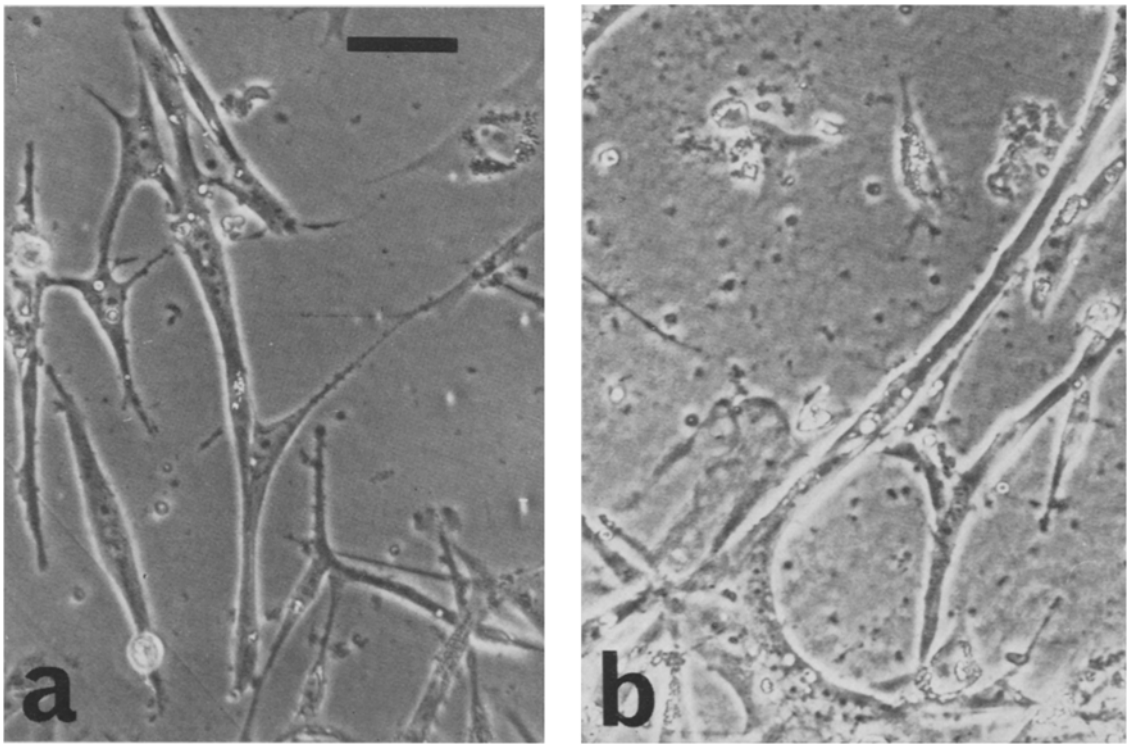

Fig. 1. Morphology of myogenic cells in culture. Phase contrast micrographs. a) 3-day control culture, showing bipolar myoblasts and small myotubes. b) 3-day culture grown in α-bungarotoxin (1 μg/ml). Calibration: 50 μm.

state of dynamic equilibrium. The snake venom component, α-bungarotoxin combines irreversibly with the acetylcholine receptor of skeletal muscle. In order to test the hypothesis that irreversible occupation of receptor sites by antagonist might influence the membrane properties of developing muscle in culture, myogenic cells were grown in the presence of α-bungarotoxin and various parameters were examined.

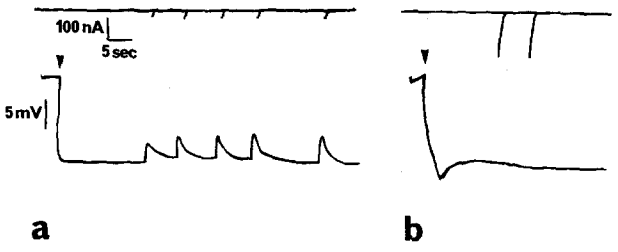

Fig. 2. Response of 3-day myotubes to iontophoretically applied acetylcholine. a) Series of depolarizations in a control fibre. Penetration of the fibre by the recording electrode is indicated by the arrow. Upper trace: iontophoresis current (each pulse is 100 msec in duration); lower trace: membrane potential of cell. b) Absence of response in a fibre grown in α-bungarotoxin (1 μg/ml), despite 10-fold increase in iontophoretic current.

