Dieses Verhalten wurde verständlich, als wir die «Scheibe» genauer untersuchten. Es zeigte sich, dass das, was man als «Imaginalscheibe» heraussezieren und transplantieren kann, aus zwei Zelltypen zusammengesetzt ist. Eine eigentliche Imaginalanlage steht dabei in engstem Kontakt mit grosskernigen Zellen, die leicht auseinanderfallen.

Zu unserer Überraschung stellten wir sodann fest, dass die Kerne dieser grossen Zellen polytäne Chromosomen enthalten. Im folgenden wird kurz über diesen Befund berichtet.

Wir verwendeten verpuppungsreife Larven des Wildstammes Sevelen von *Drosophila melanogaster*. Sie wurden mit Uhrmacherpinzetten in Holtfreter-Lösung seziert. Die mit Orcein-Essigsäure gefärbten Quetschpräparate untersuchten und photographierten wir mit dem Zeiss Ultraphot Phasenkontrastmikroskop.

Die Prothoracal-dorsal-Komplexe umgeben als rundliche Zellhaufen beiderseits die vordersten Abschnitte der Haupttracheenstämme unmittelbar hinter den fingerförmigen Spiracularpapillen. Sie liegen bei der intakten Larve in der Gegend des vierten Segmentes. Sie haften gut an den Tracheen, lassen sich daher mit diesen zusammen leicht vom Larvenkörper ablösen.

Figur 1 zeigt die Lage der locker gefügten Riesenzellen, deren Kerne die polytänen Chromosomen enthalten. Ihre Zahl variiert zwischen 30 und 50. Im vorderen Abschnitt sind sie von einem Kranz kleiner, undifferenzierter Zellen umgeben. Der ganze Komplex wird von einer Membran begrenzt.

Die kleinen Zellen stellen das eigentliche Humerusprimordium dar, dagegen sind die Riesenzellen rein larvale Bildungen, die in der Metamorphose zerfallen (LAMPRECHT[4]).

In Figur 2 ist ein Satz polytäner Chromosomen aus einem Riesenkern des Prothoracal-dorsal-Komplexes dargestellt. Der mittlere Durchmesser dieser Chromosomen beträgt 1,2 μ; das entspricht $^1/_2$ bis $^1/_3$ der Dicke von Speicheldrüsenchromosomen aus 96 h alten Larven. Die Bänderstruktur und grosse Puffs (Pfeile) sind deutlich. Es ist jedoch nicht leicht, schöne Platten herzustellen, da die Chromosomen sehr flexibel sind und oft Knäuel bilden.

Summary. Polytene chromosomes have been found in a cell complex associated with the humerus imaginal disc of *Drosophila melanogaster*.

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Inhibition of DNA Synthesis and Cell Division by Actinomycin D[1,2]

Results from this laboratory[3,4] and from that of HARDING[5,6] have shown that when amphibian or mammalian lenses are either mechanically injured or are cultured in vitro, large numbers of epithelial cells are prompted to enter DNA synthesis followed by mitosis.

In lenses of the bullfrog, *R. catesbeiana*, maintained at 24 °C, DNA synthesis commences after a period of 2 days in culture. The cells which proliferate in vitro as well as those which do so in vivo after wounding, do not do so under normal in vivo conditions. Apparently injury and culture conditions are able to call into play the whole train of events which adumbrates mitosis. Though DNA synthesis is one of the most obvious of premitotic events one has to suppose that it, too, is preceded by significant cytochemical transformations. And, in fact, there are already several published reports which indicate that the synthesis of RNA is a prerequisite for both DNA replication and cell division in injured and cultured tissue[7–9]. The experiments reported herein suggest that this is also true for lens epithelial cells. Thus it will be shown that actinomycin D, which is a potent inhibitor of the DNA primed synthesis of RNA[10], is fully capable of suppressing both of the aforementioned hyperplastic reactions.

Experimental. Lenses of adult bullfrogs were either needle injured in vivo or isolated and cultured according to methods which have been fully described elsewhere[3,4]. In the culture experiments actinomycin D (kindly supplied to us by Dr. H. B. WOODRUFF, Merck Sharpe and Dohme, Rahway, New Jersey, USA) was used at a concentration of 10^{-1} μg/ml. In the in vivo studies the actinomycin was injected intraperitoneally at concentrations varying from 0.5–1.0 μg/g body weight. Such injections were administered once a day starting on the day during which the lenses were injured. Following experimental treatment the lenses were exposed to either tritiated thymidine (5 μc/ml, 6.0 C/mM) or tritiated uridine (5 μc/ml, 20 C/mM) for 2 h and 40 min respectively. Thereafter they were fixed in Carnoy's fluid and whole

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Table I. Effect of actinomycin D on the onset of DNA synthesis and mitosis in the epithelium of cultured lenses

	Thymidine uptake between 50 and 52 h					Mitosis at 94 h			
Exposure to actinomycin begun (hours post-isolation)	0	12	24	36	48	50	65	74	90
Controls	++	++	++	++	++	+	+	++	++
Experimentals	–	–	–	+	++	–	–	–	++

Experimental lenses were exposed to antibiotic for a 4 h period starting at the times indicated above. They were then returned to normal culture medium and fixed at either 52 or 90 h. Controls were treated like the experimentals but were not subjected to actinomycin.

