Concerning the role of sympathetic and parasympathetic mechanisms in reflex dilatation of the pupil elicited by painful stimuli, there have long been many arguments. One group of authors, for example, Bechterew (1) and Braunstein (2) concluded that the pupillary dilatation resulting from painful stimuli was caused solely by inhibition of the third cranial nerve activity. Another group of investigators, for example, Lieben and Kahn (3), Bain, Irving and McSwiney (4), Ury and Gellhorn (5), Ury and Oldberg (6) and Seybold and Moore (7) were of the opinion that parasympathetic inhibition was the principal factor in the reflex dilatation while sympathetic excitation was a negligible one.

On the contrary, others, for instance, Luchsinger (8), Anderson (9), and Dechaume (10) claimed that sympathetic excitation was responsible for the pupillary reaction which was absent after cervical sympathectomy.

Weinstein and Bender (11) compared the pupillary reflex activity in cats and monkeys. They concluded that a species-difference exists: in both species pupillary dilatation is accomplished by both parasympathetic inhibitory and sympathetic excitatory mechanism. In the cat, inhibition of the parasympathetic mechanism is predominant while in the monkey excitation of the sympathetic mechanism is of greater importance.

Later Lowenstein and Loewenfeld (12) performed more detailed experiments in cats using their own pupillographic instrument; they observed that pupillary reflex dilatation was mostly due to sympathetic excitation, and was reduced to less than one-fifth of the normal control dilatation after sympathectomy.*

The author re-examined this subject with the aid of a new method devised for measuring the pupil size, simultaneously recording the contraction of the nictitating membrane and blood pressure in cats and dogs. The effects of barbiturates, pentylene-tetrazol, chlorpromazine and morphine on the responses of sciatic stimulation were studied hoping to elucidate the central mechanisms involved.

**METHODS**

Seventy-four adult cats of both sexes, weighing from 3 to 4 kg and ten dogs weighing from 6 to 10 kg were used in the experiments. Immediately after the animals were

* The large literature concerning this subject was analyzed by Loewenfeld (13).
immobilized with decamethonium bromide (0.5 mg/kg i.v.), artificial respiration was started through a tracheal cannula. The head was fixed in a stereotaxic instrument.

The size of the pupil of the left eye was recorded continuously by means of the authors' phototransistor method which was described previously (14). The movements of the nictitating membrane of the right eye were magnified with an isotonic lever and recorded on the same paper. The systemic blood pressure was recorded with a mercury manometer from the left femoral artery. The animals were immobilized by repeated injections of decamethonium at intervals of thirty to sixty minutes throughout the experiment.

The animals' eyes were illuminated by an A.C. operated fluorescent tube (20 watts), covered with red cloth from a distance of 2 meters.

The sciatic nerve was cut at the thigh, and the central end was placed on a pair of silver electrodes. It was replaced where it had been before, together with the electrodes and covered by the femoral muscles and skin.

In experiments in which the cervical sympathetic nerve was stimulated preganglionically, it was cut acutely, and its peripheral end was placed on bipolar silver wire electrodes. In this case, the sympathetic nerve was always separated from the vagus in order to avoid pupillary dilatation, possibly elicited by central vagal stimulation (15).

In order to examine the role of the sympathetic nerve in reflex dilatation, the cervical vagosympathetic trunk was cut pre-ganglionically during the experiment. The vagosympathetic trunk was very carefully separated from the surrounding tissues and vessels, and a thread was passed under the trunk. When sympathectomy was required, the vagosympathetic trunk was isolated by pulling the thread forward and cut by scissors. In this experiment, the vagosympathetic nerve was not divided into the sympathetic nerve and the vagus because, as Lowenstein et al. recognized, the sympathetic nerve is easily damaged by the handling of the nerve during the surgical procedure and, consequently, the size of the pupil is diminished, or the reflex dilatation reduced, as compared to the dilatation of the intact pupil.

In some of the experiments, the ciliary ganglion was removed in order to exclude the oculomotor innervation. The experiments were also carried out in spinal cats.

In order to stimulate the sciatic and the cervical sympathetic nerve, rectangular pulses of 1 msec duration at a rate of 100 cps were delivered for 3 seconds from a thyratoron stimulator. Voltage was varied from 0.5 to 10 volts, depending upon the sensitivity of the preparation.

Pentobarbital sodium, thiopental sodium, pentylenetetrazol, chlorpromazine hydrochloride and morphine hydrochloride were used in these experiments. They were injected through a polyethylene catheter which was inserted into the femoral vein.

Since the animals were completely unanesthetized, they were easily stimulated emotionally by any change in their environment, so that pupil size and blood pressure fluctuated markedly; therefore, the experiments were performed in a quiet atmosphere and stimuli from the environment were avoided as much as possible.
RESULTS

Since all experiments were done on animals immobilized with decamethonium, the pupillary effects of this drug were first studied: following the intravenous injection of 0.5 mg/kg of decamethonium, the pupil of the cat became small and stable. Its diameter varied among different cats between less than 1 and 3 mm. However, in the dog, the pupil did not become stable after decamethonium, but continued to oscillate. Its minimum diameter was approximately 2 to 3 mm (Fig. 1, lower part).

