ties are that: (a) reaction can occur at many sites in the enzyme without causing inactivation; or (b) repair can occur after an initial attack by radical, restoring the enzyme to its pristine condition[7].

The dose-modifying effect $F$ of added substances is defined as follows: $F = D_{37}^{m}/D_{37}^{c} = K^{c}/K^{m}$, in which the superscripts $m$ and $c$ denote respectively the values found in the presence of modifier and for the control. $E$ was $5 \times 10^{-6} M$ and the modifier was in 50-fold greater molar concentration, unless otherwise specified. For cysteamine, $F$ was 6.4, for cysteine 5.9, i.e. these substances had a pronounced protective effect. For the derivative disulfide cystamine $F$ was 3.3, for cystine 3.9; i.e. these substances were somewhat less effective, distinctly so, if compared on the basis of weight. Experiments were done with cystine-$S^{35}$ to determine the mechanism of protection[7]: the enzyme and cystine were mixed, allowed to react for some time, then separated by chromatography on Sephadex-G25. No appreciable radioactivity was incorporated into the protein in 6 h, showing that the protection realized in the present conditions was not due to formation of a mixed-disulfide product[6]; it might be due to radical-scavenging and/or to repair by the sulfur compound.

The inactivation yield was little affected by added sodium iodide at 1:50 molar ratio, but at 1:600 ratio $F$ increased to 6.4. Presumably the protection is due to competition that converts a reactive radical to a less damaging one, e.g., OH· to I·. Since iodide is a sensitizer in vivo, at least in many cases[11], the result indicates that sensitizing action cannot be directly related to some simple effect at the molecular level. $F$ for iodoacetic acid was 1.5, for 3-iodopropionic acid 1.6. Of some interest is the fact that ethyl methanesulfonate, a mutagenic and radiomimetic agent, also had little effect: $F$ was 1.0 at 50:1 ratio and only 1.1 even at very high concentration, 8000:1.

The behavior of aldolase with respect to protection and sensitization is in marked contrast to that of alcohol dehydrogenase, although this has a very similar inactivation yield[7]. Although the data so far available are not sufficient to sustain any generalization, it should be anticipated that protective and sensitizing effects may be more specific than had been suspected hitherto[12].

*Riassunto.* È stata studiata in vitro l'inattivazione da raggi x dell'aldolasi dal muscolo di coniglio. Il rendimento di inattivazione corrisponde a 0,10 (molecule/100 ev) per concentrazioni comprese tra $10^{-5}$ and $10^{-6} M$ in tampone di fosfati 0,05$M$, pH 7. Il rendimento non subisce modificazioni di rilievo variando il pH da 6 a 8 o sostituendo al tampone l'acqua bidistillata. Il rendimento é ridotto da 4 a 6 volte in presenza di cisteamina, di cisteina o dei corrispondenti disolfuri in concentrazioni corrispondenti a 50 molecule per molecola di enzima. Coll'ioduro di sodio in concentrazione di 600:1 si ottiene una riduzione di 6 volte.

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## Mannosidohydroxystreptomycin from *Streptomyces* sp.

Although the formation of mannosidostreptomycin together with streptomycin in the fermentation broths of *S. griseus* is a well-known phenomenon[1], a mannosido derivative of hydroxystreptomycin has never been described. We wish to report the isolation of mannosidohydroxystreptomycin together with hydroxystreptomycin, streptidine and toyokamycin from the cultures of *Streptomyces 86*[2].

The filtered broth (20 l) was worked up by standard absorption-elution procedures on cation exchange resins to give a mixture of sulphates which were separated by chromatography on a column of activated charcoal and filter-aid. The elution was carried out with distilled water followed by 0.1$N$ sulphuric acid in 50% aqueous methanol. The yields were the following: 1.6 g of streptidine sulphate, identified by direct comparison with an authentic specimen prepared from streptomycin[3], 5.8 g of hydroxystreptomycin sulphate, identical in all respects to an authentic sample[4], 6.4 g of sulphate of the new antibiotic. The latter was further purified through the crystalline $\beta$-naphthalenesulfonate, $C_{27}H_{49}O_{18}N_7 \cdot 3\,(C_{10}H_8O_3S) \cdot 3\,H_2O$, m.p. 182–184°, and isolated as the hydrochloride $C_{27}H_{49}O_{18}N_7 \cdot 3\,HCl \cdot {}^3/_2\,H_2O$, $[\alpha]_D^{23} -55°$ ($H_2O$), $-49°$ (methanol). Hydrogenation of this product (Pt catalyst) gave a dihydro derivative $C_{27}H_{51}O_{18}N_7 \cdot 3\,HCl \cdot 3\,H_2O$, $[\alpha]_D^{23} -62°$ ($H_2O$).

