Table III. Effect of FUdR pretreatments on the antiviral action of IUdR

FUdR[a] 2.5 μg/ml	Compounds in the medium after infection (per ml)	Counts per min and viral yields after 30 h at 37 °C in			
		Sensitive virus infected cells		Resistant virus infected cells	
		CPM	IU	CPM	IU
−	H^3 thym. 0.3 μCi	2.8×10^5		5.2×10^4	
−	H^3 IUdR 1.0 μCi	6.9×10^4		1.4×10^4	
−	—		6.6×10^7		5×10^7
−	IUdR 30 μg		$< 10^4$		3.3×10^7
+	H^3 thym. 0.3 μCi	7.1×10^5		3.2×10^5	
+	H^3 IUdR 1.0 μCi	1.5×10^5		1.1×10^5	
+	—		3.3×10^7		1.6×10^7
+	Thym. 30 μg		1.2×10^8		8.3×10^7
+	IUdR 30 μg		$< 10^4$		8.3×10^4

[a] FUdR present in the culture medium from 2 h before infection up to the end of the infection period.

HEp2 cell monolayers (10^6 cells/small petri dish) were infected with 10 infectious units (IU) per cell. After 1 h at 4 °C, monolayers were washed 3 times in Eagle MEM (pH 7.3) and incubated at 37 °C in the same medium. Metabolites, inhibitors and labelled compounds were added as indicated in the Tables. To evaluate virus yield, cell monolayers were scraped from the glass in 2 ml of medium, sonicated for 90 sec in a 9 Kc Raytheon ultrasonorator and freed of cell debris at 5000 rpm for 10 min. Infectious units were titrated in tube cultures of HEp2 cells (6 tubes for each decimal dilution) according to the end point method. Intracellular uptake of labelled compounds was evaluated as described previously[8].

Results. Data in Table I show that the resistant variant of vaccinia virus develops in the presence of concentrations of IUdR and BUdR 10 times those inhibiting the growth of the sensitive strain. Since both thymidine analogues act by being incorporated in the viral genome in the place of DNA-thymine[9], the incorporation of H^3 thymidine and H^3 IUdR under acid-insoluble form in infected cells was measured. As shown in Table II, the uptake of both thymidine and IUdR is enhanced following infection with the sensitive virus, and is, on the contrary, deeply depressed following infection with the resistant virus. The incorporation of thymidine and its analogues in DNA is strictly related to the activity of thymidine kinase. Therefore it was considered of interest to verify whether the decreased incorporation observed after infection with the resistant variant might be due to an enhancement in the feed-back regulation operated by thymidine-5-trisphosphate (TTP) on the thymidine kinase activity[10]. It was thought that, if this were true, a FUdR-induced exhaustion of the intracellular pool of TTP would increase IUdR incorporation in DNA, thus restoring the sensitivity to IUdR in the resistant strain. To test this hypothesis, cell cultures were maintained under FUdR treatment from 2 h before infection up to the end of the infection period and then incubated in a fresh medium containing either labelled or unlabelled thymidine and IUdR. Data in Table III show that, following FUdR pretreatment of cell cultures, high amounts of thymidine and IUdR are incorporated in cells infected with either viruses, and that, under these conditions, the growth of both viruses is stimulated by thymidine and inhibited by IUdR.

These results suggest that the IUdR-resistance of the vaccinia strain under study depends on a decreased incorporation of IUdR in viral DNA, due to an enhancement of the feedback inhibition of the thymidine kinase activity.

Riassunto. La IUdR resistenza che alcuni DNA virus manifestano in cellule dotate di timidino-kinasi può essere dovuta ad una accentuata retroinibizione di questo enzima ad opera di TTP.

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PRO EXPERIMENTIS

A New Vital Stain in *Drosophila*

Vital stains provide a valuable method for determining intra- and intercellular localizations of molecules and organelles in the living organism. In the course of screening *Drosophila* with compounds which might affect proline metabolism[1] we discovered that 2,2'-dipyridyl was a vital stain. We report here the age dependent and tissue specific staining properties of this compound in *Drosophila melanogaster*.

Drosophila larvae (Oregon R strain) were removed from cream of wheat media at various ages and placed

in petri dishes containing filter paper or paper pulp (Whatman CF 11) moistened with a colorless aqueous solution of saturated or 1/10 saturated 2,2'-dipyridyl (DIP). All observations were made with a Wild M-5 stereomicroscope. Dissections were performed in *Drosophila* ringers-solution. For in vitro studies Schneider's *Drosophila* media (Difco) diluted with aqueous DIP was employed.

Ingested solutions of DIP were found to produce specific pink localizations in larval tissues. Newly hatched and 2-day-old larvae placed on saturated solutions (1:200 DIP: distilled water) developed orange-pink Malpighian tubules in a few h and died. Often the color in the tubules could be seen to occur as granules.

