ERYTHROCYTOSIS OR SYMPTOMATIC POLYCYTHEMIA FOLLOWING CHRONIC CEREBRAL STIMULATION THROUGH INDWELLING ELECTRODES

J. F. KELL, JR., M.D., E. C. HOFF, M.D., AND G. R. HENNIGAR, M.D.†
Department of Neurological Science, Medical College of Virginia, Richmond, Virginia

(Received for publication October 1, 1959)

In preliminary studies to assay the effects of repeated long-term stimulation of cerebral autonomic centers, pentyletetrazol (Metrazol) given intravenously or intramuscularly evoked predominantly generalized vasopressor responses in cats and one of the most consistent concomitants of this repeated vasopressor response in the cat was hypertrophy and hyperplasia of the pulmonary arteries.15 In these chronic experiments, examination of the blood showed a notable increase in count of the red blood cells, and it was speculated that this condition might resemble the clinical entity of Ayerza’s disease (pulmonary arteriosclerosis with polycythemia).

A review of the literature reveals several reports of experiments on laboratory animals in which an erythrocytosis or reticulocytosis resulted from direct physical stimulation of the brain or ablation of cerebral areas. Schulhof and Matthies22 injected sterile siliceous earth in the region of the hypothalamus of rabbits to cause a sterile inflammatory reaction simulating an encephalitis. Three of their animals showed an increase in count of the red blood cells of 1 to 2 million. Dockhorn6 produced a reticulocytosis in human subjects by the application of diathermy to the brain stem. Mettler17 found a reticulocytosis of 0.3 to 7.4 per cent in dogs following bilateral frontal lobectomies and no such response was seen after a bilateral occipital lobectomy.

There is a wide range of normal counts of red blood cells in cats and dogs. Trautmann and Fiebiger26 gave values of 7.2 ± 1 million cells per c.mm. for the cat and 6.1 ± 1 million cells per mm. for the dog. A major cause of polycythemia in animals is exposure to high altitude, and the degree of polycythemia depends upon the severity of hypoxia and the duration of the exposure. It is of particular interest to the present study that this type of polycythemia disappears after cervical transection of the spinal cord.10

The present experiments were undertaken to determine, first, whether the medial hypertrophy and hyperplasia of cats’ pulmonary arteries, pre-
viously reported,\textsuperscript{15} were associated with an erythrocytosis; secondly, it was
desired to establish whether the arterial vascular changes and the erythro-
cytosis could be produced by chronic focal electrical stimulation of cerebral
autonomic centers through indwelling electrodes. This was pertinent since
electrical stimulation has been used so extensively as a means of eliciting
responses from both the human\textsuperscript{20} and animal\textsuperscript{8} brain.

**EXPERIMENTAL METHODS**

These investigations were conducted on 9 adult male and female dogs
(4 years or younger in apparent age) and 14 adult male and female cats.

**METHOD OF STIMULATION**

(1) *Control Studies.* To determine whether the operative procedures and inser-
tion of electrodes caused any peripheral-blood response, 1 adult cat and 1 adult dog
were studied hematologically for a period of over 1 year after implantation of the
electrode without stimulation. The remaining 13 cats were examined for an average
of 1 month and the remaining 8 dogs for an average of 4 months before operation
and/or stimulation.

(2) *Pentylenetetrazol Studies.* A series of 6 adult male and female cats were given
a subconvulsive amount of pentylenetetrazol (30 mg.) intramuscularly 4 times daily
for 4 to 6 weeks. The blood for counts was obtained from the ear every other day. The
blood for hematocrits was drawn once a week from the femoral vein.

(3) *Indwelling Electrode Studies.* In a group of 9 dogs and 8 cats, 27-gauge stain-
less-steel wire electrodes, insulated with polytetrafluoroethylene (Teflon),\textsuperscript{*}
were inserted aseptically through small trephine openings and oriented, manually or stereo-
taxically, in cortical loci of the prefrontal and pyriform areas. For this procedure the
animals were anesthetized with 2.5 per cent thiamylal sodium (Surital Sodium)\textsuperscript{†}
given intravenously. The electrodes were fixed in position by a stainless-steel holder
(described by Sheatz\textsuperscript{23}) bolted to the skull. Postoperatively, the cats received
600,000 units of aqueous procaine-penicillin, intramuscularly, daily for 1 week and
the dogs received 600,000 units of aqueous procaine-penicillin and 1 gm. of strepto-
mycin daily for 1 week.

