Erythropoiesis in Carotid Body Resected Cats

By D. B. Gillis and R. A. Mitchell

It has been reported that removal of carotid bodies in cats results in a brief, profuse reticulocytosis followed by depression to below-normal levels of reticulocytes and a progressively severe anemia. Injection of cat carotid body extract was reported to increase erythrocyte $^{59}$Fe incorporation in polycythemic rats. Other studies could find no hematological abnormality in humans after bilateral carotid body resection. We reexamined the effect of carotid body resection in the cat with serial bone marrow aspirations, hematocrit determination, and reticulocyte determination. $T_{1/2}$ for plasma $^{59}$Fe removal, erythrocyte $^{59}$Fe incorporation, and $^{51}$Cr lifespan determinations were performed on five operated and three control cats. No significant differences were found. We conclude that the carotid body has no direct effect on erythropoiesis and that the anemia reported in a prior study was secondary to sepsis resulting from an indwelling femoral vein catheter.

The kidney has long been considered the source of erythropoietin (erythropoiesis stimulating factor, ESF) in man and other mammals. Recent evidence suggests that a renal erythropoietic factor, REF, activates a serum substrate of unknown origin to produce ESF (erythropoietin). Other investigators have looked for a role for the carotid body in the regulation of erythropoiesis, usually studying the erythropoietic response to hypoxia after removal or denervation of the carotid bodies. These studies yielded no evidence of impaired erythropoietic function. Tramezzani et al., however, have recently reported marked hematocrit falls in cats following bilateral carotid body resection and increased $^{59}$Fe utilization in polycythemic cats after injection of cat carotid body homogenate or venous blood from the carotid body. Other investigators have found no change in hematocrit in humans following bilateral carotid body resection.

We have reexamined the effect of bilateral carotid body resection on erythropoiesis in the cat, using multiple indicators of marrow function, and have found no depression of erythropoiesis resulting from carotid body removal. A preliminary report of this work has been presented to the American Physiological Society (1972).

MATERIALS AND METHODS

Eight adult male cats (3.8-5.2 kg) were isolated, administered routine feline immunizations, and after 6-8 wk were considered to be in a stable, uniform metabolic state.

The eight cats were distributed into three groups: "operated" cats (five animals) who underwent bilateral carotid body resection as described below, a sham-operated control (one animal), and unoperated controls (two animals).

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We resected the carotid bodies under halothane in oxygen general anesthesia. Using sterile technique, the neck was opened ventrally in the midline. Carotid bodies were located and excised using a dissecting microscope. Surgical specimens were fixed in Bouins solution, sectioned, and stained with iron hematoxylin aniline blue for histological verification of carotid body removal. At the conclusion of the study, this group was sacrificed and the carotid bifurcation excised bilaterally and sectioned for histological study. Iron hematoxylin aniline blue stained sections were used to verify adequacy of carotid body excision.

A sham-operated control animal underwent similar anesthesia and surgical trauma; bilateral section of the superior laryngeal nerves was done instead of carotid body resection.

We performed hematocrit determinations, erythrocyte and leukocyte counts, hemoglobin by the cyanmethemoglobin method, and reticulocyte counts. Serum iron and total iron binding capacity were determined in five randomly selected cats, 1–2 wk prior to conduct of the isotope studies. The uncertainty introduced into iron-turnover calculations was accepted in order to reduce to a minimum blood sampling on day 1 of isotope studies.

Several milliliters of marrow were obtained from either femur of a lightly anesthetized animal (halothane in oxygen), and smears were prepared using standard techniques.

Isotope Studies

All cats were studied with $^{59}$Fe to determine plasma volume, plasma iron turnover, and erythrocyte iron incorporation. $^{51}$Cr was used to determine blood volume and estimate erythrocyte lifespan. The ferrokinetic studies conducted were as described by Pollycove and Mortimer with modifications for use in the cat. $^{51}$Cr studies were by the method of Small and Verloop with modifications. Detailed descriptions of these methods have been reported elsewhere and will only be outlined here. A polyethylene catheter was introduced under halothane general anesthesia into either external jugular vein and advanced to the vena cava. Blood samples were obtained from this catheter for isotope studies and all specimens withdrawn via the catheter for 24 hr. At 24 hr, we removed the catheter and further samples were obtained from venipuncture of the anterior forelimb vein in awake animals.