A single cell suspension of 2×10^5 cells/ml was obtained by trypsin dissociation of the leg musculature of 10–11-day chick embryos[3] and 2 ml added to 35 mm plastic petri dishes that had previously been coated with collagen[4,5]. Cultures were incubated at 37 °C in Eagle's Minimum Essential Medium supplemented with 5% chick embryo extract and 15% horse serum. After 2 days, when myoblast fusion had begun, cultures were routinely treated for 48 h with medium containing the DNA synthesis inhibitor cytosine arabinoside (10^{-5} *M*) in order to eliminate replicating cells such as fibroblasts. Thereafter the medium was changed every 3 days.

α-bungarotoxin was obtained from the venom of *Bungarus multicinctus* as described by DRYDEN, HARVEY

[1] D. FAMBROUGH and J. E. RASH, Devl. Biol. *26*, 55 (1971).
[2] W. F. DRYDEN, S. D. ERULKAR and G. DE LA HABA, to be published.
[3] I. R. KONIGSBERG, N. MCELVAIN, M. TOOTLE and H. HERRMAN, J. biophys. biochem. Cytol. *8*, 333 (1960).
[4] S. D. HAUSCHKA and I. R. KONIGSBERG, Proc. natn. Acad. Sci., USA *55*, 119 (1966).
[4] S. D. HAUSCHKA and I. R. KONIGSBERG, Proc. natn. Acad. Sci., USA *55*, 119 (1966).
[5] E. B. MASUROVSKY and E. R. PETERSON, Expl Cell Res. *76*, 447 (1973).

