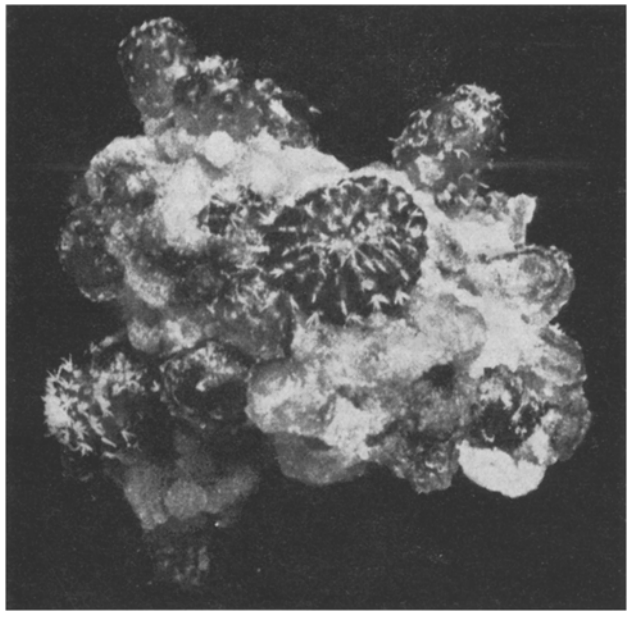

The formation of shoot regenerants in callus cultures of cactus *Mamillaria woodsii* Craig.

to the individual areoles. Finally, the epidermis together with the rests of prickles were cut off from each segment so that only the pith tissue was left from the stem.

The explants were then placed into Erlenmeyer flasks containing synthetic agar medium prepared according to MURASHIGE and SKOOG[6] with 2.0 mg · 1^{-1} of β-indolylacetic acid, and 2.0 mg · 1^{-1} of kinetin. The flasks were stored in a cultivation chamber and lit continuously by white fluorescent tube light at the temperature of 25 ± 3 °C.

Results and discussion. After approximately 1 week, the colour of explants was changed. Some of them went brown and were affected by necrosis, while in others the colouring changed to red of different shades to violet; after a few days of stagnation the latter started to produce callus, which in turn lost its reddish coloration and turned a light green. After 5 weeks of cultivation, the calluses were strong enough to be subcultured in a fresh medium, the composition of which was the same as shown above. After subcultivation an organized growth producing flat cactus shoots with apparent areoles was observed in some callus tissues. Later on the plants started shooting from areoles and subsequently whole tufts of cactus shoots with tiny prickles were produced (Figure). In one case even formation of rootlets was observed. In other cultures the callus continued growing in an unorganized way. After a 10 week's period, the other subculture could be divided into 3 groups. In one, differentiated plants from callus were produced, in the 2nd only well growing calluses without any traces of organogenesis were observed. The calluses produced in the 3rd group turned brown and were affected by necrosis.

Well differentiated cactus shoots 1 cm long were extirpated from the cultures. The cut was treated with a commercial rooting stimulator, and when dry the regenerants were placed in bowls containing humid perlite. Approximately after 4 weeks of cultivation, roots started to grow from the cut.

Mamillaria woodsii Craig thus extends the list of species in which formation of regenerants by controlled organogenesis in callus cultures in vitro was achieved. The synthetic medium chosen for the above-mentioned experiment is being used in our laboratory in routine work for induced organogenesis in the calluses of *Nicotiana tabacum*. When comparing these 2 plant species, it can be concluded that the identical nutrition and hormone conditions were well acceptable even for such systematically and ecologically remote plants as the cacti are. In order to increase the production of regenerants, the explants should be subcultivated first on media stimulating rapid and rich callus growth (e.g., by addition of 2,4-dichlorophenoxyacetic acid) with subsequent subcultivation on the media inducing organogenesis. It would perhaps be possible to obtain still better growth conditions by modifying the basal MURASHIGE and SKOOG[6] medium. By a proper balance of the synthetic medium some unfavourable effects, e.g., necrosis and anthocyanescence, could probably be avoided.

Mamillaria woodsii may be used as an example demonstrating rapid and mass propagation of selected genotypes in cacti. The development period of regenerants in tissue cultures is at least 1 year shorter as compared with the vegetation period of plants obtained from seeds. Moreover, the production of virus-free plants from tissue cultures of cacti may be of great significance. If the above clonal multiplication method is improved and adopted to other species of the Cactaceae family, it might be recommended for horticultural practice as commonly used tissue cultures in vitro in orchids or carnations.

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Brain Chopping: A New Method for the Rapid Removal of Newborn Rat Brain[1]

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Summary. A new multi-bladed air-driven guillotine is described. This device removes newborn rat heads and slices them into several thin wafers, which facilitates freezing and permits easy regional dissection.