![Figure 1. The effects of sciatic nerve stimulation on the pupil of the cat (upper records) and the dog (lower records) before and after sympathectomy.](image)

PD: Pupillary diameter of the left eye (in mm), BP: Systemic blood pressure (mm Hg), SC: Sciatic nerve stimulation (for 3 seconds).

See the text for details.

1. Role of the Sympathetic Nerve in Pupillary Reflex Dilatation

1. Influence of sympathectomy on the pupil

In response to sciatic nerve stimulation, the pupil of the cat dilated from 1 mm to 7 mm in diameter for one volt and to 8 mm for two volts (Fig. 1, upper part, A, B). The pupil was temporarily dilated by cutting of vagosympathetic nerve; thereafter, it contracted slightly in most of the cases. After sympathectomy, the pupillary response to sciatic stimulation was markedly reduced, i.e., the pupil dilated only to 1.4 mm with one volt and to 2 mm with two volts as shown in Fig. 1, upper part, D, E.

In the dogs, the pupil was oscillating continuously even when the blood pressure was stable. Nevertheless, stimulation of the sciatic nerve produced distinct pupillary dilatation, for example, from 3.5 to 5.5 mm with one volt and to 6 mm with two volts (Fig. 1, lower part, A). As in the cat, the cervical sympathectomy was followed by transient pupillary dilatation, and in a minute or so the pupil contracted to 1 mm in diameter and its size became very stable (Fig. 1, lower part, B). After vagosympathectomy, sciatic stimulation enlarged the pupil from 1 mm to 2 mm with one volt and to 3.5 mm with two volts (Fig. 1, lower part, C, D).

In both cats and dogs, then, pupillary reflex dilatation was much reduced by sym-
pathectomy. These results indicate that the sympathetic mechanism is of greater importance in reflex dilatation than the parasympathetic-inhibitory one. Even after sympathectomy, however, reflex dilatation was observed, though slightly. This remnant dilatation would be due to inhibition of the parasympathetic tone.

2. Correlation between blood pressure changes and pupillary response to sciatic stimulation

The sciatic nerve stimulation evoked blood pressure changes, i.e., weak stimuli caused slight fall in blood pressure and strong stimuli caused a rise instead. The pupil was invariably dilated by the sciatic stimuli. The stronger the stimuli, the more widely the pupil dilated and the more the blood pressure rose. However, the degree of reflex pupillary dilatation was not always paralleled by the magnitude of blood pressure changes. It was frequently observed that the reflex dilatation was ever so marked, while blood pressure rise was slight, and inversely the former was ever so weak, while the latter was marked. Even if the reflex dilatation was weak, it was always depressed by sympathectomy.

3. Experiments in spinal cats

When the sciatic nerve was stimulated, about two hours after section of the spinal cord at the level of C1-C2, reflex dilatation was not evoked in the pupil.

II. Effects of Drugs on the Pupil, Nictitating Membrane and Blood Pressure, and Their Influences on Pupillary Reflex Dilatation

1. Barbiturates (in cats)

a. Pentobarbital sodium

In normal cats, pentobarbital sodium, injected i.v. in a dose of 15 mg/kg, caused the pupil to dilate and the blood pressure to fall. This dilatation almost coincided with the blood pressure fall and reached its maximum within 30 seconds after the drug administration. In most of the cases, the pupillary dilatation due to pentobarbital was shorter in duration than the blood pressure fall. The pupillary dilatation was usually followed by its subsequent contraction.

The maximal diameter of the pupil following intravenous injection of 15-20 mg/kg of pentobarbital ranged from 2.5 mm to 3.8 mm. Usually 10-20 minutes were required for the pupil to recover its original size; thereafter, it continued to contract and remained small for a long time. In some cats, however, the pupil remained larger than it had been before the injection for 60 minutes or even longer.

The nictitating membrane showed either no change or slight relaxation after 15-20 mg/kg of pentobarbital was given.

Influence of pentobarbital on pupillary reflex dilatation: The reflex pupillary dilatation to sciatic stimulation was reduced to about 50% of the normal amplitude after 5 mg/kg of pentobarbital administration, while the contraction of the nictitating membrane due to sciatic stimulation was completely abolished. In these experiments with low doses of pentobarbital, vagosympathectomy further reduced the pupillary reflex dilatation, but was unable to abolish it completely.
As the dose of pentobarbital was increased, the pupillary reflex dilatation was more markedly depressed. The effect of intravenous injection of 20 mg/kg of pentobarbital was shown in Fig. 2. The reflex dilatation was either unaffected or only slightly diminished by vagosympathectomy. This indicates that while the reflex dilatation due to sympathetic excitement (sympathetic reflex dilatation) was almost completely depressed, that due to inhibition of the parasympathetic oculomotor nucleus was still working after 20 mg/kg of pentobarbital administration.