All electrical stimulation was carried out without anesthesia and was delivered
from a sine-wave phase-shift oscillator driving a cathode follower output (60 c./sec.;
0.2–4.0 volts). Eight dogs were stimulated in a pattern of 10 min. every hour, 6 times
times a day, 5 days a week. Stimulation was started 2 weeks postoperatively and was con-
tinued for a period ranging from 3 to 6 months. In the series of 7 electrically stimu-
lated cats, 2 animals received 10-sec. stimulations every 2 min. for about 6 hours a
day; 2 cats received 30-sec. stimulations every 5 min. for about 6 hours a day; and 3
cats received 10-min. stimulations every hour for about 6 hours a day. The cats were
stimulated for periods of 1 to 6 months.

**BLOOD AND HISTOLOGICAL STUDIES**

Both the control and indwelling-electrode groups of animals were weighed twice
a week. A blood-volume determination (T-1824 [Evans] blue dye technique) and the
hematocrit were done at least once a week. Total count of red blood cells and white

\textsuperscript{*} E. I. du Pont de Nemours and Co., Inc.

\textsuperscript{†} Parke, Davis, and Co.
blood cells, and photometric determination of the hemoglobin (with a Beckman spectrophotometer) were done 5 days a week throughout the experiment.

In the pentylenetetrazol studies, daily counts of reticulocytes were made. At the termination of all experiments, complete postmortem examinations were performed and all tissues were preserved in 10 per cent buffered formalin. Slides stained with hematoxylin and eosin were made from all tissues, and in selected cases Masson trichrome and Verhoeff-van Gieson stains were done.

RESULTS

(1) Control Studies. During the control period, all 13 cats in both the pentylenetetrazol and indwelling-electrode series revealed a variation in count of red blood cells within the normal limits reported by Trautmann and Fiebiger—26—the average prestimulation count of red blood cells being 6,000,000 to 8,000,000. The 1 control cat that had implanted electrodes without stimulation maintained, for the year observed, an average count of 6,300,000 red blood cells and the maximum never exceeded 7,500,000. This latter animal was kept in the same quarters and received the same diet as the stimulated animals.

The 8 electrically stimulated dogs and the 1 control dog observed for over 1 year had an average count of 5,900,000 red blood cells as a control value; moreover, the control animal never had a maximum over 7,000,000. These values are also consistent with those cited by Trautmann and Fiebiger. 26

Histological examinations of bone marrow from the thoracic ribs and the femurs of 15 control cats and 10 control dogs have shown no abnormalities of the blood vessels.

(2) Pentyltetrazol Studies. In 4 of the 6 cats that received pentylenetetrazol, there was a slight rise in count of the red blood cells and in concentration of hemoglobin during the first 2 or 3 days, followed by a decline. During the second week, there was an increase in count of the red blood cells and in the hematocrit, and the count of reticulocytes rose from 0.2 per cent to 5.0 per cent. After 4 to 6 weeks the animals began to have spontaneous convulsions and were sacrificed. Terminally, the hematocrit in all cases showed a definite decline and a rather pronounced poikilocytosis. Thus, the average count of red blood cells during the control period was 8,000,000 and increased to 11,000,000 and 12,500,000 within 2 weeks following administration of pentylenetetrazol. The hematocrit was 48 per cent during the control period, rose to 52 per cent, and terminally fell to 42 per cent.

(3) Indwelling Electrode Studies. It has been suggested13,14 that the mechanism for the vascular changes produced by pentylenetetrazol as well as focal stimulation of cerebral vasopressor centers is repeated peripheral arterial and arteriolar vasoconstriction. Fig. 1 illustrates the responses of the arterial blood pressure to electrical stimulation through indwelling electrodes in 2 unanesthetized dogs. In Fig. 1A the voltage was gradually increased from 0.25 volt to 2.5 volts and a right prefrontal electrode was
ERYTHROCYTOSIS AFTER CEREBRAL STIMULATION

FIG. 1. (A) Vasopressor response produced by stimulation of a right prefrontal cerebral cortical locus through an indwelling electrode in a chronic experiment (Expt. No. 905). Minimal voltage for essentially maximal response is 1.5 volts.

