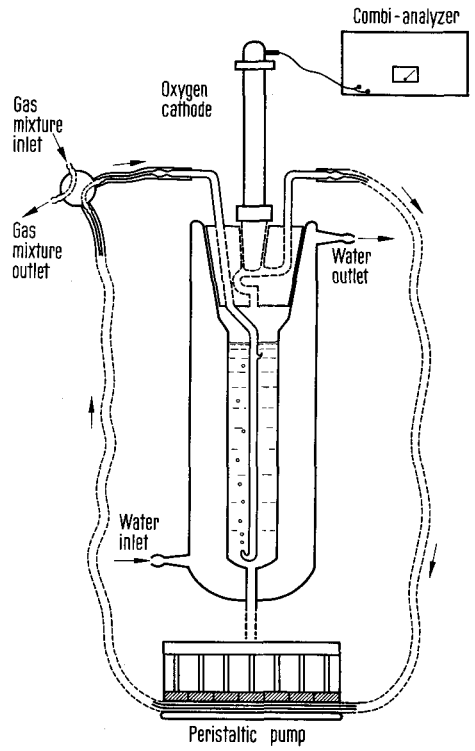
The problem of even small gas leaks in the system is very important because of the small gaseous phase used (only 20 ml) and the low oxygen consumption by the intestine. The use of a glass system and of Tygon tubes avoids gas leaks; the discrepancy found by some authors[3] between the oxygen consumption obtained by the Warburg technique and the oxygen consumption obtained by a polarographic method may have been due to the materials (Perspex and rubber) of which the perfusion chamber was made. In order to calibrate the analyser, gas mixtures, at increasing known oxygen content, are used. They are bubbled through a four-way stop-cock (these ducts can be connected in adjacent positions as shown in the Figure), into the perfusion apparatus till equilibrium is reached. The oxygen pressure of these standard mixtures is corrected by taking into account the fact that the gaseous mixture, entering the system, decreases to the atmospheric pressure and becomes saturated with aqueous vapour. At this point the gas tightness of the circuit, i.e. the the constant oxygen pressure of the system, can be checkde by simply turning the stop-cock, which isolates the system, and operating the peristaltic pump. Finally, the intestinal sac is introduced into the vessel, whose glass joint has been removed and then replaced. By operating the four-way stop-cock, oxygen at the highest concentration (95%) is made to bubble again for a few minutes to let out the air which entered during the introduction of the substrate. When the analyser goes back to its previous calibration value, another rotation of the stop-cock isolates the system and the biological experiment can start. At the end of the experiment, the volume of the gaseous phase can be easily determined by filling the whole system with water.



The complete equipment needed for the experiment.

Our first data on the oxygen consumption (58 $\pm$ 2 μmoles g^{-1} wet weight h^{-1}) by the rat small intestine (jejunal tract), incubated at 28°C, do not differ from those which can be calculated from the data reported by WILSON and WISEMAN[4] with the Warburg method under the same experimental conditions (60 μmoles g^{-1} wet weight h^{-1}).

Riassunto. Viene descritto un metodo polarografico per la determinazione continua del consumo di ossigeno in un preparato intestinale di ratto perfuso in vitro. I risultati ottenuti sono in accordo con quelli trovati da altri autori sullo stesso substrato mediante un metodo manometrico.

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Disc Electrophoresis of Proteins in the Presence of Sodium Dodecyl Sulphate

Disc electrophoresis[1] is a powerful tool for studying the purity of proteins; but, since the polyacrylamide gel acts as a molecular sieve, it is often difficult to decide whether a multiband electrophoretogram indicates an impure preparation or a complex association equilibrium.

The protein-dissociating power of several detergents is well known[2]. One of the most often studied amongst them is sodium dodecyl sulphate (SDS). Using this substance, aldolase has been shown to be the result of the association of three sub-units[3]. It occurred to us that the presence of SDS in polyacrylamide gels might be useful for the study of associations of this type. Accordingly, the procedure of ORNSTEIN and DAVIS[4] was modified as follows: (a) The acrylamide concentration in the solution for the large-pore gel layer was raised to 4% and ethylenediaminetetraacetic acid, 6 mg%, was added. (b) 0.5% SDS was added to all gels. (c) On top of the protein-containing layer, an additional large-pore gel layer was deposited. It functions as a reservoir of SDS and replaces the detergent mobilized

