This new working hypothesis has several important advantages: it explains why and when the vessel starts to grow and it explains also why and when it stops growing. Furthermore, the changes described above are in accordance with histological findings and the tangential wall stress is, under certain circumstances, a well measurable entity.

It has been shown above that the tangential wall stress can be a determining factor for both the diameter and the thickness of the wall of a blood vessel, when a chemical factor (hypoxia) forces the blood vessel to dilate.

Zusammenfassung. Die Entwicklung eines Kollateralkreislaufs wurde am Hundeherzen nach langsam erfolgendem Verschluss einer Koronararterie untersucht. An den unter Druck fixierten und histologisch aufgearbeiteten Kollateralgefässen wurde die tangentiale Wandspannung berechnet.

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PRO EXPERIMENTIS

The Preparation of Metabolically Active Suspensions of Mouse Liver Cells

The isolation of mouse liver cells has been reported by BRANSTER and MORTON[1] and by BERRY[2], the latter using the method of free cell preparation published by BRANSTER and MORTON[1]. This method involves a preliminary perfusion of the liver, in situ, followed by homogenization using a polyethylene and glass homogenizer, and finally successive filtrations through a series of filters of decreasing mesh. We wished to investigate some aspects of the nucleic acid and protein metabolism of mouse liver cell suspensions since they appeared to provide several advantages for in vitro experiments with intact mammalian cells. In the course of our initial work in the isolation of free metabolically active mouse liver cells we employed the method of BRANSTER and MORTON, as well as a number of other methods already available for the preparation of suspensions of rat liver cells[3-6]. We found that all of these methods proved to be injurious to a large proportion of the mouse hepatic tissue resulting in low yields of intact cells.

The mouse liver cells were found to be very fragile and highly susceptible to breakage during the dispersion step of the preparation, particularly when any of a number of different types of homogenizers was employed. We have developed a new method for the dispersion of liver cells which minimizes the physical treatment to the suspended cells once they are freed from the tissue.

An adult CFW mouse, fed ad libitum, is placed under ether anesthesia and the liver is perfused, in situ, via the portal vein with 15–20 ml of calcium-free Locke's solution (CFL) at room temperature at pH 7.3. This perfusion is accomplished by using a 20 ml syringe connected to a No. 24 needle shaft by a 12 inch length of polyethylene tubing. The flexible tubing aids considerably by preventing the accidental removal of the needle from the portal vein during the perfusion. The perfusion is carried out rather slowly ($1^1/_2$–2 min to completion). Sufficient pressure is maintained by means of the syringe to distend the liver. After the first few ml of fluid are administered and the liver is distended visibly, the inferior vena cava is cut to release the pressure and allow drainage. The cut is then clamped with a hemostat and the process is continued with repeated steps of pressure and release to allow adequate perfusion and complete flushing of blood from the liver.

Immediately after perfusion the gall bladder is removed and the liver excised and rinsed twice in CFL at room temperature. To provide a sufficient number of cells for metabolic studies a second mouse liver is prepared in the same manner as the above. The combined livers are rapidly weighed and then cut with fine scissors into pieces of approximately (3 mm^3) size. The tissue is then dispersed very gently by pressing it with a teflon pestle

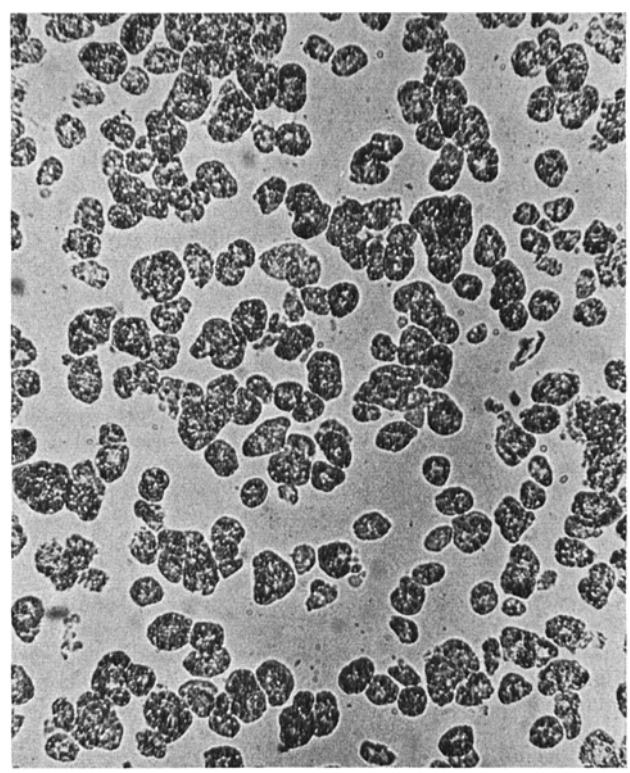

Fig. 1. Photomicrograph of mouse liver cells in suspension (× 200).