Three milliliters of whole blood was incubated for 45 min at room temperature with 25 $\mu$Ci of $^{51}$Cr as sodium chromate. Ascorbic acid, 15 mg, was then added to terminate erythrocyte labeling, and plasma and whole blood standards were prepared. Packed cell volume of the sample injected volume and activity of the standards were used to determine injected dose. Blood volume was calculated from label dilution at 15 and 30 min after injection. Additional blood samples were obtained periodically for 34 days and erythrocyte lifespan calculated as the time required for reduction of label to 50% of its activity at 1 day after injection.

For ferrokinetic studies 6 ml of heparinized whole blood was centrifuged and the plasma removed. One two microcuries per kilogram of body weight was added and the mixture incubated at 37 °C for 30 min. After preparation of a 1:500 dilution standard, the labeled specimen was injected via the jugular catheter. Injected dose was calculated from the injected volume and activity of the standard. Eight plasma samples were obtained within 24 hr, four within 4 hr of injection of the label. From this data and mean serum iron of the group we calculated plasma volume, half-life for plasma iron ($T_{1/2}$), and plasma iron turnover estimates. Whole blood samples were obtained for 34 days and used with sample hematocrit and $^{51}$Cr blood volume determination to calculate erythrocyte iron incorporation as a percent of the total injected $^{59}$Fe dose.

All isotope samples were counted with standards and controls in a two-channel gamma spectrometer.

We conducted isotope studies once on each cat. Operated cats underwent these studies 1 wk (one cat), 2 wk (two cats), 6 wk (one cat), and 8 wk (one cat) postoperatively. The sham-operated animal was studied 1 wk postoperatively.

At the conclusion of the study we sacrificed all operated cats, removed the area of the carotid bifurcation, and prepared serial sections of the specimen at 200-μ intervals.

RESULTS

Histological examination of surgical specimens confirmed successful bilateral removal in four cats and unilateral removal in cat No. 2. Histological examinations at the conclusion of the study confirmed these findings.
Table 1. Hematocrit Variations After Carotid Body Resection

<table>
<thead>
<tr>
<th>Glomectomized</th>
<th>5-7 Day Postop.</th>
<th>13-15 Day Postop.</th>
<th>20-22 Day Postop.</th>
<th>End of Study</th>
<th>Hct change from Baseline</th>
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<td>1</td>
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<td>Tromezzani et al.</td>
<td>39</td>
<td>34</td>
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</table>

*Hematocrits in baseline period are average values. Number of determinations is given in parenthesis.
†Sham-operation consisted of 3 hr Halothane anesthesia and bilateral section of superior laryngeal nerves.
§Control cat hematocrit values are postisotope studies, as no surgery was performed on controls.

Table 1 lists hematocrit variations during the course of the study. Baseline hematocrits averaged 42.5%, for unoperated controls and 42% for carotid body resected (CBR) cats. All cats, with the exception of the sham-operated control, had a fall in hematocrit in the course of the study, but by the end of the study hematocrits returned to 38.5% in controls and 38.6% in CBR cats.

At no time did bone marrow smears reveal decreased cellularity; on the contrary, there appeared to be an increase in erythroblasts. Reticulocyte counts were always below 0.5% of circulating red cells, but were always present. No increase was noted after carotid body removal, although toxic stippling did occur frequently.

For technical reasons, satisfactory plasma iron removal and plasma volume data in CBR cat No. 3 were not obtained. Data for the initial 120 min (single exponential portion) of the plasma iron clearance are plotted in Fig. 1. Mean serum iron concentration for five randomly selected cats was 92.4 ± 7.05 (SD) mg/100 ml blood. Mean total iron binding capacity was 233.6 ± 14.09 (SD) for the group. Half-time for removal of 59Fe averaged 34.3 min in the three controls and 34.9 min in four operated cats. Fractional iron transfer rate thus averaged 30.6 per day in controls and 30.2 per day in operated cats.

Erythrocyte 59Fe incorporation averaged 93% in controls and 92% in CBR cats as is shown in Fig. 2. Blood volume in the unoperated controls was 66 and 68 ml/kg of body weight and 64.8 ± 0.97 (SD) in operated cats. Red cell life-span averaged 9.5 ± 1.25 (SD) in CBR cats and 9.66 ± 0.7 (SD) in control cats, uncorrected for 51Cr elution. Some hemolysis of undetermined origin occurred in 51Cr specimens of two control cats and two operated cats, resulting in an early rapid loss of 51Cr label. After 2-3 days, rate of disappearance of 51Cr was similar in all animals.

