Whatmann No. 1 filter paper, without any treatment. The paper was developed in the upper layer of n-Butanol: Acetic acid: Water (4:1:5)[2] for 96 h without prior saturation of the chromatographic chamber. Pure solutions of known sugars were run parallel to the unknown ones for proper identification. Sugar spots were revealed by staining the dried developed chromatogram with silver nitrate reagent[3].

Results. A comparison of the unkown spots with that of the known ones revealed the unusual presence of the trisaccharide, raffinose, in the extracts of eggs, larvae, pupae and newly-emerged flies, and in the haemolymph of adult *S. calcitrans*. However, this sugar could not be detected in the anal fluid.

In comparison to the other sugars found in the various stages of this fly (unpublished), raffinose was in moderate concentration in the larvae and newly-emerged flies, in low concentration in the eggs and white pupae, and only in traces in the haemolymph.

Discussion. This study on the stable fly *S. calcitrans* has shown, for the first time, the presence of raffinose in an insect. Recent studies of McLeod[4] and Palmer[5] have shown that the trisaccharide, raffinose forms a substantial part of carbohydrate reserve of the embryo of cereal grains and that it is utilized during the early stages of germination and growth. Their findings lead us to presume that raffinose present in the freshly-laid eggs of *S. calcitrans*, is probably utilized during its embryogenesis.

The presence of raffinose in the larvae of *S. calcitrans*, in higher concentration than that in its eggs, leads us to believe that this sugar is procured afresh from the food, probably because of its nutritional value. This finds support from the recent studies of Pradhan et al.[6] who have reported that raffinose is significantly superior to several other carbohydrates, for the growth and development of the larvae of *Chilo zonellus*.

Résumé. La raffinose a été découverte pour la première fois dans l'insecte *S. calcitrans*, une mouche suceuse de sang. Le sucre est présent dans les œufs fraîchement pondus, dans les larves au troisième stage, dans les pupes blanches et dans les toutes jeunes mouches. On a aussi discuté le róle probable de ce sucre.

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5-Iodo-2-Deoxyuridine Resistance of Vaccinia Viruses in Cells Endowed with Thymidine Kinase Activity

5-iodo-2-deoxyuridine (IUdR) and 5-bromo-2-deoxyuridine (BUdR) inhibit the growth of DNA viruses by being incorporated in the viral genome[1]. For this, IUdR and BUdR must be previously phosphorilated by thymidine – and thymidilate kinases[2]. This explains why variants of Herpes and Pox virus which do not induce thymidine kinase are not inhibited by IUdR and BUdR in cells lacking this enzyme[3,4]. However, strains of Herpes and Pox virus have been obtained which develop in the presence of IUdR and BUdR in cells endowed with thymidine[5,6] kinase activity. The research referred to here was carried out to clarify the mechanism of this resistance.

Materials and methods. 5 fluoro-2-deoxyuridine (FUdR) (Calbiochem); IUdR and BUdR (K and K Labs. Plainview, USA); thymidine (Merck); ^{3}H thymidine (methyl T, 26 Ci/m*M*) and ^{3}H IUdR (3,8 Ci/m*M*) (Amersham). The experiments were carried out by using Genetron-treated, sucrose gradient purified pools of a vaccinia virus strain (I.S.M., Milan) and one of its IUdR-resistant variants[7].

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Table I. Effect of IUdR on viral growth

IUdR[a] in the medium	Infectious units produced after 30 h at 37 °C	
(μg/ml)	Sensitive strain	Resistant strain
—	8.3×10^7	5.0×10^7
6.25	1.8×10^5	1.6×10^7
12.5	7.5×10^4	3.3×10^7
25	$< 10^4$	1.6×10^7
50	$< 10^4$	1.6×10^7
100	$< 10^4$	3.3×10^6
200	$< 10^4$	1.6×10^5

[a] Added to the cell cultures after the infection period

Table II. Effect of virus infection on the incorporation of H^3 thymidine and H^3 IUdR in DNA

Labelled compound in the medium[a]	Counts per min incorporated under acid insoluble form after 30 h at 37 °C in		
(μCi/ml)	Uninfected cells	Sensitive virus infected cells	Resistant virus infected cells
H^3 thymidine 0.3	8.5×10^4	2.0×10^5	2.8×10^4
H^3 IUdR 1.0	3.8×10^4	7.6×10^4	1.1×10^4

[a] Added to the cells soon after the end of the infectious period.

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