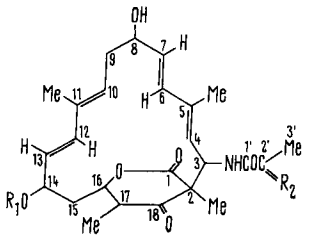
The interconversion between (III) and (IV) was the same as that between (I) and (II). (III) was deacetylated to (IV)[7] by the enzyme preparation prepared from the streptomyces, and (IV) was acetylated to (III)[7] in the presence of ethyl acetate and the enzyme.

When (II) was administered intravenously[4] to a rabbit, (IV)[7] was isolated from the bile and urine. This implies that the carbonyl group at $C_{2'}$ was reduced selectively by an enzyme in rabbits. After oral administration of (I) to rats[4] (II) and (IV) were detected in the urine and the plasma. An active fruction for the dehydrogenation of (IV) to (II)[7] was obtained from the crude enzyme solution of the streptomyces by 0.26 to 0.53 saturation of $(NH_4)_2SO_4$. The activity of enzyme is optimal at pH 3. The rate of dehydrogenation with this enzyme was increased approximately 5-fold by an external addition of $10^{-3}M$ Hg^{++} ion.



	R_1	R_2
T-2636-A(I)	-Ac	=O
T-2636-C(II)	-H	=O
T-2636-D(III)	-Ac	$-H_r$-OH
T-2636-F(IV)	-H	$-H_r$-OH

Among 3 hydroxyl group at C_8, C_{14} and $C_{2'}$ in (IV), only the hydroxyl group at $C_{2'}$ was dehydrogenated to carbonyl group. (I)[7] was detected when (III) was incubated at pH 2 with the enzyme.

From these findings, it is obvious that the culture broth of *S. rochei* var. *volubilis* contains enzymes which show deacetylation, acylation and dehydrogenation activity. The enzymes from *Aspergillus* and *Trametes* also catalyze deacetylation reversibly.

Zusammenfassung. Mit angereicherten Enzympräparaten von *Streptomyces rochei* var. *volbulis* sowie einigen Pilzen liessen sich die Antibiotica T-2636 A (I) und D (III) zu C (II) beziehungsweise F (IV) reversibel desacetylieren. Die Antibiotica (III) und (IV) wurden auch mit dem Enzym *S. rochei* var. *volubilis* dehydriert.

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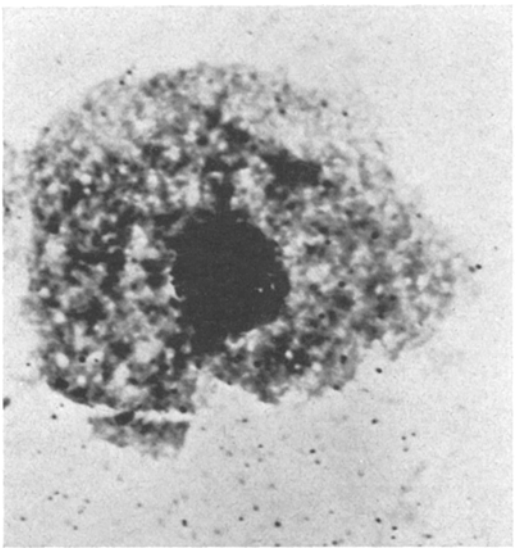
Incorporation of Macromolecules into the Salivary Cells of *Dipteran* Larvae

Since the observations of Avery, MacLeod and McCarthy[1] on the transfer of genetic information in *Pneumococcus* after treatment with DNA, others have extended their investigations to various micro-organisms and mammalian cells. In higher organisms, one would expect that the foreign DNA, after it has passed through several membranes and been transported through blood or plant sap, will be destroyed by the naturally occurring nucleases[2]. However, Gartler[3,4] found that a small amount of intact DNA is incorporated into host's (human cells) DNA, the major part being degraded by the cells.



Autoradiography of a section through a salivary gland cell after injection of foreign H^3-DNA.

Hill[5,6] has also observed a DNA uptake in bone marrow, thymus and spleen cells. A mutagenic action of foreign DNA has been claimed by Fahmy and Fahmy[7] in *Drosophila*.

Materials and methods. *Drosophila melanogaster* and *Chironomus thummi* larvae were used in the present studies. C^{14}-RNA of rat liver (300 µg/ml, specific activity 200,000 dpm/µg) and H^3-DNA of *Escherichia coli* (425 µg/ml, specific activity 1,000,000 dpm/µg) were administered into the larval body by means of microinjections. The amount of radioactive substances introduced into the larval body was of the order of 1.5 µl.

The salivary glands were dissected at certain time intervals after the injections. The glands were either squashed or sectioned after fixation and embedding. The incorporation of the radioactive molecules was followed by autoradiography[8].

Results and discussion. One hour after microinjection, the injected H^3-DNA is found in the cytoplasm. Later on, it enters the nuclei of the salvary glands. The greatest activity in the nuclei is observed 4–5 h after microinjection. Most of the radioactivity is localized on the DNA bands of the giant chromosomes of *Drosophila* and *Chironomus* (Figure). A DNAase treatment removes most of the radioactivity.

