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	$Q^{O_2}_{CO_2}$	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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In the medium described above, but without embryo extract, tegument survived in vitro only up to 4 days in good conditions, as can be judged by microscopic examination; in complete medium, with embryo extracts, explants of tegument remained in good condition up to 12 days. However, the development in vitro of the epiderm depends greatly upon the stages of the moulting cycle when the tissue is explanted. Within an instar, between one moult and the next one, epidermal cells undergo in vivo the following cycle: (1) endocuticle formation is completed, (2) epidermal cells multiply, (3) new exocuticle is secreted, (4) the old endocuticle is digested, and (5) new endocuticle begins to be formed. Finally ecdysis occurs. These processes were timed during larval development in *Locusta migratoria* by STRICH-HALBWACHS[8], and this timing corresponds almost exactly to that for *S. gregaria*.

Fragments of abdominal sterna, corresponding to 1 or 2 segments, were explanted in 0.5 ml of the media described at different times of the moulting cycle, in stoppered tubes, and incubated at 30 °C. In a typical experiment, one segment of each animal was fixed and examined as soon as severed, the others were put in culture for different lengths of time up to 8 and 12 days and fixed at various intervals. In this way it was possible to know the stage of the moulting cycle of the epidermal cell at the moment of explanting and to follow the changes occurring in culture of the tissue taken from the same larva. Segments were taken from larvae of the 4th and 5th instars (a) before the onset of multiplication of epidermal cells, (b) during the peak of mitosis, and (c) after cell multiplication. The stages (a), (b) and (c) of the moulting cycle obviously occur at different times between the moults in the 4 and 5 instar larvae.

In explants put in culture before the onset of mitosis, the peaks of mitosis were never observed in vitro; few mitoses were observed in explants cultured in complete medium (Figures 1 and 2). Even after 12 days, as it appeared on microscopic examination, epidermal cells remained in good condition, and a slight thickening of endocuticle was observed at 2–3 days of culture. A wide cell degeneration occurred after a few days of culture in explants of abdominal segments taken from the larvae during the peak of mitoses of epidermal cells. In the medium without embryo extract, cell degeneration occurred after 24 h; in the medium with embryo extract, degeneration appeared a few days later; and in both cases, it seems that mitosis did not proceed through anaphase.

[8] M. C. STRICH-HALBWACHS, Annls Sci. nat. 12e série, p. 483 (1959).

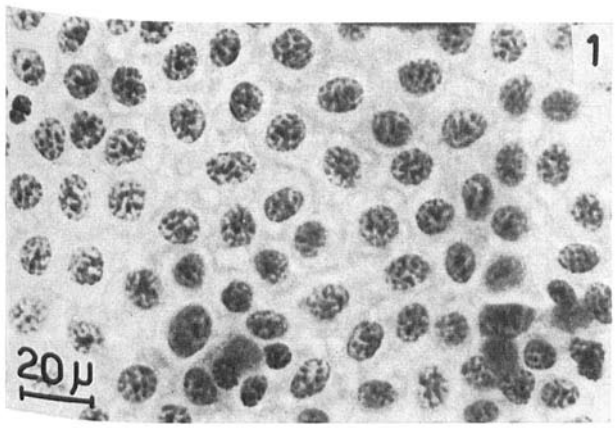

Fig. 1. Epidermal cells of an abdominal sternum of a 4th instar larva fixed soon after the moult; all the cells are in interphase. Fixed with Dubosq-Brazil and stained with hemallum.

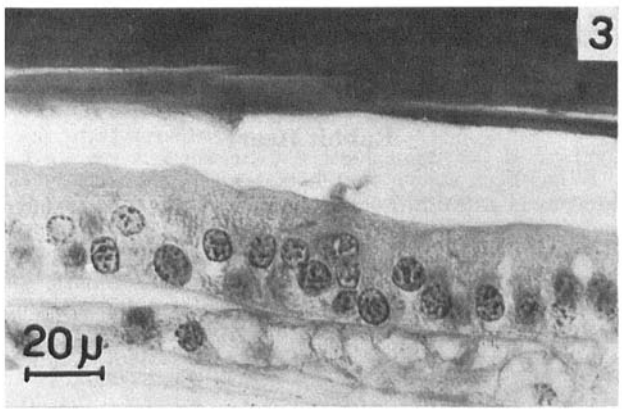

Fig. 3. Section of tegument of abdominal sternum of a 4th instar larva fixed 4 days after the moult. Fixed with Dubosq-Brazil and stained by the Azan-Mallory method.

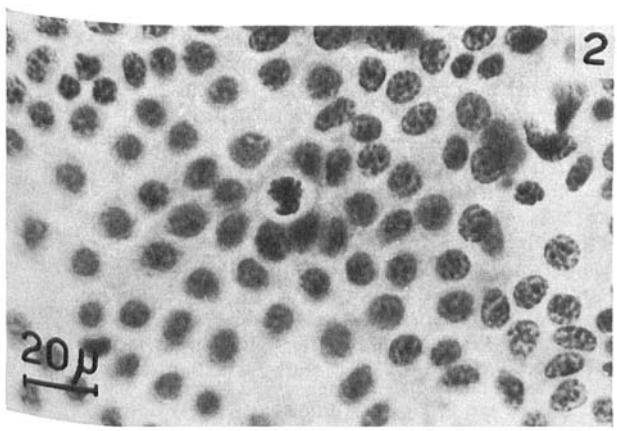

Fig. 2. Epidermal cells of an abdominal sternum of the same larva as in Figure 1, after 3 days of culture in complete medium. A metaphase is visible. Fixed with Dubosq-Brazil and stained with hemallum.