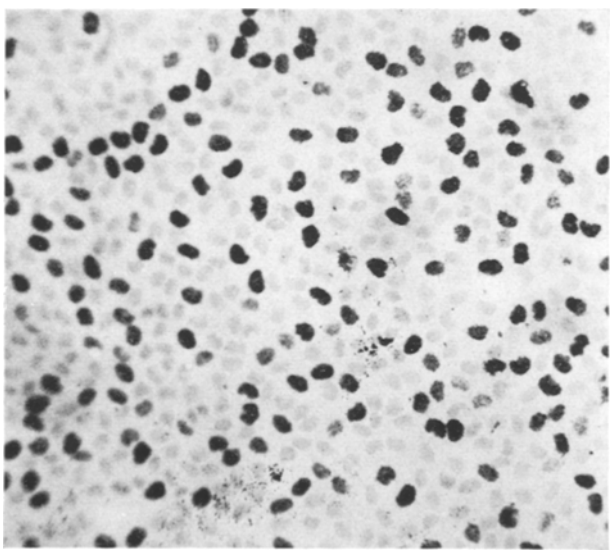
Table II. Effect of an initial exposure to actinomycin D upon uptake of H^3-uridine

Exposure to uridine begun (hours post-isolation)	4	7	24
Nuclear grain count, controls (range)	250–400	70–120	70–130
Nuclear grain count, experimentals (range)	50– 85	36– 65	30– 75

Experimental lenses were treated with actinomycin (10^{-1} µg/ml) for 4 h immediately after isolation. They were then incubated in H^3-uridine for 40 min starting at the times shown above. Controls were handled similarly except for antibiotic treatment.

mounts of the epithelium were prepared for autoradiography according to the technique of HARDING et al.[11]. The exposure time ranged between 7 and 12 days.

Results and Discussion. The Figure is an autoradiogram of a whole mount of epithelial cells from a lens which was cultured for 52 h and which was not exposed to actinomycin D. The large amount of DNA synthesis, as reflected by intense uptake of tritiated thymidine, is apparent. When lenses were exposed for 4 h to actinomycin D (10^{-1} µg/ml) between isolation time and 36 h afterwards, this reaction was almost totally suppressed (Table I). On the other hand, when they were subjected to the actinomycin after 36 h in culture, the DNA synthesis occurring between 50 and 52 h was either partially or totally uninhibited. It would thus appear that the RNA being made during the first day and a half after isolation is particularly important for the onset of DNA synthesis. When DNA synthesis was suppressed, so also was the mitosis which typically succeeds it. In fact, cell division was blocked even when actinomycin was supplied during or after the DNA synthetic period. The only period during which the drug could be added without subsequently checking mitotic activity was when that activity was actually in progress.



Autoradiogram of a whole mount of epithelial cells from a lens which had been cultured for 50 h, and which was then exposed to tritiated thymidine for 2 h and fixed.

That the experimental treatment described above neither killed the cells nor completely stopped RNA synthesis during the first day after exposure was proved through studies of the incorporation of H^3-uridine. The grain counts in Table II show that a 4 h treatment with actinomycin D, begun immediately after isolation, only partially inhibited the uptake of tritiated uridine at later times. Such uptake is DNAse resistent and occurs at times when thymidine uptake does not, hence it may be taken that it represents a true synthesis of RNA. Recent work by others indicates that the concentrations of actinomycin used here are far less than what is required to totally suppress RNA formation[12].

The data from the in vivo injury work, though not yet complete, are concordant with the above results. The normal response to trauma, which includes DNA synthesis and mitosis, is completely checked after treatment with actinomycin.

In broad outline our data agrees with that of other authors[7–9] in suggesting that cells must produce certain species of RNA in order to proliferate.

Zusammenfassung. Nach in-vitro-Kultur oder Verwundung in vivo der Froschlinsen wird zuerst meistens, im Epithel, eine intensive DNS-Synthese gefunden auf die später zahlreiche Kernteilungsfiguren folgen. Nach Behandlung mit Actinomycin D werden diese Reaktionen stark gehemmt. Autoradiographische Untersuchungen mit dem DNS-Vorläufer H-3-Thymidin und dem RNS-Vorläufer H-3 Uridin erlauben den Schluss, dass die DNS-Synthese (und damit Zellteilung) von einer früheren RNS-Synthese abhängt.

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