men reduzierten Epithelzellen auf. Die Basalzellen scheinen eine geschlossene Kette zu bilden. Der Verband der Zylinderzellen wirkt nach der Schnittrichtung und der differenten Höhenlage der Kerne mehrschichtig. Die Zellen sind durch starke Plasmalemmfalten miteinander verzahnt. Bei der Polymorphie der Kerne ist allen eine gehäufte Ansammlung und Klumpung des Chromatins, besonders an der Kernmembran, gemeinsam. Das ER ist reduziert. Die Zahl der Mitochondrien hat sich verringert. Die Golgi-Komplexe sind stark verkleinert und bläschenförmig erweitert. Die dichte apikale Zone trägt einen Stereocilienbesatz. Lipideinschlüsse werden vermisst. Cytosomen differenter Art sind hingegen noch vorhanden. Von dem skizzierten Bild der Zylinderzellen weichen einzelne Zellen ab. Diese Elemente besitzen einen bescheidenen lamellären Golgi-Apparat. Die apikale Zone ihrer Zelleiber und die Randregion beherbergt kleine Bläschen und vereinzelt Vakuolen. Der Stereocilienbesatz ist spärlich. Sie ähneln in diesem Zellabschnitt einem Zelltyp (Typ B), der im Gangepithel noch nicht geschlechtsreifer Ratten (28 Tage alt) anzutreffen ist und als Vorläufer der Nebenzellen gelten kann[3].

Die Gabe von 20 mg Testosteron führt schon nach 8 Tagen zu tiefgreifenden Veränderungen. Die *Tunica propria* und die Basalmembran verlaufen gestreckt. Die Bindegewebszellen werden in der Folge aktiviert und nehmen die Kollagenbildung auf. Die Entfaltung der Hauptzellen hat in der 2. Woche einen Grad erreicht, der dem des Normalbildes entspricht. In den Zeitabständen bis zur 9. Woche werden keine Veränderungen auffällig (Figur 2). Die Nebenzellen verhalten sich anders. Ihre Entfaltung begleiten in der Zeitfolge strukturell qualitative und quantitative Veränderungen sowie numerische Zunahme. In den Frühstadien (1. und 2. Woche nach Testosterongabe) herrscht eine strenge Lokalisation der zuerst auftretenden Lipidtropfen im basalen Zellbereich vor. In der apikalen und in der Randzone finden sich zahlreiche kleine Bläschen, vereinzelt Vakuolen und dichte Cytosomen, seltener auch lamelläre Cytosomen. Diese Region beherbergt ausserdem die Mehrzahl der Mitochondrien. Mit zunehmender Anhäufung und Vergrösserung der Fetttropfen verbindet sich eine Zunahme der Vakuolen im Randbereich, eine grössere Häufigkeit der dichten Cytosomen und die Ausbildung von lamellären Cytosomen, wobei beide Cytosomenarten in die Kernzone vordringen und später auch infranucleär anzutreffen sind (9. Woche). Von der 4. Woche ab werden in geringer Zahl Mitochondrien mit Orientierung ihrer Cristae parallel zur Längsachse beobachtet, die Veränderungen unterliegen (Figur 3). Die Basalzellen reagieren ebenfalls auf die Androgengabe.

Die beschriebene Morphodynamik des Nebenhodengangepithels lässt zwar an differente Aufgaben seiner Zellarten denken, erlaubt aber noch keine endgültigen Aussagen[4].

Summary. The epithelium of the epididymal duct (corpus region) of the rat after hypophysectomy and after androgen substitution has been examined by light and electron microscopy. The epithelium consists of 3 different cell types: principal, accessory (Nebenzellen), and basal cells. The characteristics of these cells disappear after hypophysectomy and redifferentiate after androgen application. The alterations of the principal and accessory cells in course of time (from the 1st week up to 9 weeks after injection of 20 mg testosterone propionate) are the subject of this report.

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Aerobic Glycolysis of Normal Tissues Suspended in Thoracic Duct Lymph

About 40 years ago Warburg[1] stated that the formation of lactic acid from carbohydrate sources in the presence of a supply of oxygen (that is, aerobic glycolysis) is one of the most general and distinctive features of cancerous tissues. However, such a property, though closely associated with the neoplastic process, cannot be said to be essential for malignancy, since it is known that aerobic glycolysis is displayed also by some normal tissues, for instance brain, retina, kidney medulla and embryonic cells[2].

Nevertheless, according to Warburg, almost all the data showing that normal tissues glycolyse aerobically in vitro are fallacious, because they have been obtained with cells incubated in simple saline media, instead of more physiological media or similar body fluids. As a matter of fact, he has recently reported that normal rat or mouse tissue slices respectively suspended in homologous sera and in ascitic fluid of Ehrlich tumour, produce almost no lactic acid aerobically[3].

However, neither fluids can be regarded as fully physiological media because their chemical composition is very different from that of the interstitial lymph in which the cells are normally immersed. Since interstitial lymph may be considered equivalent to the thoracic duct lymph, we thought it worth investigating the glycolytic behaviour of some normal rat tissues incubated in such body fluid.

Wistar rats of both sexes, weighing about 200 g each, were used throughout these experiments. The lymph was collected by cannulation of the thoracic duct according to the method of Bollman, Cain and Grindlay[4],

[1] O. Warburg, Biochem. Z. *142*, 317 (1923).

