

Hyaluronate adsorbs water more than collagen, but the difference is not large. The effect of depolymerization is seen in the change of the constant C.

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## Purification and Properties of a Bacterial Glucose Dehydrogenase

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A bacterium possessing a glucose dehydrogenase was isolated from a secondary culture infection. It has provisionally been assigned to the genus *Alcaligenes*. 100 g quantities (dry wt.) of the organism could be grown in a simple 20 liter continuous culture apparatus in a normal work day. Resting cells oxidize glucose rapidly to the level of 1 atom oxygen consumed per molecule glucose, and from then on at a reduced rate. In this first period CO<sub>2</sub> evolution is slight. Cell-free extracts, prepared by grinding with Ballotini glass beads, also carry out oxidation of glucose with oxygen as acceptor. The primary dehydrogenation could be studied with 2,6-dichlorophenol indophenol as hydrogen acceptor. Using this assay method, the enzyme was purified 250 fold with protamine sulphate, ammonium sulphate, DEAE cellulose, and calcium phosphate gel. At this stage the enzyme catalyzes the reduction of 40 μmoles dye per min per mg protein.

Cytochrome c or DPN are not reduced at a significant rate, and reoxidation with oxygen is not measurable, unless phenazine methosulphate is also added. In the presence of phenazine methosulphate and oxygen, the immediate product of the reaction was shown to be gluconolactone. The absorption spectrum of the enzyme preparation shows the presence of a b-type hemoprotein, partially reducible by glucose. The spectrum also suggests the presence of functional flavin, and preliminary inhibition and fluorescence data support this evidence. This soluble dehydrogenase appears to represent a fragment of the particulate glucose oxidation system found in the cells and the extract.

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## Collagen Fractions in Experimental Lathyrism

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The collagen fractions of pooled skins of lathyrictic and control rats were obtained by a modified procedure by Harkness *et al.*<sup>1</sup>. In the disodium phosphate-soluble fractions the collagen was estimated from the content of hydroxyproline<sup>2</sup>, the acid-soluble and insoluble fractions from the nitrogen content. Four extraction series were performed in both groups.

The insoluble collagen was nearly same in lathyrictic animals as in controls. The acid-soluble collagen was higher in lathyrictic samples (content 0.48 % and 0.18 % from the air-dried skin samples of lathyrictic and normal rats, respectively;  $P > 0.10$ ).

The most marked difference was in the disodium phosphate-soluble collagen. Calculated from the hydroxyproline contents of the extracts, the averages were 3.15 % and 0.62 % in the lathyrictic and normal samples, respectively ( $P < 0.001$ ) and after precipitation the alkali-soluble collagen amounted to 1.07 % and 0.30 % ( $P < 0.01$ ). It is concluded that in experimental lathyrism the alkali-soluble collagen is increased but there is a defect in the fibre formation.

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## A New Sensitive Detector for Gas Chromatography

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We constructed Lovelock's type of gas chromatography detector<sup>1</sup> of Pyrex-glass using the <sup>90</sup>Sr-covered silver plate as anode. Some incidental observations prompted us to replace the anode by a non-radioactive aluminium plate.

This modification of the detector was equally useful for analysis of methyl esters of fatty acids. It seemed to possess the same characteristics of response as the  $^{90}\text{Sr}$ -detector, with some advantages in the operation.

The response of this detector was studied in a gas-chromatography apparatus made in our laboratory according to James<sup>2</sup>, using a 4 feet glass column of 4 mm diameter filled with Celite 545 of 100–120 mesh containing 20% (w/w) Apiezon L grease as stationary phase. Commercial argon (99.8%) at an inlet pressure of 0.5 atm was used as carrier gas. The column and the detector were maintained at a temperature of 180°C. The ionization current was amplified by a feedback electrometer amplifier with 5 000 M $\Omega$  input resistance and recorded by a Honeywell recorder. Different anode-voltages ranging from 450 V to 2 000 V could be applied. Using a potential of 2 000 V and applying 0.1  $\mu\text{l}$  of a 1:200 000 dilution (w/v) of methyl laurate in chloroform, the detector still gave a well detectable response, which corresponds to  $5 \times 10^{-4}$   $\mu\text{g}$  of methyl laurate.

The sensitivity of this detector is at least of the same order as that of Lovelock's detector. The absence of the strontium plate causes a considerable fall of the basic ionization current and increases stability of the detector at higher anode potentials and gives a low noise level.

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## The Mechanism of Inactivation of SH-Enzymes by X-Rays

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Previous work in this laboratory has established<sup>1,2</sup> that the SH-enzymes muscle glyceraldehyde dehydrogenase (GAPDH) and yeast alcohol dehydrogenase (ADH) are inactivated

by X-rays with ionic yields which fall in the same range as those of non-SH-enzymes. This finding, which stands in contrast to previous claims<sup>3,4</sup>, raises the questions as to the radio-sensitivity of protein SH-groups and the role of such groups in the radiation induced inactivation of SH-enzymes. In order to throw light on these questions the X-ray inactivation of GAPDH and ADH in dilute solution has been correlated with the concomitant disappearance of titratable SH-groups.

In the case of GAPDH, the disappearance of the protein SH-groups as well as the enzyme inactivation was found to be a linear function of the radiation dose. Complete inactivation of the enzyme occurred when 3 SH-groups per enzyme molecule had been destroyed. Since GAPDH is completely inactivated by the addition of 3 moles of *p*-chloromercuriphenyl sulphonate<sup>5</sup> the present data strongly indicate that the radiation induced inactivation of the enzyme is entirely due to radiochemical destruction of SH-groups.

The ADH was inactivated by X-ray to a somewhat greater extent than could be expected on the basis of the disappearance of the titratable SH-groups. Thus, from a comparison of the experimentally determined number of SH-groups with the theoretical number calculated on the basis of the remaining enzymatic activity<sup>6</sup>, it was estimated that approximately 60–80% of the enzyme inactivation can be accounted for by the radiochemical destruction of SH-groups.

In both enzymes the disappearance of SH-groups proceeds with a low ionic yield compared with that observed on irradiation of low molecular thiols<sup>7</sup>. This finding indicates that the enzyme SH-groups are protected by the rest of the protein molecule and that the major part of the radiation energy is dissipated in reactions not leading to the loss of thiol-groups. The consequent alterations of the enzyme molecule are apparently of little significance for the enzymatic activity.

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