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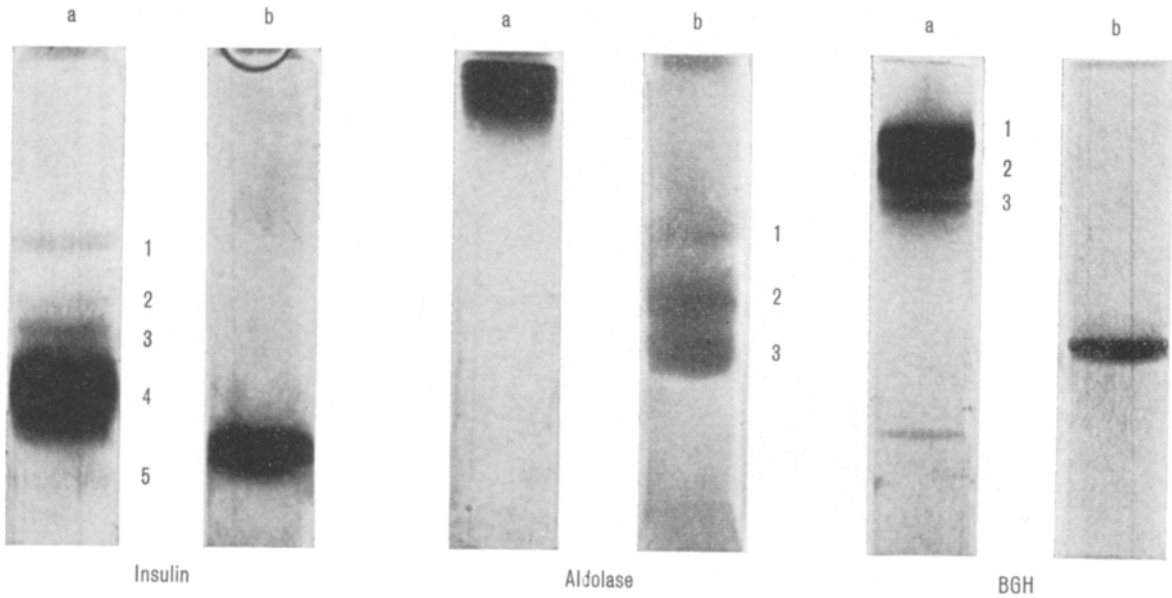

Disc electrophoresis of insulin, aldolase and bovine growth hormone (BGH) without SDS (a), and in the presence of SDS (b).

by the electric current. If the SDS is not kept above a minimum concentration during the run, the protein may show association phenomena in that region. SDS added to the buffer solution foams excessively. (d) The staining procedure was carried out under more rigorous conditions: the gel was placed for 4 h in a mixture of equal volumes of methanol and 20% acetic acid, and then transferred to the staining bath prepared by mixing equal volumes of 2% Amidoschwarz in 10% acetic acid and 10% acetic acid in methanol. The gel was kept in this solution for 12 h before proceeding to the next step.

The method with the present modifications was applied to insulin (E. Lilly, 25.2 U/mg), aldolase (Sigma, crystalline suspension from rabbit muscle), and bovine growth hormone prepared according to the method of DELLACHA and SONENBERG[5].

The electrophoresis of insulin in a gel without SDS (Figure, insulin a) gives a multicomponent pattern formed by a diffuse fast-migrating band and several slower minor ones. In the presence of SDS (Figure, insulin b), only one compact band is apparent.

Aldolase migrates very little when SDS is absent (Figure, aldolase a) but is resolved in three faster-migrating bands when the detergent is added to the gel (Figure, aldolase b).

Bovine growth hormone gives three closely-migrating bands on the polyacrylamide gel (Figure, BGH a). The electrophoresis of the same protein in the presence of SDS indicates only one component with greater mobility (Figure, BGH b).

The results obtained with insulin and aldolase can be correlated with what is known about these molecules: insulin preparations are complex equilibrium mixtures of several polymeric forms[2]; and RAMEL et al.[6] have indicated that, when aldolase is degraded by SDS, three components are found depending on the ratio of SDS to protein.

The bovine growth hormone preparation used in the present experiments is homogeneous as judged by ultracentrifugation and free-electrophoresis analysis[5], as well as end-group determinations[7,8]. Nevertheless, starch-gel[9] or polyacrylamide-gel electrophoresis indicate the presence of a complex mixture. The results obtained here suggest that association phenomena may explain the observed facts. In this connection, it can be mentioned that DELLACHA et al.[10] have observed that the sedimentation coefficient of bovine growth hormone is appreciably decreased when 0.5% of SDS is added to the buffer. Also, the enzymatic action of carboxypeptidase on bovine growth hormone is appreciably favoured by the presence of 0.1% of detergent[7] in the incubation medium[11,12].

Zusammenfassung. Die elektrophoretische Trennungsmethode an Polyacrilamidgelen von ORNSTEIN und DAVIS[4] wurde so abgeändert, dass sie im Anwesenheit von Natrium-dodecyl-sulfat zu gebrauchen ist. Dadurch ist es möglich, Assoziationen und Dissoziationen von Proteinen zu bestimmen, wie Resultate mit Insulin, Aldolase und Rinderwachstumshormon beweisen.

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[11] This work was supported in part by Grant No. 2029 from the Consejo Nacional de Investigaciones Científicas y Técnicas de la República Argentina.
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