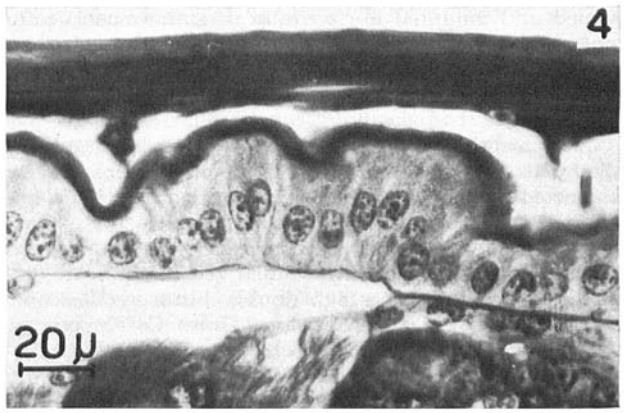

Fig. 4. Section of tegument of an abdominal sternum of the same larva as in Figure 3, fixed after 4 days of culture. New cuticle is clearly visible. Fixed with Dubosq-Brazil, and stained by the Azan-Mallory method.

Tegument, after the multiplications of the epidermal cells, was kept in vitro up to 12 days without signs of degeneration, and this was also found in the poorer medium (without embryo extract). Moreover, in explants of abdominal segments of 4 instar larvae, new cuticle (eso- and andocuticle) was found to be formed in vitro with lysis of the old endocuticle. The synthesis of the new cuticle occurs in the first 1–2 days of culture but thickening of the cuticle, as in vivo, was not observed after 8 days of culture (Figures 3 and 4).

The results of the described experiments emphasize the statements reported above about the difficulties of the in vitro culture of insect tissue, i.e. the need for improved media and the importance of hormonal control. For locust tissues, the medium tested in these experiments appears to be a relatively good one, mainly because of the addition of extract of locust embryos. However, multiplication of epidermal cells was not sustained, and it is difficult to discriminate whether some chemical compounds or hormones are lacking.

A result worthwile mentioning is the synthesis of new cuticle obtained in vitro by the culturing tegument of the 4th instar larvae taken after the multiplication of epidermal cells. At this stage of the moulting cycle, epidermal cells have either received hormonal 'information', or synthesis of new cuticle by the cells after multiplication is out of hormone control.

Riassunto. Negli espianti presi prima della moltiplicazione delle cellule epidermiche non si è avuto in vivo il picco di mitosi, ma solo ispessimento dell'endocuticola; nei frammenti espiantati al momento del picco delle mitosi si ha dopo alcuni giorni degenerazione delle cellule epidermiche; negli espianti di tegumento messi in coltura dopo la moltiplicazione delle cellule epidermiche si è ottenuta in vitro la sintesi della nuova cuticola (eso- ed endocuticola).

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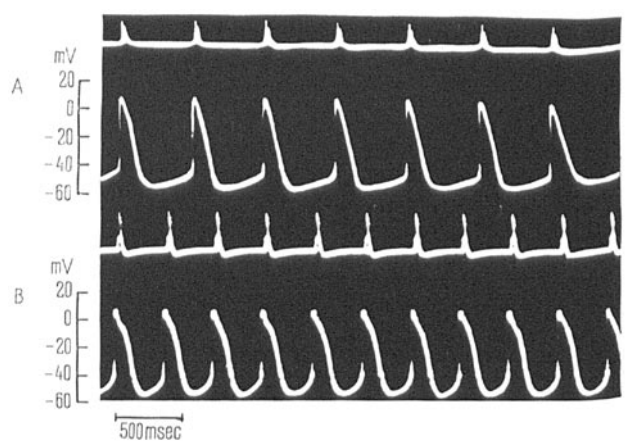
Effect of Histamine on Sinoatrial Node Cells of Rabbit Heart

It has been shown that histamine stimulates the isolated atria of rabbit[1] and guinea-pig[2]. Good pharmacological evidence assigns to histamine a direct cardiac effect not mediated through release of adrenergic substances[2–4]. Moreover, histamine initiates automatic activity in rabbit[5] and guinea-pig[6] isolated left atrium. Transmembrane potentials from non-pace-maker cells have been recorded during histamine treatment of rabbit atria[7].

The present study was undertaken to investigate the effects of histamine on cardiac automaticity, by directly studying its action on the pace-maker cells of rabbit sinoatrial node. Rabbits of either sex weighing approximately 1.5 kg were killed by cervical dislocation. Their hearts were removed immediately, and the right atria excised, opened and mounted horizontally in a thermostatically controlled perspex chamber. The preparation was bathed in a continuously-flowing pre-warmed Tyrode solution (30 °C), through which 95% O_2 and 5% CO_2 (pH 7.2) was bubbled.

Glass microelectrodes (0.5 μ diameter, 10–20 megohms) filled with 3.0 M KCl, and flexibly mounted, were directly connected to the input grid of a feed-back cathode follower. A unipolar electrode was also used in the experiments in order to record extracellularly from the atrial roof. Extracellular and intracellular recordings were displayed on a Tektronix 502 double beam oscilloscope. Photographs were taken with a Grass C4-K camera. Histamine (histamine dihydrochloride, Roche) was dissolved in the perfusion fluid and used for periods of 10 min at a concentration of 10^{-6} g/ml, expressed as the salt. The rabbit sinoatrial node area was identified by the recorded pace-maker potentials, which showed an appreciable diastolic depolarization and preceded muscular excitation by 10–30 msec. The photographic records were enlarged



Effect of histamine 10^{-6} g/ml on sinoatrial node cells of rabbit heart: (A) control; (B) during histamine treatment. Upper tracings: extracellular recordings (retouched). Lower tracings: intracellular recordings.

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