SUMMARY The ill effects of carotid artery occlusion or ligation vary in man and experimental animals according to surgical techniques, environmental conditions, diet, stress, sex, age, strain of the animal, blood pressure, the state of cardiopulmonary system, and other diverse factors. Unilateral carotid artery ligation in most small laboratory animals is an innocuous procedure. The gerbil, a desert rat, has been found to be quite sensitive to unilateral carotid artery ligation. The present experiments were designed to study the influence of age, sex, and castration on end results of the unilateral carotid artery ligation in gerbils.

It appears that the young animals prior to sexual maturity and the sexually mature female are more resistant than the sexually mature male. This difference is assumed to be due to the influence of the gonads on the blood flow of the brain.

THE PRINCIPAL ill effect of occlusion of the common or internal carotid artery (hereinafter referred to as the carotid) is ischemic brain damage. Arteriosclerotic occlusive disease of the carotid is quite common. However, these lesions are not associated with brain damage in every case, most probably because of the slow progress of the disease. In contrast, acute thromboembolic lesions and surgical ligations of the carotid are frequently accompanied by dramatic physiological changes. Ligations of the carotid artery is used occasionally as a therapeutic measure for certain human pathological states, such as head and neck tumors and aneurysms. The eventual outcome of the carotid artery obstruction depends on many factors. Fields et al. report six cases of bilateral arteriosclerotic occlusions of the carotid arteries with no brain damage. This is unusual however. The more common picture, even with a unilateral carotid obstruction, is infarction of the brain and at times death.

The subject has also been investigated by those interested in experimental neurological sciences. The results of carotid artery ligation in experimental animals vary according to many factors including species. Chang and Liu noticed 100% mortality in rats after bilateral carotid ligation. Jilek reported 40% mortality in adult rats, whereas Levine and Klein found 8 to 56% survival depending on environmental conditions. The mortality and morbidity of the carotid artery ligation in rats are also influenced by the strain and age of the animal. It should be noticed, however, that these reports are the result of bilateral carotid ligation. Unilateral carotid ligation is almost always innocuous in rats and other small laboratory animals. The only exception to this observation is the Mongolian gerbil (Meriones unguiculatus). The gerbil, a desert rat, is a valuable small laboratory animal used by many investigators in the fields of endocrinology, infectious diseases, temperature regulation, and water metabolism. The susceptibility of the gerbil to carotid artery ligation makes the animal unique for research in brain ischemia. The present experiments were designed to study the influence of age, sex, and castration on the final outcome of the unilateral carotid artery ligation in gerbils. It appears that the young animals prior to sexual maturity and sexually mature females are more resistant than the sexually mature male.

Methods

Gerbils varying in initial body weight, age, and sex, as indicated in table 1, were divided into eight experimental groups. Subgroups of four to five were placed in plastic cages with free access to commercial laboratory chow and tap water. Carotid artery ligation and castration were done according to a prescribed time schedule (table 1). Ether was used for anesthesia. A small incision was made above the sternal notch. The carotid artery on the left side was dissected, freed from the adjacent structures, particularly the nerves, doubly ligated, and cut. The brains were fixed in Bouin's solution for 48 hours and sectioned. The frontal and occipital poles were discarded, and the rest of the cerebral hemispheres were divided into four equal parts to be embedded in paraffin. Slides were stained with hematoxylin-eosin and Luxol-fast blue for microscopic examination.

Results

The mortality rate for mature noncastrated males and females was 57% and 23%, respectively. The rate of combined mortality and morbidity (brain ischemic lesions) for mature male animals was twice that of mature females. The same significant difference was true for the animals which were castrated after sexual maturity. However, if the carotid artery ligation was performed prior to sexual maturity (table 1, groups 5 and 6), the difference in mortality between the male and female was lost. In the last two groups (7 and 8), the animals were castrated prior to sexual maturity but allowed to grow by age and weight (10 to 12 weeks, 50 gm or more) to maturity before undergoing carotid artery ligation. The results were similar to those of groups 5 and 6 with no significant difference in mortality rate between the male and female (fig. 1). The number of surviving gerbils with brain ischemic damage, as well as the mortality rates, is shown in table 1.

Histopathology

There was a total of 38 animals with ischemic lesions in the brain. Fourteen of them had massive cerebral infarction of the left side. Of the remaining 24 which had focal lesions, 13 involved the cortex, 12 basal ganglia, 14 hippocampus, and 4 corpus callosum (figs. 2 and 3). Some of the animals

\*Purchased from Tumblebrook Farm, West Brookfield, Massachusetts.

