wurden jeweils fünf unabhängige Inkubationsproben mit dem Extrakt aus 3–5 frischen Pinealisdrüsen versetzt; fünf weitere Proben dienten als Kontrollen. Nach Extraktion der Inkubationsansätze, Reinigung der Extrakte und papierchromatographischer Isolierung der Corticosteroide wurde der C^{14}-Progesteronumsatz und der Radioaktivitätseinbau in Cortexon (DOC), Corticosteron (B), Aldosteron (ALD), 17α-OH-Cortexon (S), Cortisol (F) und Cortison (E) durch Flüssigszintillationsmessung bestimmt. Die verwendete Versuchsanordnung wurde an anderer Stelle eingehend beschrieben[7].

Die Abbildung zeigt Veränderungen des Corticosteroid-Synthesemusters, die nach Inkubation mit den FARRELL-schen Pinealis-Hexanextrakt beobachtet wurden. Die Ergebnisse sind als prozentuale Unterschiede von den entsprechenden Kontrollwerten dargestellt. Es fällt auf, dass die Bildung der 11β-Hydroxysteroide Corticosteron (B), Aldosteron (ALD), Cortisol (F) und Cortison (E) unter der Einwirkung des Pinealisextraktes signifikant vermindert wurde. Die Radioaktivitätsausbeute in den 11-Desoxysteroiden Cortexon (DOC) und 17α-OH-Cortexon (S) war dagegen signifikant erhöht. Die mässige Erhöhung des Progesteronumsatzes (P turnover) lag nicht im Signifikanzbereich.

Ganz ähnliche Veränderungen des Corticosteroid-Synthesemusters wurden unter der Einwirkung von Metopiron (2-Methyl-1, 2-bis-(3-pyridyl)-propanon), eines Inhibitors der biologischen Steroid-11β-hydroxylierung, beobachtet[7].

Aus den Befunden ist zu schliessen, dass corpus pineale von Rindern eine Substanz (Substanzen) enthält, welche ebenso wie Metopiron die biologische Steroid-11β-hydroxylierung spezifisch hemmt (hemmen). Die Beobachtungen von BARBOUR, SLATER, CASPER und BARTTER[6] könnten danach so erklärt werden, dass nicht neurale Mechanismen, sondern humorale Wirkstoffe des corpus pineale die Sekretion von Aldosteron, Corticosteron und Cortisol drosseln, und zwar durch Hemmung der 11β-Hydroxylierung. Nach neueren Anschauungen von FARRELL[8] ist Ubichinon (Coenzym Q) möglicherweise einer dieser Wirkstoffe[9].

Summary. A hexane extract of bovine pineal glands reduced the production of 11β-OH-corticosteroids and augmented the formation of 11-desoxycorticosteroids in slices of bovine adrenal cortex. It is suggested, therefore, that bovine pineal glands contain one or several substances which inhibit the 11β-hydroxylation of corticosteroids.

D. LOMMER

II. Medizinische Klinik und Poliklinik der Universität des Saarlandes, Homburg (Saar, Deutschland), 20. September 1965.

[7] D. LOMMER and H. P. WOLFF, Steroids, in Vorbereitung.
[8] G. FARRELL, *Aldosterone* (Symposium Prague 1963) (Eds., E. E. BAULIEU and P. ROBEL; Blackwell Scientific Publications, Oxford, England 1964), p. 243.
[9] Mit Unterstützung der Deutschen Forschungsgemeinschaft. Die Pinealisextrakte wurden von der Firma Organon (Oss/Niederlande) zur Verfügung gestellt. Die Durchführung der Untersuchungen wurde ermöglicht und gefördert durch Herrn Professor Dr. H. P. WOLFF (II. Medizinische Klinik der Universität des Saarlandes) und Herrn Professor Dr. W. LAMPRECHT (Biochemisches Laboratorium der Technischen Hochschule München).

PRO EXPERIMENTIS

A Method for the Contemporaneous Determination of Oxygen Consumption and of Other Chemical Parameters in the Isolated Intestine

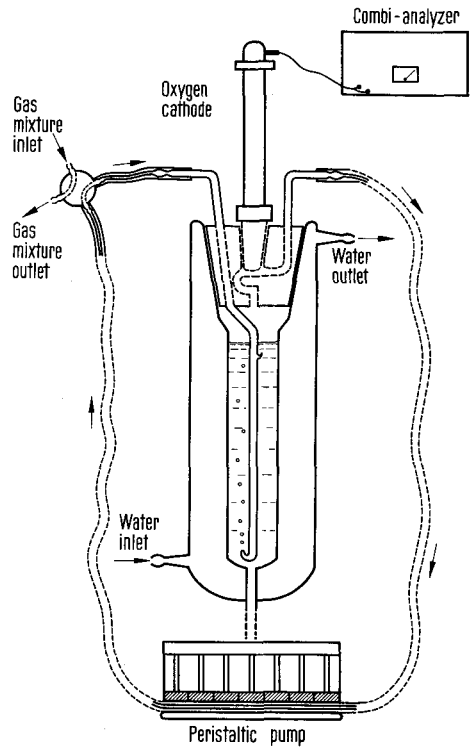
A widely employed method to determine the oxygen consumption of in vitro perfused biological substrates is the well-known Warburg manometric technique. Another method is based on the determination of oxygen concentration in samples withdrawn from the perfusing fluid at given times during the experiment. A polarographic method was used by us for a continuous measurement of oxygen consumption by inserting the oxygen cathode into a gaseous phase in equilibrium with the perfusing fluid. This method is suitable for following the oxygen consumption in the sacs of rat intestine, everted according to the WILSON and WISEMAN technique[1], and, at the same time, for determining both the transport of sodium and glucose across the intestinal wall as well as the lactic acid production.

The apparatus (see Figure) consists of a glass vessel which is enclosed in an outer jacket through which warm water can be circulated at the requisite temperature. Inside the vessel, the intestinal sac is dipped into the perfusion fluid containing streptomycin at a concentration of 0.01 mg/ml in order to avoid the oxygen consumption due to the presence of microorganisms. At the top of the vessel, a ground-glass joint holding glass tubes for the inflow and outflow of the gas mixture is inserted.

Such a gas mixture flows through the tube which reaches the bottom of the vessel and flows out through an S-shaped glass tube. The oxygen cathode[2] is inserted along this S-shaped tube (see Figure) and connected with a Combi-Analyzer. By connecting the analyzer to a Metrawatt recording device, it is possible to record the oxygen pressure continuously. The cathode has been deliberately put in this position in order to avoid the effects of slight pressure changes in the gas flowing into the system and to save the oxygen cathode from incidental spray of perfusion fluid. The connection between the inlet and outlet gas tubes is made by means of a completely gas-tight Tygon tube, which is elastic enough to allow the use of a peristaltic pump for the gas recirculation (20 ml/min under our experimental conditions).

[1] T. H. WILSON, J. Physiol. *123*, 116 (1954).
[2] U. GLEICHMANN and D. W. LUEBBERS, Pflügers Arch. ges. Physiol. *271*, 456 (1960).

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.

A. FAELLI and G. ESPOSITO

Istituto di Fisologia Generale dell'Università di Milano (Italy), September 13, 1965.

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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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[3] E. STELLWAGEN and H. K. SCHACHMAN, Biochemistry *1*, 1056 (1962).
[4] L. ORNSTEIN and B. J. DAVIS, *Disc Electrophoresis* (Preprint distributed by Distillation Products Industries, Rochester, New York 1962).