In neurochemical investigations it is frequently desirable to measure both labile metabolites and enzymes in the same sample from various regions of the brain. Decapitation or submersion of the intact animal in liquid nitrogen results in a delay of many seconds before subcortical brain regions freeze[2–5], which may result in significant

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Comparison of various metabolites in newborn rat brain obtained by submersion in liquid nitrogen and by the new brain-chopping technique[a]

	Submersed		Chopped	
	Cortex	Brainstem	Cortex	Brainstem
Glucose	1.83 ± 0.10	1.95 ± 0.08	1.52 ± 0.09	1.86 ± 0.12
Glucose-6-PO_4	0.181 ± 0.022	0.152 ± 0.009	0.143 ± 0.010	0.151 ± 0.009
Lactate	1.36 ± 0.23	1.28 ± 0.23	0.62 ± 0.04[b]	0.72 ± 0.07[b]
Pyruvate	0.039 ± 0.008	0.060 ± 0.012	0.051 ± 0.013	0.038 ± 0.006
ATP	3.65 ± 0.30	3.33 ± 0.11	3.58 ± 0.17	3.56 ± 0.14
ADP	1.57 ± 0.17	1.74 ± 0.14	1.36 ± 0.15	1.58 ± 0.14
AMP	0.29 ± 0.04	0.40 ± 0.07	0.40 ± 0.05	0.50 ± 0.03

[a] Data expressed as m moles/kg wet weight. Each mean ± SE is derived from 5 to 8 animals. [b] $p < 0.05$ as calculated by the Mann-Whitney U-test.

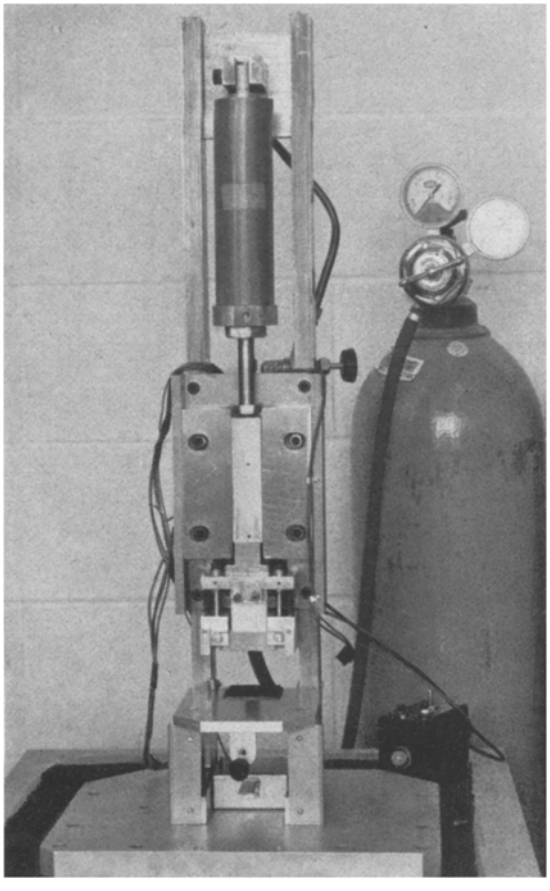
changes in the levels of highly labile metabolites[6]. Further, the subsequent regional dissection of the frozen brain is difficult, especially in newborn rats since the low temperature makes the brain fragile and brittle.

The method of freeze-blowing permits removal and freezing of the supra-tentorial portion of the brain more rapidly than any other method[7]. Unfortunately, regional studies are currently not possible with this method, and it is only suitable for adult rats. A method in which a 14 mm slab is removed from a rat head by rotating blades and then clamped between 2 pre-chilled aluminum pistons is also rapid but does not permit regional analysis[8]. Perhaps the most effective method for rapid inactivation of labile metabolites and subsequent regional dissection is microwave irradiation, which effectively fixes labile metabolites, and regional dissection is readily accomplished. However, since many enzymes are irreversibly inactivated by the heat, it is impossible to measure both metabolites and enzymes in the same sample[9].

We, therefore, have developed a new apparatus for rapidly fixing newborn rat brain. This method facilitates easy regional dissection, and permits the measurement of both enzymes and metabolites in the same samples. This device is an air-driven multi-bladed guillotine which, in 200 msec, slices the animal's head into several thin wafers. These wafers are then dropped in a container of 2-methylbutane brought to its freezing point with liquid nitrogen. The thin wafers freeze rapidly, and can be readily dissected into various regions.

In experiments to test the brain chopping apparatus, pregnant Sprague-Dawley rats were obtained commercially, housed individually, and fed and watered ad libitum. Newborn animals were sacrificed when 1–2 days old; litters were split into 2 groups, 1 group was sacrificed by submersion, the other by the new chopping technique. Animals sacrificed by submersion were grasped lightly by the tail with forceps, and quickly plunged into either liquid nitrogen or 2-methylbutane brought to its freezing point with liquid nitrogen. The animals were held under the coolant until bubbling stopped.

Animals sacrificed by the air-driven multi-bladed guillotine (Figure) were positioned with their heads on the trap door. Operating at 125 pounds per square inch, a piston carrying the cutting blades is driven downward toward the trap door. When the blades have passed through the head of the rat, the trap door opens, and the 3–4 6 mm thick coronal wafers are mechanically stripped from between the blades, whereupon they drop into the coolant. The entire cutting and stripping process takes about 200 msec. With 2-methylbutane as the coolant, freezing of the 6 mm wafers is achieved in less than 4 sec. The wafers are usually intact anatomically, and most brain regions can be visualized. In addition, when placed on dry ice slabs, these wafers are readily trimmed of skin and bone, and appropriate brain regions can be isolated.