![Fig. 2. The effect of pentobarbital on the pupil. Sixty-three minutes after pentobarbital 20 mg/kg, sympathectomy was performed.](image)

A : Control responses to sciatic stimulation, B : Pentobarbital (PENTO) 20 mg/kg was injected intravenously, and the sciatic nerve was stimulated 5 minutes later, C, D, F : Sciatic nerve stimulations 30, 60, and 70 minutes respectively after the drug. Cervical sympathectomy was performed between D and E, i.e., 63 minutes after the drug.

Abbreviations are the same as in Fig. 1.

The usual effect of pentobarbital upon the responses of the acutely sympathectomized pupil to sciatic stimulation is shown in Fig. 3. In this case, pentobarbital was successively administered at intervals of 15-20 minutes as indicated in the figure. The pupillary dilatation due to inhibition of parasympathetic activity was not reduced, but in fact, was rather increased in magnitude. This increased response usually occurred in the mydriatic as well as in the miotic period of pentobarbital treatment, until the last response shown, when it was reduced by the cumulative influence of several small doses. However, in one of four cats, reflex dilatation was decreased already by the first administration of 5 mg/kg and was completely depressed after the third injection.

Large doses of pentobarbital always depressed the pupillary dilatation due to parasympathetic inhibition.

The blood pressure rise in response to sciatic stimulation was reduced by pentobarbital. The larger the dose, the more diminished was the pressor response and after larger doses, blood pressure changes were scarcely observed and/or only blood pressure falls were registered.
FIG. 3. The effect of pentobarbital on the sympathectomized pupil.

NM: The response of the right nictitating membrane with the intact sympathetic nerve, PD: Pupillary diameter of the left eye (sympathectomized pupil), BP: Systemic blood pressure.

Pentobarbital was successively injected in doses indicated in the figure, and the responses to sciatic nerve stimulation (B-I) were examined. Note the nictitating membrane response was abolished.

A: Control responses to sciatic stimulation, B, C: Ten and fifteen minutes after pentobarbital 4 mg/kg, D: Fifteen minutes after the second injection, E: Ten minutes after the third injection, F, G, H: Five, twenty, and thirty minutes after the fourth injection respectively, I: Five minutes after the fifth injection.

FIG. 4. The effect of thiopental.

Reflex dilatation was completely abolished 12 and 20 minutes after thiopental 10 mg/kg i.v., and almost recovered an hour after the drug.

b. Thiopental sodium

The effects of thiopental on the pupil, blood pressure and the nictitating membrane were, in general, similar to those of pentobarbital. However, the mydriasis following the injection of thiopental was more rapid and marked but of shorter duration (Fig. 4), i.e., the mydriasis after intravenous injection of 15-20 mg/kg thiopental, subsided within ten minutes and, subsequently, the pupil contracted.

The reflex dilatation was either unaffected or only slightly diminished by cervical sympathectomy 20 minutes after intravenous injection of 20 mg/kg of thiopental, while after only 10 mg/kg i.v. of thiopental, sympathectomy reduced the magnitude of reflex dilatation more markedly.

The increase of reflex dilatation due to parasympathetic inhibition after thiopental was smaller than after pentobarbital. In some cats when thiopental was successively
administered in doses of 3 mg/kg at intervals of ten minutes, pupillary dilatation due to parasympathetic inhibition was, as in the case of pentobarbital, gradually reduced by every administration and was completely abolished after 9 mg/kg in total dose.

The pressor response and the contraction of the nictitating membrane to sciatic stimulation were similar to those after pentobarbital.

2. Pentylenetetrazol (Metrazol) (in cats)

The effects of Metrazol on the pupil, nictitating membrane and blood pressure were examined in cats. Following the injection of Metrazol in doses larger than 10 mg/kg, the blood pressure rose; it fluctuated markedly and then gradually declined. Within 30 minutes, its original level was recovered. The changes in pupil size and tone of the nictitating membrane were almost parallel to those in blood pressure, i.e., the pupil dilated and the nictitating membrane contracted when blood pressure rose. The pupil recovered its original diameter one to two hours after administration of the drug. The tone of the nictitating membrane returned to the control level within 30 minutes in the majority of experiments, but was rather relaxed afterwards in a few cases.

The acutely sympathectomized pupil also dilated after 10 mg/kg of Metrazol, but the extent of this dilatation was definitely smaller than that of the intact pupil. For a minute or two after Metrazol injection, the pupil fluctuated in parallel with changes in the nictitating membrane and blood pressure, but soon only dilatation occurred without any fluctuation; the pupil recovered its original diameter more than sixty minutes later.