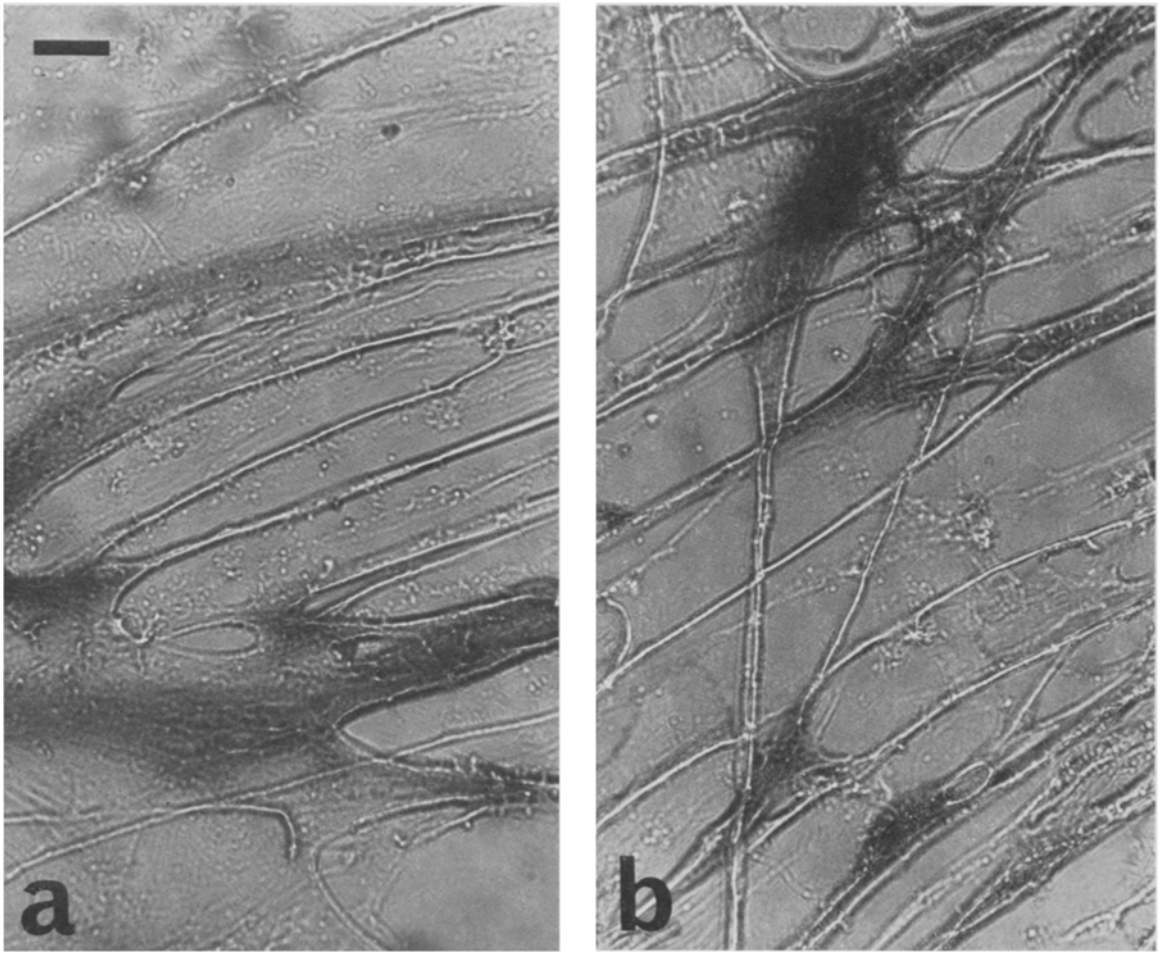

Fig. 3. 8-day muscle cultures stained for cholinesterase activity. Stain shows as black. a) Control culture. b) Culture grown in α-bungarotoxin (1 μg/ml) Calibration: 50 μm.

and MARSHALL[6]. Test cultures were incubated continuously in medium containing 1 µg/ml α-bungarotoxin, a concentration known to cause irreversible blockade of cholinoreceptors in cultured skeletal muscle[6,7]. Parallel cultures without the toxin were used as controls. Cultures were examined at various stages up to 12 days in vitro. Morphology was observed by phase contrast microscopy; resting membrane potentials and acetylcholine sensitivity were measured by standard electrophysiological techniques; and cholinesterase activity was monitored by a modification of the histological staining method of KOELLE and FRIEDENWALD[8] using acetylthiocholine as substrate.

During the first 2 days development proceeded normally, myoblasts multiplied and fusion began on the second day. At 3 days there was no obvious difference between control and α-bungarotoxin treated cultures (Figure 1, a and b). Fusion of myoblasts and formation of myotubes was unimpaired by the presence of the toxin. Subsequent observation showed that the continued morphological development of muscle fibres in culture was not affected by the toxin.

Resting membrane potential recordings made on myotubes at various stages of development showed the expected rise in mean resting potential in both control and toxin grown cells. There was no significant difference between the pairs of measurements (Table).

The control muscle fibres responded at all stages of development to iontophoretically applied acetylcholine, whereas fibres grown in the presence of α-bungarotoxin were not depolarized by acetylcholine even when the iontophoresis current was increased by a factor of 10 (Figure 2). 5 min exposure to 10^{-3} *M* acetylcholine did not produce any depolarization in a 10-day-culture grown in 1 µg/ml α-bungarotoxin. The absence of response in the α-bungarotoxin treated fibres indicates that the toxin retains its blocking activity throughout the incubation period. It has been shown by others that in cultured muscle receptor synthesis occurs in the presence of α-bungarotoxin and that such newly synthesized receptors are blocked as they occur[9].

The intensity and distribution of the cholinesterase stain in toxin treated fibres was similar to controls at all stages (Figure 3), although at 8 days some of the fibres grown in α-bungarotoxin had more distinctly localized regions of high enzyme activity than did the control fibres. At 10 days localization of enzyme was the same in both control and α-bungarotoxin treated cultures although the intensity of staining in both sets of cultures diminished. Localized areas of cholinesterase activity found in aneural muscle cultures may represent regions which would form motor endplates if innervation were to take place[10].