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O. Warburg, K. Gawehn, and A. W. Geissler, *Weiterentwicklung der zellphysiologischen Methoden* (Thieme, Stuttgart 1962).

[4] J. L. Bollman, J. C. Cain, and J. H. Grindlay, J. Lab. clin. Med. *33*, 1349 (1948).

except for the use of a drop of plastic adhesive (methyl-2-cyanoacrylate; Eastman 910)[5] instead of ligatures to hold in place the plastic tube slipped into the duct. Lymph was collected for 2 successive days from each animal in sterile recipients containing Hank's solution plus antibiotics and heparin, as suggested by GOWANS[6]; samples from 6 different rats were pooled, centrifuged cell-free, heated at 56 °C for 30 min and used immediately or stored at − 20 °C. Fed animals were decapitated and the organs immediately removed and prepared according to the conventional techniques. Tissues were suspended in 3 ml of lymph and incubated for 1 h at 37 °C, in air, in vessels of about 40 ml capacity with one large side arm containing 3 ml of a carbonate-bicarbonate buffer 3 *M* pH 10.8 and 2 ml of carbonic anhydrase (Serva, Heidelberg), as described by WARBURG, GEISSLER and LORENZ[7]. Readings of the O_2 uptake were taken every 10 min; after incubation, the lactic acid content of each vessel was determined by the 'lactate-test' (Boehringer, TC-B 15972). All the data were referred to the dry weight of the tissues and corrected according to the blanks obtained at 0 time.

Aerobic glycolysis and respiratory rate of normal rat tissues suspended in thoracic duct lymph

Tissue	$Q_{CO_2}^{O_2}$	Q_{O_2}
Brain white matter	+ 0.16	− 5.73
Diaphragm	+ 0.25	− 1.55
Liver	+ 0.29	− 5.13
Mucous membrane (jejunum)	+ 0.96	− 1.94
Kidney cortex	+ 1.14	− 10.17
Testicle	+ 1.39	− 6.30
Spleen	+ 2.08	− 6.84
Kidney medulla	+ 2.48	− 6.68
Brain cortex	+ 3.00	− 5.76
Uterus (gravid)	+ 3.50	− 1.34
Amnion	+ 8.40	− 5.96
Retina	+ 22.35	− 7.19

As shown in the Table, all the normal tissues tested produce lactic acid aerobically in spite of their being suspended in thoracic duct lymph; that is, in the most physiological medium so far available. The glycolytic activity is reasonably within the range of the data usually reported in the literature[2] for each tissue incubated in a wide variety of saline media or body fluids.

Therefore, our present results are at variance with the above mentioned findings of WARBURG[3]. On the contrary, these data further support the generally held opinion that (1) aerobic production of lactic acid by many normal tissues is not an artifact dependent on experimental variables but a true biological property of their own, and that (2) the correlation between aerobic glycolysis and malignancy is not universal.

Riassunto. La produzione aerobia di acido lattico da parte di tessuti normali di ratto non viene ridotta o inibita dalla loro incubazione in un mezzo fisiologico quale la linfa di dotto toracico.

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Istituto di Patologia Generale dell'Università di Milano (Italy), August 24, 1966.

[5] Supplied through the courtesy of Ethicon Inc., Somerville (N.J., USA).

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Experiments of in vitro Cultures of Larval Epiderm of Desert Locust (*Schistocerca gregaria* Forsk)

The difficulties in the in vitro culture of tissues and organs of insects derive mainly from (a) the very limited knowledge of the composition of the insect fluids, and (b) the fact that the activity of almost all the cells of insects are under hormonal control[1-3]. The first difficulty is beginning to be overcome, and media are proposed for cell cultures of different orders of insects which allow development of cell lineages of fibroblasts[4].

As for organs and tissues, only gonad maturation seems to be out of the hormonal control, and the differentiations of testes and ovaries were obtained in vitro[5]. However, in order to investigate the hormonal control of morphogenesis, tissues which are strictly under hormonal control, such as epiderm[6], appear to be much more interesting when cultivated in vitro.

In vitro cultures of tegument of desert locust (*Schistocerca gregaria* Forsk) larvae were tried, and the results of these experiments are reported here. Tests conducted in this laboratory on the saline solution proposed by HOYLE[7], showed that it was capable of maintaining embryos and embryonic tissues of locust alive for a long time; therefore it was used throughout these experiments. Other experiments on the in vitro development of embryos of *S. gregaria* without yolk suggested to us the following medium, which seems suitable also for the culture of epidermal cells: 100 ml of HOYLE's saline (KCl 0.075 g; $NaCl_2$ 0.75 g; $MgCl_2$ 0.041 g; $NaKCO_3$ 0.034 g; NaH_2PO_4 0.083 g; distilled water 100 ml); glucose 0.1 g; trehalose 0.1 g; lactalbumine hydrolysate 1 g; locust hemolymph 10 ml; and locust embryo extract 10 ml. Hemolymph was heated at 60 °C for 5 min, centrifuged, and the clear supernatant used. Embryo extract was obtained from eggs after 8 days of incubation, homogenated, centrifuged at 20,000 *g* for 30 min, and the clear supernatant used. Media supplemented with antibiotics were sterilized by filtration before use.

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