Localized Cerebral Hypothermia.* (23686)

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A number of technics have been developed for localized cooling of the brain. They have in common either total vascular isolation of the perfused brain, as described by Malméjac for instance(1), or irrigation of the brain with cooled blood through the carotid arteries with an unimpeded efferent flow into the systemic circulation of an intact animal(2-7). In several experimental applications of the latter design and in one clinical trial(8), a significant lowering of body temperature has also been noted, and brain-body circulatory mixing must be considerable. The pattern of the present study includes an intact systemic circulation and a localized extracorporeal cerebral circulation whose afferent and efferent channels are controlled through the carotid arteries and jugular veins by a dual channel, independently variable, torque conversion Sigmamotor pump.

Methods. The perfusion unit consisted of a 500 cc bubble oxygenator into which blood and 100% oxygen were pumped, a Monel metal helix heat-exchanger immersed in ice water and stainless steel filters, included in the circuit to remove fibrin clots. Bead thermistors were used to record temperature gradients in the extradural space, the esophagus and rectum. An electromagnetic Rotameter was used to record perfusion flow and perfusion pressure was measured by a Sanborn electromanometer. Paired silver electrodes were placed in the frontal and occipital extradural areas and standard limb EKG leads were used. The systemic arterial pressure was measured by a Statham strain gauge. These observations were recorded on a 6 channel Offner Polygraph and a 4 channel Sanborn Polyviso. The systemic venous pressure was measured on a standard water manometer. Hematocrit, clotting-time and arterio-venous blood oxygen samples were obtained from appropriate areas of both circulations. Mongrel dogs were used and 43 studies have been carried out in the continuing development of this technic. Each animal was anesthetized with nembutal, 20 mg per kg, and placed on a Palmer respirator giving 400 cc of 100% oxygen per minute through an endotracheal tube. After operative preparation of the recording sites, both carotid arteries and both external jugular veins were prepared for cannulation. The extracorporeal circulation unit was primed with 1500 cc of donor blood secured 24 hours previously by exsanguination. Both jugular veins were cannulated and connected with a venous reservoir through gravitational flow. The carotid arteries were cannulated in sequence and the perfusion started. Each animal received 25 mg of heparin. At the end of the perfusion period, ranging from 40 minutes to 3 hours and 55 minutes, the jugular veins were ligated, the carotid arteries repaired and rewarming occurred through the systemic circulation. The extradural electrodes were left in place. EEG recordings were taken at survival periods of 5 to 33 days, the animals sacrificed and their brains studied by standard neuropathological methods.

Results. The localized brain cooling produced by this technic possessed the properties of rapid induction and ready reversibility and results obtained were reproducible in all parameters of study within the limit of technical errors. Perfusion flow, or perfusion pressure, was the single important factor in terms of survival. Flow rates above 18 to 20 cc per minute per kg resulted in high systemic venous pressures, cardiac failure and autopsy evidence of cerebral edema. In the normothermic animal, perfusion flows of 6 to 7 cc per minute per kg allowed survival. In the hypothermic brain, flow values of 10 cc per minute per kg permitted the perfusion pressure to approximate the value of the systemic carotid pressure and these animals did well.

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With perfusion flows of 6 to 10 cc per min. per kg, brain temperature dropped an average of 10 degrees in 30 minutes and reached levels of 21° to 20°C after 60 minutes of perfusion. There was a linear relationship between brain cooling and the systemic blood pressure and at brain temperatures of 15° to 20°C, the mean systemic arterial pressure might range between 30 and 50 mm of mercury. The changes in cortical electroactivity noted in this form of localized brain cooling were identical to those described in generalized hypothermia(9) or in states of hibernation(10). Slow wave activity became apparent after cooling the brain to 32°C. There was a concomitant fall in amplitude as the temperature was reduced and the recordings became flat at 21° to 20°C. The cortex remained electrically silent below this temperature and when the gradient was reversed, small bursts of low amplitude activity reappeared at 21°C. At 30°C, fast activity dominated the pattern and the record became normal again at the pre-cooling temperature level. No abnormal changes were noted in surviving animals.

