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 $[\text{H}]$-arginine or $[\text{H}]$-lysine to have high concentrations of those amino acids. These findings are in agreement with previous studies which employed different embryos and developmental stages.

Both enucleated eggs and ovarian oocytes are also actively synthesizing histones (Table II). Previous studies on HeLa cells 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, 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 $18^\circ C$ the number of cells doubles approximately once every 2 h.

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

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Differentiation of Cultured Muscle in the Presence of $\alpha$-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. 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 $\alpha$-tubocurarine: no effect on development was seen during the first 48 h in vitro. Although $\alpha$-tubocurarine has a high affinity for the cholinoreceptor, its action is reversible and the receptor-antagonist complex is, therefore, in a
state of dynamic equilibrium. The snake venom component, \( \alpha \)-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 \( \alpha \)-bungarotoxin and various parameters were examined.

A single cell suspension of \( 2 \times 10^5 \) cells/ml was obtained by trypsin dissociation of the leg musculature of 10-11-day chick embryos and 2 ml added to 35 mm plastic petri dishes that had previously been coated with collagen. 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^{-6} M) in order to eliminate replicating cells such as fibroblasts. Thereafter the medium was changed every 3 days.

\( \alpha \)-bungarotoxin was obtained from the venom of \textit{Bungarus multicinctus} as described by Dryden, Harvey

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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 \( \alpha \)-bungarotoxin (1 \( \mu \)g/ml), despite 10-fold increase in iontophoretic current.

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