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(A. H. Thomas Co., size A) through a sac made of hard finish dacron mesh (marquisette, 24 threads/inch in one dimension by 30 threads/inch in the other) into a Thomas grinding vessel, size B containing only 30 ml of CFL at room temperature. While the tissue is pressed gently the individual cells disperse into the bulk of the suspension medium thereby avoiding any additional contact between the free cells and the pestle. When most of the tissue ($\sim 85\%$) has been dispersed in this way the tissue still remaining in the mesh sac (largely connective tissue) is discarded. The suspension is filtered once through a filter of fine mesh rayon (80 threads/inch by 84 threads/inch) to remove any large clumps of cells. The suspended cells are gently sedimented by centrifugation at 50 *g* for 4 min at 4 °C using an International refrigerated centrifuge. The supernatant is removed by suction and the sedimented cells are carefully resuspended in 20 ml of CFL using a pipette having an orifice of 0.90 mm, or larger. The use of a pipette with a finer bore appears to result in rupture of some of the cells during resuspension. The cells are sedimented again for 3.5 min at 50 *g* using the same type of centrifuge. After removal of the supernatant by suction, the cells are resuspended in 15 ml of CFL. The entire procedure can be completed in approximately 35 min (Figure 1).

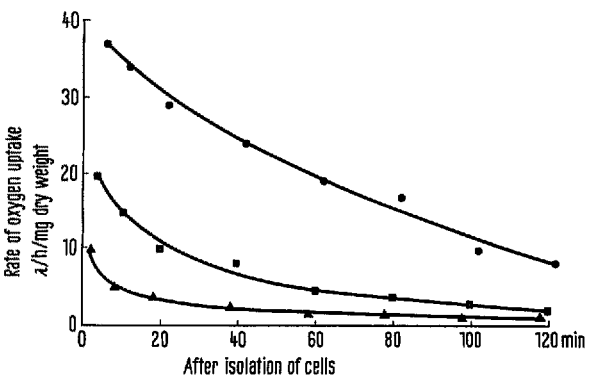

Fig. 2. The effect of increased time intervals between the isolation of mouse liver cells and the measurement of their rate of oxygen uptake. The cells were maintained at 25 °C in the absence of additional substrate during this time. Oxygen consumption was measured in the Gilson Oxygraph at 37 °C, the chamber containing 2.0 ml of calcium-free Locke's solution, 0.3 ml of the cell suspension (3.0 mg dry weight), and, when added, 0.1 ml of the substrate to give a final concentration of $10^{-2}M$. ▲ no addition; ■ pyruvate; ● succinate.

This method has been found to give consistently a yield of from 40–50% of the original tissue mass recovered as intact free cells. Two mouse livers dispersed in the manner described yield 1.8–2.2 ml of packed cells which have a dry weight of 160–200 mg. The suspensions have less than 3% of free nuclei apparent microscopically. There are also very few erythrocytes present. The number of cells in the suspension was determined in the Coulter Counter (Coulter Electronics, Inc.). The yield was found repeatedly to be $35 \cdot 10^6 \pm 10\%$ of cells/g of dispersed liver.

It was found that the free cells maintained at 25 °C in the absence of additional substrates (other than the $5.5 \cdot 10^{-3}M$ glucose present in CFL) exhibit a slowly decreasing capacity to respire. This activity was measured at 37 °C with the Gilson Oxygraph, both with and without additional substrates. The rates of respiration observed in the presence of $10^{-2}M$ glucose are essentially identical to those shown in Figure 2 for cells respiring in the presence of CFL alone. The presence or absence of $5.5 \cdot 10^{-3}$ to $10^{-2}M$ glucose had no effect on the rate of respiration observed when pyruvate or succinate was added to the cell suspension in the Oxygraph chamber. The metabolism of nucleic acids, protein, and lipids of mouse liver cells prepared by this technique is currently under investigation[7].

Zusammenfassung. Einfache und schnelle Methode zur Isolierung freier Mäuse-Leberzellen. Freie Hepatozyten (bis 50% des Gewebes) zeigen so aktiven Stoffwechsel.

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Electrophoresis of Mitochondrial α-L-Glycerophosphate Dehydrogenase on Acrylamide Gels

2 enzymes catalysing the interconversion of α-L-glycerophosphate and dihydroxyacetone phosphate exist in the mammalian cell. 1 form, localized predominantly in the cytoplasm, requires NAD for its function[1,2], the other, bound tightly to mitochondrial structure, does not[3]. The 2 enzymes differ further in that the activity of the mitochondrial but not the cytoplasmic form is stimulated several fold in certain tissues of the rat by thyroxine and some of its analogues[4–6]. Although, at least in rat kidney, the soluble, or cytoplasmic form has been found to be heterogeneous by starch gel electrophoresis[7], the bound or mitochondrial form has thus far resisted attempts at its resolution by electrophoresis in solid or semi solid media. This is apparently due to its high degree of adsorption to the medium and consequent failure to migrate[8]. The present paper reports the successful resolution of the mitochondrial enzyme into 2 active units by treatment with urea and electrophoresis in acrylamide gels of special (2.5% acrylamide-bisacrylamide) composition.

Methods. Acetone powders[9] of mitochondria from various tissues were extracted once with de-ionized water and twice with $0.03M$ potassium phosphate buffer, pH 7.5. α-L-glycerophosphate dehydrogenase (α-GPDH) activity was solubilized from the residue by incubation with *crotalus terrificus* venom under the conditions of RINGLER and SINGER[8]. The resulting suspension was centrifuged at 25,000 rpm in the No. 30 rotor of the Spinco preparative ultracentrifuge for 60 min. Portions