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C^{14}-RNA is mostly incorporated into the cytoplasm after 1 h following the injection and, to a very small extent, into the nucleus and the chromosomal regions. After 6 h, the activity has increased in cytoplasm and also the nucleolus. Many observations suggest that the ingested foreign DNA becomes part of the nuclear chromatin[9,10].

In our experiments, the labelling, after H^3-DNA injection, is found in the cytoplasm; later on, it accumulates in the nucleus, on the polytene chromosomes. KONG and FICQ[11] observed that after injection of phage C^{14}-DNA into newts, radioactivity can be found in the lampbrush chromosomes of the oocytes. It is reported that when H^3-DNA (from *E. coli*) is introduced into the blastomeres of *Pleurodeles* embryos, radioactivity is soon found in the nuclei and can later be distributed between the daughter nuclei[12].

The incorporation of macrophage RNA into lymph nodes has been studied by FISHMAN et al.[13]. LACOUR et al.[14] have observed that Ehrlich ascites tumor cells absorb large amounts of exogenous RNA without apparent destruction. Our autoradiographic analyses show that rat liver C^{14}-RNA is mainly found in the cytoplasm and in the nucleoli of the salivary gland cells of *Dipteran* larvae.

Zusammenfassung. Autoradiographisch wurde festgestellt, dass sich injizierte DNS mit der DNS der polytonen Chromosomen verbindet und in die Zellkerne der Speicheldrüse eingebaut wird.

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Presence and Adaptive Changes of Citrate Cleavage Enzyme in the Yeast *Rhodotorula gracilis*[1]

Citrate cleavage enzyme[2] (ATP citrate lyase: E.C. 4.1.3.8) is widely assumed to be a first regulator enzyme of the fat storage sequence from citrate in animal tissues, where its level depends on the nutritional state of the organism[3]. This communication deals with the presence of a citrate cleavage enzyme also in a yeast species and with its long-term changes in response to different external carbon sources. The yeast *Rhodotorula gracilis* is an obligate aerobe fat-storing organism, which in adequate conditions can develop a true 'obesity'[4]. In the environmental conditions of fat storage a high level of citrate cleavage enzyme has been observed, and this suggests an interpretation of function and regulation of this enzyme in some yeast cells analogous to that proposed for animal cells.

Experimental. The organism used was a strain *Pan* of *Rhodotorula gracilis*, obtained from the Istituto di Microbiologia Agraria of the University of Milan. From the same collection were also obtained other yeast strains of *Saccharomyces cerevisiae* and *Candida utilis*. Yeasts were cultivated in a liquid synthetic medium having the following composition, per liter: $(NH_4)_2SO_4$, 15 mmoles; K_2HPO_4, 5.7 mmoles; NaCl, 8.6 mmoles; $MgSO_4$, 4 mmoles; $CaCl_2$, 2.3 mmoles; $FeCl_3$, 0.018 mmoles; Ca pantothenate, 5 mg; thiamine-HCl, 5 mg; glucose or other carbon sources, as indicated in the Tables and in the Figure; final pH was 4.5. Such a medium, rich in sugar, was described by LUNDIN[5] as particularly suitable to obtain a 'fat-yeast': extensive fat depots are microscopically visible in *Rhodotorula gracilis* cells after cultivation under these conditions in presence of 20–200 m*M* glucose.

Growth took place in well aerated flasks with eccentric agitation, at 30 °C. For the larger cultures a New Brunswick Microferm Fermentor was used.

For enzyme determinations cells were collected by centrifugation and disrupted in a Braun glass-beads homogenizer (5 g of beads per g of yeast; 30 sec at 70 rev/sec) using as suspending medium 100 m*M* potassium phosphate + 10 m*M* 2-mercaptoethanol + 10 m*M* $MgCl_2$, at pH 7.0. The suspension was held 30 min at 4 °C with stirring and then centrifuged at 20,000 *g* for 30 min to obtain a clear extract.

A partial purification of citrate cleavage enzyme was achieved by precipitation with $(NH_4)_2SO_4$ (fraction 0.7–1.6*M*, pH 6.8) and passage through a Sepharose 4B column. Fractions after gel filtration step contained minimal or no malate dehydrogenase activity. Stability of enzyme preparations depends very strongly on pH, optimal conservation being obtained at pH's between 6.5 and 7.0.

Identification and activity measurements of citrate cleavage enzyme were carried out in reaction mixtures having the following basic composition: 200 m*M* Tris-HCl buffer, 10 m*M* $MgSO_4$, 10 m*M* glutathione-SH, 10 m*M* sodium citrate, 0.2 m*M* CoA-SH, 5 m*M* ATP, 0.2 m*M* NADH, 3 IU cryst. malate dehydrogenase, 20–50 µl/ml enzyme preparation. Oxalacetate formation was followed by continuous monitoring of optical extinction change at 366 nm in an Eppendorf recording photometer, thermoregulated at 30 °C. Routine tests were carried out at pH 8.4 and started with ATP.

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