The influence of Metrazol on pupillary reflex dilatation: In the early stage of the Metrazol effect, when the nictitating membrane was intensely contracted and the pupil widely dilated, it was difficult to produce reflex responses to sciatic stimulation. When the size of the pupil and the tone of the nictitating membrane became stable and began to decline, the responses were clearly observed and usually were even more marked than the control response (Fig. 5). However, in rare cases, as shown in Fig. 6, the reflex dilatation was reduced after Metrazol administration.

![Fig. 5. The effect of pentylenetetrazol on the pupil.](image)

A: Control responses to sciatic stimulation, B, C, D, E, F: Sciatic nerve stimulations 15, 20, 25, 35, and 40 minutes after pentylenetetrazol 10 mg/kg i.v. respectively.

Sympathectomy: Only the left vagosympathetic was cut 30 minutes after the drug.

NM: The right nictitating membrane, PD: The size of the left pupil.
The contraction of the nictitating membrane due to sciatic stimulation was decreased twenty and twenty-five minutes after Metrazol (Fig. 5, C, D), but more than thirty minutes later, it returned to almost the same magnitude as in the control reaction (Fig. 5 E, F).

The pressor response to sciatic stimulation was either unaltered or slightly increased for a certain period after the administration of Metrazol, but it was subsequently decreased in six of eight cases (Figs. 6 and 7). Depression of the response of the nictitating membrane to sciatic stimulation usually occurred much earlier and was of shorter duration than that of the pressor response (Fig. 6).

When, thirty minutes after injection of 10 mg/kg of Metrazol, the left vagosympathetic nerve was cut, the sympathectomy had no influence on the pupil size (Figs. 5 and 7), nor on the magnitude of reflex dilatation in four out of five cases (Fig. 7). In the remaining case, sympathectomy reduced the reflex dilatation (Fig. 5). These observations suggested that Metrazol excited the sympathetic nervous system, but inhibited it afterwards when administered in large doses.

Comparing the results in Figs. 5 and 7, it seems that when the sympathetic nerve innervating the pupil was inhibited intensely by Metrazol, the pressor response to sciatic stimulation was also decreased and when the sympathetic was inhibited weakly, the pressor response was not decreased. However, it was also observed, in some cases, that even if Metrazol did not alter the pressor response it inhibited completely the sympathetic nerve innervating the pupil, because sympathectomy caused no reduction of the reflex dilation. Therefore, it is supposed that the sympathetic nerve fibers innervating the pupil and those responsible for the pressor response have different sensitivity to Metrazol.

The vagosympathetic was cut prior to the administration of Metrazol. After 10
mg/kg of Metrazol, pupillary dilatation due to inhibition of parasympathetic activity was increased for more than sixty minutes in all three cats. After only 5 mg/kg of Metrazol were given, in 3 other cats, the responses to sciatic stimulation of the sympathectomized pupil, nictitating membrane, and blood pressure were increased in two, but in the third animal, unaffected. In some cases, Metrazol, even in a dose of 2 mg/kg, increased these responses. In all cases, the reflex dilatation due to inhibition of the third cranial nerve was never reduced, whereas the contraction of the nictitating membrane and the rise of the blood pressure were usually suppressed by Metrazol in doses more than 10 mg/kg.

The parasympathectomized pupil dilated after Metrazol 10 mg/kg was given. This dilatation was followed by a gradual contraction, and the diameter became smaller than it had been before the drug administration. The pupil recovered its control diameter sixty minutes after injection of the drug. Vagotomy, at this time, contracted the pupil and abolished the responses to sciatic stimulation. This observation indicated that the sympathetic nerve supply of the pupil had been suppressed by large doses of Metrazol.

3. Chlorpromazine (in cats)

The effects of chlorpromazine on the pupil, nictitating membrane and blood pressure were investigated in ten cats. When chlorpromazine was injected i.v. in a dose of 0.5 mg/kg, the pupil dilated from 1.1 mm to about 3.0 mm in diameter, the nictitating membrane protruded slightly and systemic blood pressure was lowered by approximately 40 mm Hg (Fig. 8, B).

Influence of chlorpromazine on pupillary reflex dilatation: Since chlorpromazine has an adrenolytic action, the effects of chlorpromazine on the responses to injected adrenaline as well as to sciatic stimulation, were investigated. One of the results is shown in Fig. 8. Before the drug administration, the cat's pupil dilated from 1.1 mm
to 3.4 mm in response to sciatic stimulation, and to 5.2 mm upon injection of adrenaline (5 pg/kg, Fig. 8, A). Five minutes after i.v. injection of chlorpromazine (0.5 mg/kg), the pupil had dilated to 3.1 mm; it was further enlarged up to 4.0 mm by sciatic stimulation. Ten minutes after chlorpromazine, the pupil dilated from 3.5 mm to 5.8 mm in response to injection of adrenaline (5 pg/kg, Fig. 8, B). The pressor responses to sciatic stimulation as well as to adrenaline were much reduced in magnitude and the nictitating membrane response was abolished completely by chlorpromazine.