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Table 1 Influence of Age, Sex, and Castration on Carotid Artery Ligation in Gerbils

<table>
<thead>
<tr>
<th>Group</th>
<th>No.</th>
<th>Maturity (sex)</th>
<th>Castration</th>
<th>Carotid artery ligation (body wt, gm)</th>
<th>Mortality</th>
<th>Brain lesions in survivors</th>
<th>Mortality and morbidity*</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>65</td>
<td>Mature (M)</td>
<td>—</td>
<td>Day 1 (60)</td>
<td>37/65 = 57%</td>
<td>2/28 = 7%</td>
<td>39/65 = 60%</td>
</tr>
<tr>
<td>2</td>
<td>51</td>
<td>Mature (F)</td>
<td>—</td>
<td>Day 1 (52)</td>
<td>12/51 = 23%</td>
<td>3/39 = 7%</td>
<td>15/51 = 29%</td>
</tr>
<tr>
<td>3</td>
<td>40</td>
<td>Mature (M)</td>
<td>Day 1 (60)</td>
<td>Day 31 (66)</td>
<td>19/40 = 49%</td>
<td>5/21 = 24%</td>
<td>24/40 = 60%</td>
</tr>
<tr>
<td>4</td>
<td>40</td>
<td>Mature (F)</td>
<td>Day 1 (51)</td>
<td>Day 31 (58)</td>
<td>8/40 = 20%</td>
<td>4/33 = 12%</td>
<td>12/40 = 30%</td>
</tr>
<tr>
<td>5</td>
<td>50</td>
<td>Immature (M)</td>
<td>—</td>
<td>Day 1 (42)</td>
<td>15/50 = 30%</td>
<td>9/35 = 26%</td>
<td>24/50 = 48%</td>
</tr>
<tr>
<td>6</td>
<td>48</td>
<td>Immature (F)</td>
<td>—</td>
<td>Day 1 (38)</td>
<td>15/48 = 32%</td>
<td>4/33 = 12%</td>
<td>19/48 = 39%</td>
</tr>
<tr>
<td>7</td>
<td>40</td>
<td>Immature (M)</td>
<td>Day 1 (34)</td>
<td>Day 31 (63)</td>
<td>14/40 = 35%</td>
<td>5/26 = 19%</td>
<td>19/40 = 47%</td>
</tr>
<tr>
<td>8</td>
<td>40</td>
<td>Immature (F)</td>
<td>Day 1 (37)</td>
<td>Day 31 (51)</td>
<td>16/40 = 40%</td>
<td>6/23 = 26%</td>
<td>22/40 = 55%</td>
</tr>
</tbody>
</table>

*Morbidity (ischemic brain lesions).

had more than one lesion. The ischemia of the different areas of the brain revealed a higher percentage of involvement of the cortex in males (10 out of 21) than in females (3 out of 17). Comparison of the other focal lesions of the brain evinced no significant difference.

Additional Experiments

A few other experiments not included in table 1 were also carried out. Ten male and ten female animals had both carotid arteries ligated within a few minutes; all died in four hours. Sections of their brains revealed massive edema.

Several other groups of animals (216 gerbils), both male and female, were used for bilateral carotid artery ligation at intervals of 1 day to 18 weeks. Virtually all these animals (95%) died within a few hours after the second ligation. In a third group of experiments, animals were used to show the difference in mortality rate after unilateral ligation of the right and left carotid arteries. No significant difference was found. Regardless of the experimental design, all animals which died had generalized, spontaneous convulsions prior to death.

Discussion

The significant difference between the mortality rate of the male and female gerbils, as well as that between the mature male and sexually immature male, is probably related to gonadal hormones. The influence of the gonads on the blood flow of the brain was described by Gordan. Working with humans and rats, he noticed that the amount of cerebral blood flow in adults is 54 ml/100 gm of brain weight/min. This value, however, is 105 ml for children. He further stated that resection of the gonads will not influence this value in adults unless it is done prior to sexual maturity. In a study of 500 carotid strokes in man, Jennett and Cross discovered that pregnant women with stroke had a considerably higher mortality rate than men or nonpregnant women. Neurological catastrophes related to oral contraceptives have been described on many occasions.

Experimentally, female mice have been reported to be more resistant to hypoxia of the brain. Subcutaneous injection of 50 mg/kg of benzoate estradiol to neonatal male mice reduced their mortality rate to the same level as the female. Resection of the adrenal glands in mature rats with or without removal of gonads is accompanied by a significant increase in mortality and morbidity associated with carotid artery ligation. The mortality, however, is greater when adrenals are removed prior to sexual maturity, particularly when combined with gonadectomy. The results of the present experiments seem to be in agreement with the hypothesis that gonadal hormones influence the blood flow of the brain, and therefore, the end result of the carotid artery ligation could vary, as it does in gerbils, according to sex and sexual maturity.

It appears that at the time of sexual maturity, gonadal hormones influence and reduce the amount of the blood flow.
to the brain. Resection of the gonads will prevent the alteration of the blood flow of the brain provided it is done prior to sexual maturity.14 Apparently, once the change in the brain blood flow is established by gonadal hormones at the time of sexual maturity, castration will not increase or alter the blood flow to the original childhood level. For this reason castration of the gerbils after sexual maturity has minimal influence on the end result of the carotid artery ligation.

References

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