The principal finding in the electrocardiogram was a relative bradycardia that developed with progressive cooling. The average cardiac rate at the beginning of perfusion was 156 and was reduced to 88 when electrocortical activity ceased. Associated with the decrease in rate, the QT interval was prolonged from a pre-cooling average of .23 second to an average of .43 second when the brain temperature reached 21°C. There were no instances of ventricular fibrillations or other cardiac arrhythmias. The rectal temperature fell from an average of 38°C to one of 32°C and was dependent upon the length of perfusion. Low body temperatures usually occurred at the end of perfusion periods when rewarming of the brain was carried out by the animal's systemic circulation.

The arterio-venous oxygen difference in the perfusion blood of the cerebral circulation fell in a linear fashion with decreasing brain temperatures and reached 0.0 at a brain temperature of 21° to 20°C. There was little change in AVO difference in the systemic circulation unless the animal's body temperature at the stage of rewarming fell below 34°C. Brain-body circulatory mixing could be demonstrated by the addition of 30 μc of radioiodine labelled serum albumen in the blood volume to be circulated through the brain. By conventional radioanalysis methods, the percentage of circulatory mixing could be determined and, in 3 animals, this measured between 15 and 20%. In 6 animals, the blood perfusate was replaced by Ringer's solution at a brain temperature of 20°C, and venous return hematocrits from the brain measured at 5-minute intervals at a constant perfusion pressure. These fell from 14% during the first 5 minutes of perfusion to 5% at the end of 30 minutes, again suggesting a collateral circulation approximating 15%. At these flow levels, no evidence of air embolism as a result of blood oxygenation could be demonstrated.

Conclusion. Localized cerebral hypothermia has been produced in the dog by an extracorporeal circulation utilizing the carotid arteries and external jugular veins. During the acute stage of perfusion, various parameters of study have been recorded and in general, these results are in accord with those noted in states of generalized hypothermia. Survival is predicated upon low perfusion flows and development of perfusion pressure closely aligned with the systemic arterial pressure. Such long-term survivals allow the further investigation of the effect of various drugs and chemical substances upon the normothermic and hypothermic brain whose circulation is isolated in a measurable manner from the systemic circulation.

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Modification of Sex Differentiation by Steroid Hormones in a Tree Frog (*Pseudacris nigrita triseriata Wied*).* (23687)

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Amphibians have been used most successfully in experiments on hormonal modification of the processes of sex differentiation. While results obtained with different taxonomic orders and families first seemed irregular or even contradictory, accumulation of a considerable body of data is now suggesting a correlation between genic sex type and mode of response(1). In view of the possible bearing of this relationship on the theory of gene action, verification by further extension of the experiments to still untested groups is desirable. The present report on a tree frog bears on a family (hylidae) which, according to Yosida (2) and the senior author's own observations, seems chromosomally related to the family of the common frogs (ranidae).

Material and methods. Normally deposited eggs were collected near Iowa City and kept in finger bowls until hatching of the tiny larvae. On the day when feeding started, groups were separated according to the planned hormone treatments. The experimental lots were placed and reared in steroid solutions of the concentrations indicated in Table I. Mortality was moderate, too low to affect the outcome of the tests.

Results. Testosterone propionate is completely masculinizing the genetically female half of the treated groups. The used concentrations of 50 and 500 μg/l obviously are not limit values, but they are sufficiently apart to permit the conclusion that all effective dosages are masculinizing. The study of normal sex differentiation of the same species by Deal (3) revealed that all male gonads are invaded by numerous pigment cells during and immediately following initial sex differentiation (larvae 15 to 18 mm). They move along with ingrowing mesonephric blastema cells. Ovaries never are pigmented. In the experimental transformation of genetic females into males, in the present experiments, the testes became as darkly pigmented as those of genetic males. In contrast to the persistently masculinizing action of testosterone, the effect of estradiol (and estrone) treatments depends on the dosage level; it is feminization at 50 μg/l, but an almost normal sex ratio at the higher level (Table I).

Discussion. The described experiments with treefrogs give results that are essentially identical with those obtained in more extensive tests with *Rana sylvatica*(4). The slowly but steadily accumulating data on reactions of amphibians belonging to various taxonomic orders and families permit now to distinguish

TABLE I. Effect of Continuous Steroid Hormone Administrations on Sex Differentiation in Pseudacris. Treatments started after closure of gill sacs (Stage 25). All animals preserved and sexed at end of metamorphosis (Stage 33).

Hormone		Females	Intersexes	Males
Testosterone	50 μg/l			27
"	500 "			209
Estradiol	50 "	16		
Estradiol + Estrone	100 " 200 "	104	1	96
Controls		37		33

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