When further doses of chlorpromazine (0.5 mg/kg) were added successively, the pupil dilated further as the cumulative dose was increased (Fig. 8, C and D). After a total dose of 1.5 mg/kg of chlorpromazine, the vagosympathetic trunk was cut, but the reflex dilatation was unchanged (Fig. 8, E).

In another cat injections of chlorpromazine were repeated at intervals of 15-20 minutes, and the responses to sciatic stimulation and to injection of 5 pg/kg of adrenaline were examined (Table 1). The nictitating membrane failed to respond to sciatic stimulation after the first administration of chlorpromazine (0.5 mg/kg). After the cumulative dose of chlorpromazine 3.0 mg/kg, vagosympathectomy was performed without causing a change in pupil size. Unlike the previously described case, however, reflex dilatation was slightly decreased. After sympathectomy, further injections of 5 and 10 mg/kg of chlorpromazine were added; the pupil now dilated to 7.0 mm but no pupillary dilatation was elicited by either sciatic stimulation (10 volts) or injection of adrenaline (20 pg/kg). Similar results were observed in four other cats. It was noticed in this series of experiments that the effect of chlorpromazine on the pupillary responses to sciatic stimulation and to adrenaline was most marked after the first administration.
TABLE 1. The effects of additional administration of chlorpromazine on reflex dilatation and adrenaline dilatation.

<table>
<thead>
<tr>
<th>Injection of chlorpromazine (mg/kg)</th>
<th>Pupil size (mm)</th>
<th>Reflex dilatation elicited by sciatic stimulation (2 volts)</th>
<th>Response to adrenaline (10 μg/kg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.5 mg/kg</td>
<td>1.2</td>
<td>1.2-9.1(7.9)</td>
<td>1.2-5.7(4.5)</td>
</tr>
<tr>
<td>0.5</td>
<td>1.2</td>
<td>1.2-5.7(4.5)</td>
<td>1.2-2.6(1.4)</td>
</tr>
<tr>
<td>2.0</td>
<td>1.7</td>
<td>1.7-3.8(2.1)</td>
<td>1.7-2.4(0.7)</td>
</tr>
<tr>
<td>Sympathectomy</td>
<td>2.0</td>
<td>2.0-3.5(1.5)</td>
<td>2.0-2.9(0.8)</td>
</tr>
<tr>
<td>5.0</td>
<td>3.1</td>
<td>3.1-4.1(1.0)*</td>
<td>3.1-4.6(1.5)**</td>
</tr>
<tr>
<td>10.0</td>
<td>7.0</td>
<td>No dilatation</td>
<td>No dilatation**</td>
</tr>
</tbody>
</table>

* Sciatic stimulation in 10 volts, ** Adrenaline 20 μg/kg.

Fig. 9 shows the effects of chlorpromazine upon the responses of the acutely sympathectomized pupil to sciatic stimulation, to electrical stimulation of the peripheral end of the cut sympathetic nerve and, to injections of adrenaline. In the control reaction, the pupil dilated from 0.5 mm to 2.8 mm to sciatic stimulation, to 5.5 mm upon preganglionic cervical sympathetic stimulation and to 5.1 mm after i.v. injection of adrenaline (10 μg/kg) (Fig. 9, A, B, and C). When chlorpromazine 1.5 mg/kg was injected i.v., the pupil dilated to about 1.5 mm. Ten minutes after chlorpromazine, sciatic stimulation caused pupillary dilatation from 1.0 mm to 3.4 mm (Fig. 9, D). Fifteen minutes after chlorpromazine administration, the pupil dilated from 1.7 mm to 4.2 mm to sympathetic stimulation (Fig. 9, E). Twenty minutes after chlorpromazine, 10 μg/kg of adrenaline elicited dilatation from 1.3 mm to 4.2 mm (Fig. 9, F). These results indicate that the extent of the pupillary dilatation due to inhibition of oculomotor activity, elicited by sciatic stimulation, was not reduced, although the pupillary responses to sympathetic stimulation as well as to injection of adrenaline were significantly decreased.

Thirty minutes after the first administration of chlorpromazine, an additional injection of 3 mg/kg of chlorpromazine was given. As can be seen in Fig. 9, the pupillary

(0.5 mg/kg) and less marked upon successive injections.

SY : Preganglionic sympathetic stimulation.
Other abbreviations are the same as in the previous figures.
See the text for details.
responses to sciatic stimulation and to injection of adrenaline were comparable to those obtained before (Fig. 9, D-G, H and F-J), while the dilatation to sympathetic stimulation was decreased (Fig. 9, E-I). Similar results were obtained in other cats. These observations suggest that chlorpromazine decreases more intensely the pupillary dilatation evoked by preganglionic sympathetic stimulation than that evoked by injection of adrenaline.