With one exception, all animals tolerated removal of carotid bodies well,
maintaining weight and erythropoietic function. One cat, No. 5, experienced a severe postoperative infection with fever to 105°F and leukocytosis to 35,000 WBC/cu mm. With intensive fluid and antibiotic therapy he recovered fully. Cats No. 1 and 2 died suddenly of urethral calculus at 102 and 144 days postoperatively, respectively. Such a demise is a frequent occurrence in male cats at this institution, and is presumably related to diet.

DISCUSSION

After removal of the carotid sinus and denervation of the aortic arch in rabbits, Latner concluded that the area of the carotid sinus played a role in determining the normal blood picture. A transient anemia and a brief, profuse reticulocytosis was noted postoperatively. Although not specifically stated, it may be concluded that ether was the anesthetic used. One-third of the animals died as a result of the surgical procedure.

Later, Schafer performed similar experiments using the dog and found eventual increases in hematocrit and red cell mass. Grant considered the likely organ involved to be the carotid chemoreceptors, which he removed or ligated.
The aortic arch was denervated, and the rabbits exposed to hypoxia in a low-pressure chamber, to bleeding, or to cobalt administration. No differences were observed between control and operated except in response to hypoxia. Chemoreceptor-denervated animals showed an augmented erythropoietic response, presumably secondary to absence of a ventilatory response to hypoxia, and consequently more severe hypoxia, than control animals.

In 1955, Terzioglu et al. reported a similar study in which chemoreceptor denervation of rabbits with sodium pentobarbital anesthesia showed a transient reticulocytosis and an hypoxic erythropoietic response greater than that of controls. In 1966, Gilfillan et al. performed chemoreceptor denervation in the dog and noted that exposure to high altitude resulted in a marked increase in erythrocyte volume and hematocrit. Thus, most evidence indicated that erythropoiesis was not impaired as a result of chemoreceptor denervation, and, indeed, marked increases in hematocrit resulted from an hypoxic stimulus.

Recently, Tramezzani et al. reported that removal of the carotid bodies resulted in an immediate, profuse reticulocytosis followed by disappearance of reticulocytes and progressive, severe anemia. The injection of carotid body homogenate was reported to stimulate reticulocytosis in cats and increased incorporation by erythrocytes in the polycythemic rat. Few of the operated cats survived for more than 3 wk. Tramezzani et al. concluded that the carotid body in the cat secreted one or more substances with erythropoietin activity.

Lugliani et al. studied 57 patients who had undergone bilateral carotid body removal and found no anemia. In five of the patients the reticulocyte response to acute phlebotomy of 1 liter of blood was equivalent to that of a control group. They concluded that in man carotid bodies were not an important source of erythropoietin. Fogh et al. have found carotid body removal to result in serum concentrations of erythropoietin greater than that of controls in response to hypoxia. They were unable to detect erythropoietin in carotid body homogenates.

We have found no anemia of significance after bilateral carotid body removal when compared to controls, and suggest that the severe anemia reported in Tramezzani's study was secondary to sepsis resulting from their use of a chronic indwelling femoral vein catheter. By obtaining our samples by venipuncture we avoided this complication. Neither did we observe reticulocytosis after carotid body removal. We attribute the profuse reticulocytosis of Tramezzani et al. to severe hypoxia during surgery under sodium pentobarbital anesthesia. Since barbiturates depress the central chemoreceptor response to carbon dioxide, the hypoxic response of peripheral chemoreceptors becomes important in maintaining adequate spontaneous respiration. When the carotid bodies are removed under these circumstances, significant under-ventilation can be anticipated. By using halothane in oxygen, surgical hypoxia was avoided, and there was no reticulocyte response. The absence of significant alteration of bone marrow function studies after carotid body removal argues strongly against any direct role of chemoreceptors in erythropoiesis in normal cats. Carotid body deprived cats and other animals have a more vigorous erythropoietic response to hypoxic environments since they lack the normal ventilatory response and are, in
fact, more hypoxic than intact animals in the same environment. Thus the carotid body acts only indirectly on erythropoiesis through its effect on ventilation.

REFERENCES

16. Tramezzani JH: Personal communication
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