(B) Vasopressor response in the same animal (Expt. No. 905) when the same electrode was stimulated 1 week after the recording in (1A) with 1.5 volts for 10 sec. with a maximum rise in blood pressure to 300 systolic and 200 diastolic.

(C) Increase in vasopressor response obtained after intermittent stimulation over a period of 6 months in a dog (Expt. No. 900). This response was obtained with 2.5 volts for 15 sec. and the blood pressure rose to 450 systolic and 200 diastolic.

stimulated. The blood pressure began to rise (from a resting level of 150 systolic and 100 diastolic) at 1.25 volts, and at 1.5 volts reached a maximum pressure of 300 systolic and 200 diastolic and only changed slightly when the voltage was increased to 2.5 volts during the total stimulation period of 60 sec. Fig 1B records the vasopressor response when the same electrode was stimulated in the same animal 1 week later with a stimulus of 1.5 volts for 20 sec. Again, the maximum blood pressure attained was 300 systolic and 200 diastolic. Fig. 1C demonstrates one of the differences found in the pressor response after several months of repeated stimulation. This dog had been stimulated intermittently for 6 months and the stimulation at the time of this recording was 2.5 volts for 15 sec. to a right prefrontal electrode and the blood pressure rose to 450 systolic and 200 diastolic. In the early stimulation sessions of this, or any dog, the blood pressure never exceeded a peak of 350 systolic pressure. These vasopressor responses were obtained without convulsive movements; in fact, convulsive seizures were not associated with as pronounced elevations of blood pressure as were caused by maximal electrical stimulation of a cerebral pressor locus.
Six of the 8 dogs showed results similar to those in Expt. No. 901. These are illustrated in Fig. 2. During the control period, the average hematocrit was 42 per cent as shown by the solid bar; the count of the red blood cells was approximately 5,000,000 (solid lower curve). Each cross on this curve represents the average of 5 counts of red blood cells taken on 5 consecutive days. The interrupted curve represents the individual extremes of the counts of the red blood cells, and the solid upper curve, the total blood volume. Each solid square on this curve denotes the average of 3 different determina-

![Graph showing hematocrit and blood volume changes](image)

**Fig. 2.** Increase in hematocrit (solid vertical bars), changes in count of red blood cells (solid lower curve with crosses), and changes in total blood volume (solid upper curve with squares) resulting from chronic, intermittent stimulation of indwelling electrodes in the prefrontal and temporal cortex bilaterally over a period of 25 days in a dog (Expt. No. 901). There was a maximum increase of 10 per cent in the hematocrit, an increase of 4,000,000 in count of red blood cells, and an increase of 600 cc. in the total blood volume. Each cross (in lower curve) represents average count of red blood cells for a 5-day period. On the interrupted line, A is the peak low (r.b.c.) before stimulation, B is peak high during stimulation, and C is peak low after stimulation was discontinued. The squares (upper curve) denote average blood volume (3 determinations in a 15-day period). Vertical arrows mark starting and stopping of stimulation.

tions of blood volume done during a 15-day period. The left arrow marks the beginning of 10-min. stimulations once every hour 6 times a day. At the end of 18 days, there was an increase of 10 per cent in the hematocrit, an increase of 4,000,000 in count of the red blood cells, and an increase of 600 cc. in the total blood volume. The right arrow shows the time at which the stimulation was stopped; there followed a decrease in all values.

Fig. 3 is a graph of the same determinations in a cat (Expt. No. 783) that received a similar pattern of stimulation followed by increase in all values. In this case, the stimulation was stopped for 14 days and the values decreased; when the stimulation was resumed the values became elevated again. Determinations of the total blood volume show that these results are not the effects of hemoconcentration. Four of the 7 cats and 6 of the 8 dogs showed similar changes in the peripheral blood.
ERYTHROCYTOSIS AFTER CEREBRAL STIMULATION

There was approximately a 100 per cent increase of the mass of red cells in these animals when calculated on the basis of cc. per kg. of body weight. A comparison of the plasma volume, red-cell mass, total blood volume, and hematocrit values of the 2 control animals and 2 stimulated animals (before and after stimulation) are summarized in Table 1. The response of erythrocytosis indicated by these values compares favorably with that reported by Schafer.21

The bone marrow of all animals given pentylenetetrazol or electrical stimulation revealed a slight erythroid response, and there was also a suggestion of medial thickening of the arteries of the bone marrow. The vascular changes were more prominent in the cats than in the dogs. Fig. 4 illustrates suggestive thickening of the arteriole as well as an increase in the normoblasts in the bone marrow of a cat that received pentylenetetrazol.