Forty minutes after the second administration of chlorpromazine, a further dose of chlorpromazine was added (7 mg/kg). The pupil dilated further to about 3.5 mm, and it now showed very slight response to sciatic and sympathetic stimulation and to injection of adrenaline (Fig. 9, K, L, and M).

The effects of chlorpromazine on the contractions of the nictitating membrane in response to adrenaline injections and to preganglionic sympathetic stimulation were examined. The intensity of the sympathetic stimuli was adjusted to induce the same magnitude of contraction of the nictitating membrane as that which followed injections of 5 μg/kg of adrenaline. While the response of the nictitating membrane to adrenaline was depressed completely by chlorpromazine 0.5 mg/kg, that to sympathetic stimulation was not abolished by the doses smaller than 1.5 mg/kg.

In cervically sympathectomized cats, the effects of atropine on pupillary reflex dilatation were similar to those of chlorpromazine (Fig. 10). In the control reactions the pupil dilated from 1.1 mm to 2.9 mm upon sciatic stimulation and from 0.6 mm to 5.5 mm after injection of 10 μg/kg of adrenaline. After atropine 0.01 mg/kg i.v. pupillodilatation occurred. Sciatic stimulation caused pupillary dilatation of about the same magnitude as that of the control (1.8 mm, Fig. 10, C). 10 μg/kg of adrenaline elicited slightly less dilatation than in the control reaction (from 3.3 mm to 7.5 mm, Fig. 11, D). When atropine (0.02 mg/kg) was added, sciatic stimulation dilated the pupil from 5.2 mm to 6.0 mm, i.e., the magnitude of dilatation was considerably decreased (0.8 mm, Fig. 10, E). Thereafter, when atropine administrations were repeated, the pupil dilated more and more, and the magnitude to reflex dilatation faded away gradually (Fig. 10, F and G). After a total dose of 0.06 mg/kg of atropine administration, the reflex dilatation was scarcely discernible (Fig. 10, F and J). However, adrenaline was still able
to elicit a marked dilatation, only slightly reduced in magnitude compared to the control (from 7 mm to 10.5 mm, Fig. 10, I).

When chlorpromazine (2.0 mg/kg) was given to cats with intact sympathetic innervation but whose ciliary ganglion had been removed acutely, sciatic stimulation failed to elicit pupillary reflex dilatation. In these animals, injection of adrenaline (10 µg/kg) continued to cause pupillary dilatation, though less extensively than in the control reaction.

The pupil of the cats in which both the ciliary ganglion and the cervical sympathetic nerve were extirpated was unaffected by chlorpromazine.

4. Morphine (in cats and dogs)

The effects of morphine were examined in cats and dogs.

a) Cat

When morphine was injected in a dose of 5 mg/kg in the cat, the pupil dilated; the extent of this dilatation varied from 0.7 mm to 5 mm. In 8 of 10 cats, it caused the blood pressure to fall about 10 mm Hg; in the remaining 2 cats, the blood pressure fell more steeply and extensively. In all cases, the blood pressure effect was only short-lasting, and it recovered its control level within five minutes. Occasionally, the blood pressure fall was followed by a subsequent slight rise. The nictitating membrane invariably contracted after morphine. The effects on the pupil and the nictitating membrane were of much longer duration than those upon the blood pressure.

Influence of morphine on pupillary reflex dilatation: Five minutes and 20 minutes after morphine injection (5 mg/kg), the pupillary dilatation in response to sciatic stimulation was increased. Seventy minutes after the injection, the pupil recovered its original diameter and the reflex dilatation returned to the same magnitude as in the control reactions. The pressor response to sciatic stimulation was usually unaffected by mor-
Phine; occasionally it increased slightly.

In most cats, the response of the nictitating membrane to sciatic stimulation was increased when the animals were treated with morphine (5 mg/kg). However, in some instances, the nictitating membrane was strongly retracted after the administration of morphine. Due to this already existing high level of contraction, the response to sciatic stimulation was not increased in magnitude (Fig. 11).

After acute preganglionic sympathectomy, the cat's pupil dilated from 1.7 mm to about 4 mm in response to 5 mg/kg of morphine (Fig. 11). In most cats, this dilatation was less extensive than that of the normal pupil; in a few instances, the dilatation was equal in the two eyes. After the morphine injection, the reflex dilatation was increased in extent and duration (Fig. 11; five, fifteen and thirty minutes, respectively). Two hours after the drug, the pupil almost recovered its control diameter, and the magnitude of the pupillary reflex dilatation was almost the same as in the control. In some cats, 3 mg/kg of morphine sufficed to dilate the pupil too widely to allow further reflex dilatation.

The parasympathectomized cat's pupil dilated very slightly after 5 mg/kg of morphine was given, but reflex dilatation was not increased. This appeared to be due to the intense mydriasis which was caused by ciliary ganglionectomy.