A 1/10 saturated DIP solution (1:2000, DIP: distilled water) was found to reduce mortality in larvae fed at 2 and 3 days after hatching. Such larvae showed additional sites of staining when observed the day following feeding. Larvae fed at 2 days showed only slight or no staining within the proximal portion of the Malpighian tubules. The gut was tinged pink with heavier staining occurring in variable positions, the gut contents were deeply stained. In all larvae examined the proventriculus remained unstained. In addition to gut and Malpighian tubule structures, some of the imaginal discs and the brain were stained pink. The eye and antennal disc were often visibly pink as was the anterior cap on the male gonad (probably the apical cells). The female gonad was not identified in these small larvae. As with the Malpighian tubules colored granules could usually be detected as the source of color on the brain surface. Larvae fed at 3 days showed the same localization pattern as described with the exception that the ring gland stained much more deeply than the other structures and could be seen as a cherry red organ on a pink background even in the intact larva. At this time the discs were all distinctly pink so that the location of the wing, haltere and third leg discs were readily seen without dissection. The staining described was very distinct and by comparison there was no staining in salivary glands, fat body or muscle.

When larvae were fed DIP at 3 days pupation was somewhat delayed compared to water controls. Shortly after pupation larval type localizations were observed, however, by this time both anterior and posterior portions of gonads of both sexes were stained. It would appear that the stained tissue is the connective tissue of mesodermal origin which was stained in the apical region in 2-day larvae and which eventually enveloped the gonad[2].

As pupation proceeded the whole pupa took on a pink cast as more tissues became involved. Adults which ingested DIP as larvae retained this coloration. When normal adults from a dry culture were transferred to media moistened with DIP, ingestion occurred. Of particular interest was the preferential staining of testicular tips under such circumstances. This is compatible with CARSON's[3] demonstration of the persistence of apical cells in adult Diptera.

In vitro incubation studies were undertaken to determine if DIP combined first with substances in the hemolymph which were then picked up by cells or if DIP combined initially with intracellular molecules. For these studies organs from 3 day larvae were incubated at room temperature in Schneider's media diluted 1:1 with saturated DIP. In Malpighian tubules colored granules could be seen within 20 min suggesting that DIP reacted directed with cell components. Aside from the gut and its contents no staining was detected among other cultured organs (imaginal discs, brain, ring gland, fat body, salivary gland).

The nature of the cell component stained by DIP is of interest. As DIP is a chelating agent which specifically binds to ferrous ions producing a colored product it is most likely that the stained tissues contain a high concentration of ferrous ions either free or in bound form.

At the present time we can envision the use of DIP vital staining as a rapid method of screening for mutants which have small ring glands or which lack particular discs. As the nature of the stained molecules is elucidated, it may be possible in the future to detect more subtle alterations in the chemical composition of these organs.

Résumé. On décrit l'utilisation du 2,2'-dipyridyle comme colorant vital chez *Drosophila*. Si l'on en nourrit les formes larvaires. les disques imaginaux, la glande annulaire, les tubules de Malpighi, l'intestin et les pointes des gonades embryonnaires deviennent roses.

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Ultramikromethoden[1]. Darstellung von Diazomethan; Methylierung von Fettsäuren mittlerer Kettenlänge

Kleine Mengen Diazomethan, wie sie für die unten beschriebenen Methylierungsexperimente benötigt werden, lassen sich mit Hilfe der in Figur 1 skizzierten Vorrichtung erzeugen. In dem durchbohrten Korkzapfen A steht ein mit einem Stiel versehenes Glasröhrchen B, das als Stielröhrchen bezeichnet werden soll (Gesamtlänge 80 mm, ohne Stiel 20 mm, ä. ⌀ 7 mm). Das Glasröhrchen D (Länge 80 mm, ä. ⌀ 3,3 mm, i. ⌀ 1.8 mm) führt durch einen Polypropylenbecher E (Zentrifugenbecher, auf 40 mm gekürzt, ⌀ 35 mm) und eine Silicongummimembran C (GC-Einspritzmembran). Der Becher E wird an einem Stativ befestigt. Man füllt ihn mit Eis und gibt nacheinander 1 mg fein pulverisiertes N-Methyl-N-nitroso-p-toluolsulfonamid, 1 Tropfen Aethanol und 1 Tropfen 40% (G/G) KOH in das Stielröhrchen B. In das inzwischen vorgekühlte Glasröhrchen D lässt man eine 3–4 mm hohe Säule Äther aufsteigen und hebt anschliessend das Stielröhrchen B so weit an, bis seine Öffnung durch die Gummimembran C verschlossen wird. Das entstehende Diazomethan löst sich in dem im Röhrchen D befindlichen