A more consistent and definite degenerative change in the blood vessels of the bone marrow of cats stimulated through indwelling cerebral electrodes is exemplified in Fig. 5 which illustrates reduplication and splitting of the internal elastic membrane. This change in the arterial wall is also seen in hypertensive vascular disease in man.18

In those experiments in which cats were stimulated through indwelling electrodes over a period of several months and an erythrocytosis resulted, there were also pulmonary arterial changes. Fig. 6 demonstrates both medial hypertrophy and hyperplasia as well as intimal proliferation inside the internal elastic membrane of a pulmonary artery in such a cat. None of the dogs has shown similar pulmonary arterial changes.
TABLE 1

Prestimulation values and poststimulation values of an electrically stimulated dog (Expt. No. 901) and a stimulated cat (Expt. No. 783) and observations on a control dog (Expt. No. 780) and a control cat (Expt. No. 785)*

<table>
<thead>
<tr>
<th>Experiment</th>
<th>Plasma Volume</th>
<th>Red-Cell Mass</th>
<th>Total Blood Volume</th>
<th>Hematocrit</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Total (cc.) cc./kg.</td>
<td>Total (cc.) cc./kg.</td>
<td>Total (cc.) cc./kg.</td>
<td></td>
</tr>
<tr>
<td>Dog (Electically stimulated)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Expt. No. 901</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Averages before stimulation</td>
<td>613.0</td>
<td>916.0</td>
<td>1529.0</td>
<td></td>
</tr>
<tr>
<td>Averages after stimulation</td>
<td>810.0</td>
<td>1086.5</td>
<td>1996.5</td>
<td></td>
</tr>
<tr>
<td>Dog (Electrodes inserted but not stimulated)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Expt. No. 780</td>
<td>964.0</td>
<td>1086.5</td>
<td>2050.0</td>
<td></td>
</tr>
<tr>
<td>Cat (Electrically stimulated)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Expt. No. 783</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Averages before stimulation</td>
<td>91.5</td>
<td>51.5</td>
<td>142.0</td>
<td></td>
</tr>
<tr>
<td>Averages after stimulation</td>
<td>160.5</td>
<td>160.5</td>
<td>321.0</td>
<td></td>
</tr>
<tr>
<td>Cat (Electrodes inserted but not stimulated)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Expt. No. 785</td>
<td>125.8</td>
<td>77.1</td>
<td>203.0</td>
<td></td>
</tr>
</tbody>
</table>

* Each figure in this table indicates an average of all the values determined on the individual animal during the period designated and represents the average of at least 5 different determinations in each of the stimulated animals. In the control animals, the values are the averages of examinations made for a 1-year period and at no time did the control animals show values of red-cell mass (in cc./kg.) as high as for the stimulated animals after stimulation.

Fig. 4. Suggestive medial thickening of the arteriole as well as an increase in the normoblast series of red blood cells in the bone marrow of a cat (Expt. No. 340) receiving subconvulsive injections of pentylenetetrazol (Metrazol) 4 times a day for 42 days. Hematoxylin and eosin stain, X450.
ERYTHROCYTOSIS AFTER CEREBRAL STIMULATION

1035

FIG. 5. Arrows point to reduplication and splitting of the internal elastic membrane in an artery of the bone marrow of a cat (Expt. No. 783) that received 3564 10-sec. periods of stimulation on 51 different days over a 6-month period. Verhoeff-van Gieson stain, ×270.