These effects of morphine (5 mg/kg) were of fairly long duration, ranging from 30 minutes to 3 hours and usually lasting about 1 hour.

2) Dog

When 0.5 mg/kg of morphine was injected i.v. in the dog, the systemic blood pressure fell approximately 25 mm Hg, and the pupil showed a fluctuating and gradually increasing contraction from a beginning diameter of 5 mm to 1.5 mm (Fig. 12, B). Before morphine was given, sciatic stimulation produced long-lasting reflex dilatation (from 5.0 mm to 7.0 mm, Fig. 12, A). The pupil dilated from 0.5 mm to 6.5 mm, and from 0.5 mm to 6.5 mm, 34 and 46 minutes respectively after morphine administration (Fig. 12, C and D); i.e., dilatation was as extensive as before morphine, although the duration became shorter. Fifty-one minutes after the morphine injection, the vagosympathetic trunk was cut. The sciatic response was markedly reduced five minutes after sympathectomy, i.e., the pupil dilated from 0.5 mm to only 2.0 mm (Fig. 12, E). After an additional dose of 1.0 mg/kg of morphine was given, the reflex dilatation was further decreased (Fig. 12, F).

3) Antagonism between morphine and levallorphan

Fig. 13 shows the antagonism of levallorphan to the pupillary effect of morphine in the dog. Before morphine was given, the pupil enlarged from 1.0 mm to 3.0 mm in response to sciatic stimulation. Morphine, in a dose of 2.0 mg/kg, contracted the pupil to 0.5 mm after transient dilatation; it practically abolished the reflex dilatation to sciatic stimulation. When 0.25 mg/kg of levallorphan was injected i.v., seventeen minutes after morphine was given, the pupil began to dilate step by step. Nine minutes
later, the reflex dilatation became again distinctly observable; thereafter, the pupil gradually declined in diameter.

The antagonism of levallorphane to the pupillary effects of morphine was not observed in the cats.

**DISCUSSION**

1) The effect of decamethonium on the pupil

Since decamethonium was used in all experiments to immobilize the animals, the effect of this drug should be considered first. Decamethonium (C₁₀) invariably caused the pupil of the cat and of the dog to contract. In cats, the miotic pupil remained quite stable, while in dogs it showed marked oscillations. This miosis might be due...
to the direct effect of \(C_{10}\) on the sphincter muscle. It has been known that the intraocular pressure is markedly decreased after the extraocular muscles are relaxed by curare (Duke-Elder) or the tendons are sectioned [Schoenberg; Salbati; and Ishikawa (16)]. It is conceivable that the decrease in the intraocular pressure resulted from the similar muscle relaxant effect of \(C_{10}\) contributes to the miosis. The pupil size of the unanesthetized dog immobilized with \(C_{10}\) was unstable, and the dogs were sensitive to psychosensory stimuli in this experimental condition.

Even though the pupil was contracted by \(C_{10}\), the pupillary responses to sciatic stimulation and to administration of central nervous system stimulants or depressants were marked. Therefore, it appeared that immobilization of the animals with \(C_{10}\) had little influence on pupillary reflex dilatation.

2) Mechanism of pupillary reflex dilatation

It had long been a predominant view that pupillary reflex dilatation was brought about solely by inhibition of the parasympathetic tone, as being approved in some recent text books (17, 18). Lowenstein et al., demonstrated that in awake animals, the main component of reflex pupillary dilatation was sympathetic activity. In agreement with their finding, the author confirmed that in both awake cats and dogs, reflex dilatation was greatly reduced by cutting the cervical sympathetic trunk. Further, Lowenstein et al. have not found any difference between pupillary dilatation responses elicited by different kinds of sensory stimuli such as sound, painful stimuli applied to the skin, electrical stimulation of the sciatic nerve, or even spontaneous emotional excitement.

A number of authors, for example Arieff (19), assumed that the afferent stimuli in the spinal cord were transmitted directly to the peripheral sympathetic chain without reaching higher brain centers. But, in agreement with many earlier findings [for example, Braunstein (2)], the author's experiments showed that pupillary reflex dilatation was abolished after transection of the spinal cord above Budge's center (C1-C4).

The midbrain obviously must be the site of pupillary dilatation due to inhibition of the oculomotor nucleus. Harris, Hodes, and Magoun (20) found that the reflex dilatation evoked by electrical stimulation of the sciatic and splanchnic nerves in anesthetized or in decerebrated cats was evidently completed at the midbrain level, because destruction of the parts rostral to the oculomotor nucleus did not impair the response. They further demonstrated that this pathway ascended through the lateral funiculus of the spinal cord, though it is distinct from the lateral spinothalamic tract, traversed the reticular formation of the medulla, gained a paramedian position in the dorsal pontile tegmentum, and ascended through the midbrain in or near the ventral central grey of the aqueduct. They gave no comment to sympathetic pupillary reflex dilatation. This may be presumably due to the fact that their experiments were performed under pentobarbital or chloralose anesthesia. It has long been known that barbiturates depress the hypothalamus [Masserman (21); Bremer (22); Laidlaw and Kennard (23)]. Hodes and Magoun (24) observed that under pentobarbital anesthesia, electrical stimulation of the forebrain elicited neither pupil-
lodilatation of the parasympathectomized pupil nor piloerection. The author also observed that barbiturates depressed the sympathetic component of reflex dilatation. Chloralose, urethane, and ether, even in small doses, depress the activity of the cerebral cortex. It is reasonable that the majority of investigators who performed their experiments under anesthesia with these drugs deny the sympathetic component of reflex dilatation.