Brain chopping apparatus. See text for details of operation.

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Parietal cortex and brainstem were dissected and rapidly weighed on a Roller-Smith balance and subsequently homogenized in 1.0 ml of cold 0.3 *N* perchloric acid containing 1.0 m*M* EDTA. After centrifuging at 25,000 *g*/30 min, the supernatants were neutralized with 3.0 *N* $KHCO_3$.

The intermediates glucose, glucose-6-phosphate, lactate, pyruvate, ATP, ADP, and AMP were measured by the methods of LOWRY and PASSONEAU[10]. The Table compares the net levels of these intermediates in parietal cortex and brainstem of animals sacrificed by the two methods. Animals sacrificed by submersion in liquid nitrogen or in 2-methylbutane cooled to its freezing point with liquid nitrogen had similar metabolite values, and so these control data have been pooled.

Note that the lactate concentration was found to be significantly lower in both parietal cortex and brainstem of the chopped as compared to the submersed animals. Higher values as measured in the submersed group are associated with anoxia due to slow freezing of tissue[6]. The levels of the other metabolites measured were comparable in newborn rats sacrificed by the two methods.

In this study, we have developed and validated a new air-driven multi-bladed guillotine which rapidly sacrifices newborn rats, and fixes their brains such that regional studies are possible. We have compared commonly measured metabolites in animals sacrificed by the conventional submersion technique, and by the new chopping method. We find that only lactate, the most labile intermediate measured, was significantly lower in animals sacrificed by the chopping method. Since the small newborn rats have minimal insulative skin and bone covering their brains, submersed animals freeze almost as rapidly as the wafers. Major differences in the levels of all metabolites might be expected when comparing submersion vs. chopped adult animals. Modifications to the chopping apparatus which will allow accomodation of adult rats are currently being made.

The major advantage of this technique is that it facilitates regional studies in newborn rats. Thin, flat coronal wafers are readily dissected on dry ice into various anatomical regions. It is also sometimes advantageous to measure both enzymes and metabolites on the same sample. An advantage of the chopping method over sacrifice by microwave oven is that enzymes are not inactivated.

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Technical Note About Simultaneous Recording of Oxygen Partial Pressure and Neuronal Activity in Cat Cortex

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Summary. A method is described for simultaneous measurements of pO_2 and action potentials with microelectrodes using prefabricated gold wires. The construction and measuring with such gold electrodes is easier than with traditionally used platinum electrodes. Single electrode recording is done with a specially adapted electronic circuit, allowing the separation of both signals (pO_2 and action potentials. A measurement in cat brain illustrates this procedure.

In brain as in other tissues the local pO_2, when measured on a microscale, shows considerable spatial nonhomogeneities depending on the distance from the nearby capillaries, pO_2 in those capillaries, and O_2 consumption of the region under investigation. The following method allows a direct determination of pO_2 and action potentials (APs) simultaneously in the range of intercapillary dimensions. This requires a miniaturization of the measuring elements, which gives rise to certain problems described in detail.

A) *The gold microelectrode.* 1. *Construction.* The construction of the electrode, based on the principle published by ERDMANN[2], uses glass insulated gold wires, manufactured by the Battelle-Institute (Frankfurt/Main, Germany). After connecting of the gold wire to a copper wire by a conductive adhesive, it is threaded in a glass capillary so that it protrudes from the peripheral end of the capillary. To ensure mechanical stability, both wires are fixed at their respective ends of the glass with Araldit AY103 (Ciba-Geigy, Basel, Switzerland). The electrode tip is then abraded obliquely on diamond paste, so that the diameter of the tip is no more than 1–2 µm. This allows a good penetration of the electrode into the tissue, without gross damage to cellular structures. Finally, by dipping the tip in a 2% polystyrol-CCl_4 solution and fixing at 70 °C, the gold wire is coated with an O_2 permeable membrane. The loss of sensitivity which is often observed after the abrading process can be abolished by etching the gold on a length of about 20 µm in a KCN solution and new gilding of the tip in a gold chloride bath by applying 50 nA for 45 sec under microscopic control.

2. *Physical parameters.* Before use, the physical parameters of the electrodes have to be examined to control the quality of both wire and membrane. Examinations of polarographic curves confirm the finding of other authors[3] that the 'plateau' lies between 700 and 1200 mV. Therefore, we perform our pO_2 measurements at a voltage of (−1000 mV) against a silver-silver chloride reference electrode. At this voltage, the pO_2 dependent current curve has to be linear and the residual current at zero O_2 tension very low. It is recommended to apply a 1V potential for 24 h before use, thus permitting a suitable stabilization of the electrode. The current is only slightly temperature-dependent (0.03 nA/°C), while it is not affected by pH variations in the physiological

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