have been brought into an aqueous hydrophilic environment, all the fluorescence spectra of proteins become the same (maxima at 350 nm)[3]. As tryptic digestion causes a similar change in the micro-environment of tryptophan residues, probably this change is responsible for the observed long-wave shift of the fluorescence spectrum. The decrease of the fluorescence intensity shown in Figure 2 may also be due to this change, since the quantum yield of phosphorylase b is reduced by urea. A disturbance in the energy of migration may also play a role.

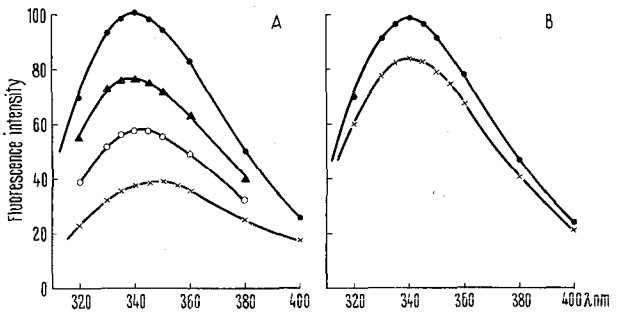


Fig. 2. Changes in the fluorescence spectrum of phosphorylase b during tryptic digestion. (A) Without Glc-6-P; (B) with 3.6 m*M* Glc-6-P. The spectra were determined after 0-min (●—●), 10-min (▲—▲), 20-min (○—○) and 50-min (×—×) incubation. The fluorescence was excited at 290 nm.

As is known, phosphorylase b is inactive in the absence of AMP. Earlier data indicate an effect of AMP on the conformation of the enzyme[1,4,5]. The demonstrated protective effect of ATP[1], glucose and Glc-6-P against tryptic digestion strongly suggest that allosteric inhibitors do not favour the native inactive state of the enzyme, but, like AMP, cause conformational changes in phosphorylase b. Since substrates tested by us did not influence the tryptic digestibility of phosphorylase b, the protective effects demonstrated seem to be specific of allosteric transitions.

*Zusammenfassung.* Glukose und Glc-6-P hemmen die Trypsinhydrolyse von Phosphorylase b konzentrationsabhängig, was darauf hinweist, dass die allosterischen Inhibitoren von Phosphorylase b die native Konfiguration des Enzyms verändern.

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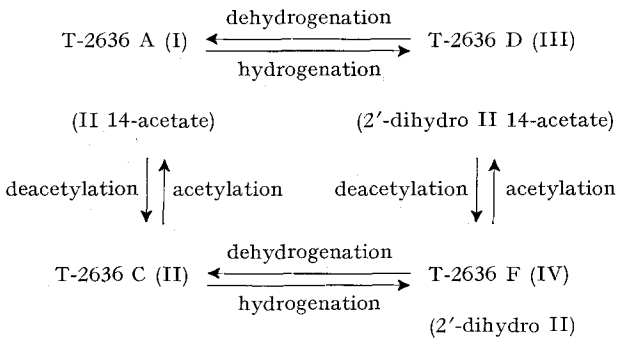
## Interconversion of T-2636 Antibiotics Produced by *Streptomyces rochei* var. *volubilis*

In a previous paper[1,2], we reported the isolation and structures of T-2636 antibiotics produced by *Streptomyces rochei* var. *volubilis*[3]. The structural relations of these antibiotics are as follows.




In this paper we describe the enzymatic interconversion of these 4 antibiotics. The enzymatic deacetylation occurred when (I) was treated with rat liver homogenate[4] or the enzymes from the streptomyces, *Aspergillus sojae*[5], *Asp. niger* and *Trametes sanguinea*[6]. The acetyl group at $C_{14}$ of (I) was deacetylated enzymatically[7].

The activity of the enzyme obtained from the fermented broth of the streptomyces by fractional precipitation with ethanol (30–60%) followed by chromatography on DEAE cellulose was remarkably much stronger than other enzymes when (I) was used as substrate.

The activity of the enzyme is optimal at pH 7 and at 40 °C, and can be kept stable in an aqueous solution at 33 °C for 30 min in the range of pH 4.0 to 9.0, but treatment of the enzyme at pH 3 and pH 10 under similar conditions leads to 25% and 85% decrease of the activity, respectively. By heating the solution at 80 °C for 10 min at pH 7, 75% inactivation is observed. The activity is inhibited (60%) by $10^{-3}M$ of $NaAsO_2$.

The enzymatic reaction is reversible as well. When ethyl acetate, ethyl formate and ethyl propionate were used as acyl donor, (II) was converted by the enzyme to (I)[7], (II) 14-formate $C_{26}H_{33}NO_8$, mp 175–177 °C (dec.), $[\alpha]_D^{22} - 259$ (c = 1.0, MeOH), UV: $\lambda_{max}^{EtOH}$ 227 nm ($\varepsilon$ = 49,200) and (II) 14-propionate, respectively. The acyl groups introduced at $C_{14}$ were determined by the NMR- and IR-spectra.

Deacetylation of (II) 8,14-diacetate and (II) 8,14-dipropionate by the enzyme gave the corresponding 8-acetate and 8-propionate in good yield, respectively. The enzyme deacylates the acyl groups at $C_{14}$ of the substrates selectively.

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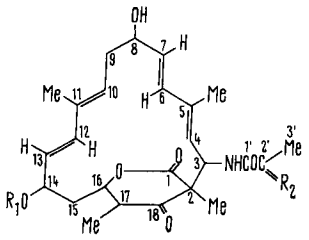
[7] The compounds (I), (II), (III) and (IV), obtained by enzymatic reaction, were identified with authentic samples by the comparison of their IR- and UV-spectra, mp, Rf value in TLC and antimicrobial activities.

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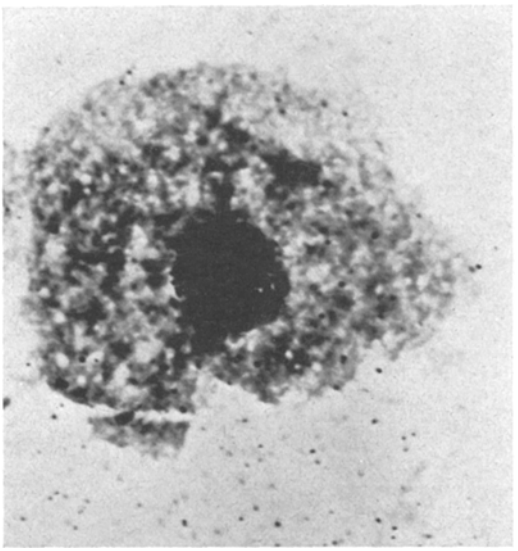
## Incorporation of Macromolecules into the Salivary Cells of *Dipteran* Larvae

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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