DISCUSSION

The exact mechanism of the fundamental stimulus for erythropoiesis is still obscure; however, Grant and Root\textsuperscript{10} pointed out that experimental evidence indicates that hypoxia in the bone marrow is the primary factor. The hypoxia may be produced (1) by reduction of O\textsubscript{2} tension in inspired air (as in exposure to high altitude) or (2) by fibrosclerotic pulmonary arterial change as in Ayerza’s disease or (3) by inhibition of the respiratory function of immature red cells in the bone marrow as postulated in cobalt-produced polycythemia. (4) Vasoconstriction of the blood vessels of the marrow by

FIG. 6. Medial hypertrophy and hyperplasia as well as intimal proliferation seen inside the internal elastic membrane of a pulmonary artery of a cat (Expt. No. 783) described in Fig. 5. Verhoeff-van Gieson stain, ×100.
epinephrine, other vasoconstrictor drugs,\textsuperscript{3--5} or stimulation of central sympathetic centers may also cause the hypoxia. The involvement of higher autonomic centers is inferred from Mettler's findings\textsuperscript{17} of reticulocytosis after bilateral frontal lobectomies and by the polycythemia developed in dogs following bilateral depressor neurotomy and reversed by total sympathectomy (Schafer\textsuperscript{21}).

The present experiments offer a possible explanation of the polycythemia associated with clinical lesions of the central nervous system. Since repeated stimulation of vasopressor centers in the prefrontal and limbic areas are seen experimentally to produce an erythrocytosis, it seems likely that brain lesions act by central sympathetic stimulation. There is evidence that the nature of the lesion itself is not contributory. Thus, Silver and Hennigar\textsuperscript{24} as well as others\textsuperscript{1, 2, 13, 19, 27--29} have found no definite evidence of hemopoiesis within posterior-fossa neoplasms themselves. Furthermore, reviews of this subject by Lucia and Marasse\textsuperscript{16} and Drew and Grant\textsuperscript{7} indicate that erythrocytosis can result from inflammatory and non-neoplastic lesions located in the supratentorial region as well as in the posterior fossa. Also, Starr \textit{et al.}\textsuperscript{25} cited a clinical case in which adhesions producing internal hydrocephalus caused a recurrence of polycythemia after complete removal of a posterior-fossa tumor.

It may be hypothesized that erythrocytosis and transient hypertension seen in clinical cases with involvement of the central nervous system are produced by a slowly expanding irritative lesion which stimulates centers or pathways of the central sympathetic nervous system in the frontal lobe or limbic system, the posterior hypothalamus\textsuperscript{9} with secondary stimulation of the hypophysial system,\textsuperscript{11} or the posterior longitudinal fasciculus of Schütz and other pathways. Since these latter pathways are more concentrated in the posterior fossa, peripheral vasoconstriction is more easily initiated at this level than in the cerebrum where sympathetic nervous centers are more widely separated and more diffusely represented.

**SUMMARY**

1. Chronic stimulation of vasomotor centers in the cat brain by convulsive or subconvulsive injections of pentyleinetetrazol (Metrazol) produced pronounced medial hypertrophy and hyperplasia as well as intimal proliferation of the pulmonary arteries.

2. A series of 6 cats received repeated administrations of pentylene-tetrazol (Metrazol) intramuscularly in subconvulsive amounts and in 4 of these cats there developed an increase in count of red blood cells of 3 to 5 million, a rise in blood hematocrit, and hyperplastic bone marrow.

3. The blood and bone marrow have been studied in 7 cats and 8 dogs that received stimulations focally to the pressor areas of the cerebral cortex through indwelling electrodes in chronic experiments. Four of the 7 cats and 6 of the 8 dogs exhibited erythrocytosis, hyperplastic bone marrow, and an increase in blood volume.
4. All of the 6 cats injected with pentylenetetrazol (Metrazol) showed some degree of pulmonary vascular change and 3 of these showed pronounced changes. Four of the cats stimulated through indwelling electrodes revealed medial hypertrophy and hyperplasia as well as intimal proliferation of pulmonary arteries. None of the control or stimulated dogs showed changes in the pulmonary arteries.

5. It is postulated that the polycythemia associated with brain tumors in man is caused by chronic stimulation of vasomotor centers in the brain and is not produced by some distinctive characteristic of the neoplasm itself.

REFERENCES

21. Schaffer, P. W. The etiology and treatment of polycythemia rubra vera. Observations based upon studies of body fluid changes in dogs subjected to proprioceptor depressor neurotomy and ex-