It appears, therefore, that although the rate of receptor incorporation into cultured skeletal muscle is unaffected by α-bungarotoxin[9], the irreversible blockade of these receptors has little effect on the development of the fibre itself, its resting membrane potential, or its content of cholinesterase.

In contrast to our findings in culture, blockade of cholinoreceptors in developing chick embryos resulted in pronounced dystrophic effects[11-13]. It is possible that in the whole embryo the acetylcholine receptor plays a role in the formation of neuromuscular junctions[10], and that the blockade of the receptor interrupts the functional relationship between nerve and muscle which seems essential for normal development of embryonic muscle in situ. However, in a nerve-free culture system, we have shown that, despite irreversible blockade of acetylcholine receptors, early muscle development continues normally. Our results indicate that myogenesis in culture is not dependent on the presence of functional acetylcholine receptors.

Resting membrane potentials in control and α-bungarotoxin treated cultures

Age of cultures (days)	Resting potentials in control cultures (-mV)	Resting potentials in α-bungarotoxin (-mV)
3	15.3 ± 2.1 (6)	15.1 ± 1.1 (15)[a]
6	28.3 ± 1.3 (19)	30.3 ± 2.1 (14)[a]
10	31.9 ± 1.3 (25)	28.1 ± 0.9 (59)[a]
12	25.7 ± 2.9 (15)	22.2 ± 1.8 (20)[a]

Values are mean ± S.E.M. of the number of fibres shown in parentheses. [a] Difference between control and toxin values not significant at $P > 0.025$, Student's *t*-test.

Résumé. Nous avons étudié le rôle du récepteur cholinergique dans la différentiation du muscle de squelette en culture. En présence continuelle de la α-bungarotoxine, les cellules n'ont pas réagi à l'acétylcholine, mais la myogénèse et l'acétylcholinestérase ont été normales. Nous concluons que la myogénèse en culture ne dépend pas de la présence des récepteurs cholinergiques fonctionnels.

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[6] W. F. DRYDEN, A. L. HARVEY and I. G. MARSHALL, Europ. J. Pharmac., in press (1974).
[7] A. L. HARVEY, W. F. DRYDEN and I. G. MARSHALL, J. Pharm. Pharmac. *25*, Suppl., 126P (1973).
[8] G. B. KOELLE and J. S. FRIEDENWALD, Proc. Soc. exp. Biol. Med. *70*, 617 (1949).
[9] H. C. HARTZELL and D. M. FAMBROUGH, Devl. Biol. *30*, 153 (1973).
[10] A. J. SYTKOWSKI, Z. VOGEL and M. W. NIRENBERG, Proc. natn. Acad. Sci., USA *70*, 270 (1973).
[11] Y. Y. AHMED, Anat. Rec. *155*, 133 (1966).
[12] D. B. DRACHMAN, Archs Neurol., Chicago *17*, 206 (1967).
[13] G. GIACOBINI, G. FILOGAMO, M. WEBER, P. BOQUET and J. P. CHANGEUX, Proc. natn. Acad. Sci. ,USA *70*, 1708 (1973).
[14] We gratefully acknowledge financial support from the Medical Research Council (to A.L.H.) and from the Scottish Home and Health Department. (to W.F.D.). We thank Mrs. B. HAMILTON for skilled technical assistance.

Inhibition of Metamorphosis by Juvenoids in *Nauphoeta cinerea* (Olivier)

It has been repeatedly proved that juvenoids or juvenile hormone analogues inhibit imaginal differentiation when applied before a critical period in insect metamorphosis. The present article examines the period of sensitivity to juvenoids in the cockroach *Nauphoeta cinerea* (Olivier) (Blattodea, Blaberidae) and compares activities of 18 selected juvenoids on this species.

The stock of *N. cinerea* was maintained at 25 °C and 45–50% r.h. on Larsen's diet and fresh carrots. Groups of 10 last instar nymphs of an age known within 2 days