Paper chromatography of the hydrolyzate obtained by treatment of the antibiotic with $N$ aqueous sulphuric acid on the boiling water bath showed spots corresponding to streptidine, $N$-methylglucosamine and mannose. Treatment of the dihydro derivative of the antibiotic with 1.6$N$ hydrogen chloride in methanol gave, after 130 h at room temperature, streptidine, α-methyldihydrohydroxystreptobiosaminide (isolated as the exaacetate which was identical in all respects with the same product obtained from

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dihydrohydroxystreptomycin[5]), and 2 additional compounds behaving on paper chromatograms as the anomeric α- and β-methylmannopyranosides. The presence of the hydroxystreptose moiety was further confirmed by the formation of 2-hydroxymethyl 3-hydroxy-γ-pyrone on treatment of the antibiotic with *N* sodium hydroxide on the boiling water bath, as already known for hydroxystreptomycin[6,7].

The presence of the D-mannose moiety was definitely established by the preparation of this hexose, m.p. 130° dec., $[\alpha]_D^{23} + 13°$ (at equilibrium, $H_2O$), on hydrolysis of the antibiotic with 0.05 *N* aqueous hydrogen chloride for 20 h at 100° in the presence of a sulphonic exchange resin, followed by chromatography on a column of a mixture of charcoal and filter-aid. The phenylosazone of the hexose, m.p. 198–200°, was identical with that prepared from an authentic sample of D-mannose by mixed m.p., UV- and IR-spectra. The similarity of the linkage of D-mannose to hydroxystreptomycin with that present in mannosidostreptomycin is shown by the easy enzimic splitting of the 2 moieties on incubation of the antibiotic with the mannosidase preparation of *S. griseus*[8] in pH 6.5 phosphate buffer at 28°.

The new antibiotic mannosidohydroxystreptomycin and its dihydro derivative show an antimicrobial activity in vitro qualitatively similar to that displayed by streptomycin and by hydroxystreptomycin; they are however quantitatively less active on a weight basis.

In addition to the said compounds the microorganism produces a quite different antibiotic substance, $C_{12}H_{13}O_4N_5$, $[\alpha]_D^{23} - 43°$ (0.1 *N* HCl), which was easily recovered from the mycelium (yield 60 mg/l), and which was identified with the nucleoside-type antibiotic toyokamycin[9,10] on the basis of its elemental composition and physicochemical properties.

All compounds whose isolation is described in this communication gave correct elemental analyses and showed satisfactory spectroscopic properties.

*Riassunto.* Viene descritto l'isolamento e lo studio chimico della mannosidoossistreptomicina, presente, insieme con ossistreptomicina, streptidina e toiocamicina, nelle colture dello *Streptomyces 86*. Il nuovo antibiotico ed il suo diidroderivato presentano una attività antibatterica paragonabile a quella di streptomicina e di ossistreptomicina.

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## Monoamines in the Glomus Pulmonale

Various authors describe the glomus tissue in the vicinity of the pulmonary artery and the ligamentum arteriosum (BARNARD[1], VERITY[2], KRAHL[3], BOYD[4], HEYERS[5]). None of those authors offers proof of the presence of chromaffine tissue in this organ. Examining the monoaminergic innervation of the ductus arteriosus[6], we have found clusters of small cells situated in the vicinity of the pulmonary artery and giving an intensive specific fluorescence. They may be regarded as the pulmonary glomus. We therefore began a close examination of this organ.

Six foetuses from 4 gravid guinea-pig females were examined with histochemical fluorescence technique according to FALCK[7]. The mature foetuses were taken out by means of the Caesarean section. The ductus arteriosus was dissected with a part of the pulmonary artery and of the aorta and quenched in propan at the temperature of liquid nitrogen. For 1 week lyophilization was performed at the temperature decreasing from − 60° to − 30 °C. Afterwards for 1 h the tissue was condensed with formaldehyde at 80 °C. The paraformaldehyde used in this reaction was standardized in the atmosphere with diluted sulphuric acid (1 part of sulphuric acid, 4 parts of water) according to HAMBERGER[8]. The 15 μ thick serial sections of paraffin blocks were mounted in liquid paraffin and examined with the fluorescence microscope (cardioid condenser, HBO 50 bulb, BG 12/4, OG 13/2 activating filters, OG 4/1 barrier filter).

After the photographic exposure in the fluorescence microscopy, some sections of the condensed tissue were stained with the haematoxylin-eosin or impregnated with protargol according to BODIAN[9,10]. The paraffin was removed with benzine, the section was rehydrated and mounted with albumen on the microscope slide and fixed with the BODIAN formol trichloracetic acid fixation for 10 h. The incubation in the copper protargol mixture was performed for 2 weeks at the temperature of 37°C[11]. The fine perivascular nerves were sufficiently impregnated in the sections with this method.

In all cases we have found clusters of cells giving an intensive specific fluorescence. The glomus cells were situated in the perivascular tissue of the angle between the pulmonary artery, ductus arteriosus and the aorta. In one case 2 glomuses were found surrounding the pulmonary artery. Similar solitary cells were dispersed in the vicinity

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