As mentioned in the introduction of this paper, Weinstein and Bender found a species-difference between cats (little sympathetic dilatation) and monkeys (marked sympathetic dilatation). It will be noted from their protocols, however, that these authors used light ether anesthesia for the monkeys, but experimented on cats anesthetized by nembutal. It is, therefore, probable that the apparent species-difference was really due to the difference in the kind and level of the anesthetics used.

In addition to the afferent path described by Harris, Hodes, and Magoun (see above), afferent pain impulses evoked by sciatic stimulation ascend in the lateral spinothalamic tract. In conscious animal, it is probable that these impulses reach the cerebral cortex through the diencephalon and then descend to the sympathetic center of the hypothalamus. The hypothalamic center may receive also afferent impulses through polysynaptic relays in the midbrain reticular formation.

Pupillary dilatation by humoral transmission poured into the blood by the adrenal glands was also considered as a mechanism of pupillary reflex dilatation. However, McDowall (25) and Bain et al. (4) observed that removal of the adrenals did not alter the pupillary response. This means the adrenaline, released from the adrenals, plays an insignificant role in reflex dilatation. It had, however, been known for a long time that some time after cervical sympathetic ganglionectomy, the dilatation response to sensory stimulation was increased on the operated side [for example, Langendorff (26)]. In response to moderate stimulation, this "paradoz_ical" pupillary dilatation has been shown to be caused by noradrenaline from many structures, especially the heart and the smooth muscle tissue of the arterial tree [see (13) and older literature contained therein; (27)]. Because of the relative insensitivity of the normal iris—compared to, for example, normal blood vessels—humoral pupillary dilatation probably is negligible in the normal pupillary reactions to moderate stimuli, although adrenaline, released from the adrenals under conditions of severe stress, may have an influence on reflex dilatation of the pupil.

Pupillary reflex dilatation in cats and dogs is predominantly due to the sympathetic mechanism, since it is greatly reduced by cervical sympathectomy. The same results were demonstrated as well in rabbits [for example, Gullberg (28)], monkeys [for example, Weinstein and Bender (11)], goats [Luchsinger (8)], and frogs [Schipolow (29)].

Barbiturates: Pentobarbital and thiopental caused an immediate dilatation of the pupil followed by subsequent contraction. This primary dilatation was observed in the sympathectomized pupil as well as in the intact one. In this mydriatic stage, the tone of the nictitating membrane was unchanged. This observation suggests that the pupil-
lary dilatation is due to parasympathetic inhibition. Long-lasting contraction following the initial dilatation seems to be due to an excitation of the miotic center in the midbrain. The following explanation is most likely: supranuclear structures, lying above the midbrain, have an inhibitory effect upon the oculomotor nucleus; when they are depressed by barbiturates, the oculomotor nucleus is disinhibited, that is, secondarily excited and miosis occurs. Inhibition of the sympathetic system takes part in the mechanism of this miosis. Although the primary dilatation after the injection of barbiturates appears to be due to the inhibitory effect of barbiturates on the oculomotor nucleus, it cannot be interpreted as a direct action on the oculomotor constrictor activity, because the dilatation is transient and is not in parallel with the anesthetic level of barbiturates. Barbiturates always lowered blood pressure for a while. The initial mydriatic effect of barbiturates corresponds well with the blood pressure fall in both temporal and spatial patterns, whereby thiopental cause more marked but short-lasting hypotension and mydriasis than pentobarbital.

The sympathetic component of pupillary reflex dilatation is always decreased by barbiturates, whereas the parasympathetic-inhibitory one is often increased after smaller doses of barbiturates. Barbiturates seem to accelerate the afferent conduction for parasympathetic reflex dilatation since this potentiating effect is observed not only in the mydriatic stage but also in the miotic stage after barbiturate administration. Nevertheless, the parasympathetic-inhibitory reflex dilatation is reduced progressively and finally abolished by large doses of barbiturates. This loss of pupillary dilatation is due to the depressing effects of barbiturates on the supranuclear structures which normally inhibit the oculomotor nucleus.

**Pentylenetetrazol (Metrazol):** Metrazol dilates the cat’s pupil as described in the results. The dilatation is a little less extensive in the sympathectomized pupil than in the normal one, although the sympathectomized pupil dilates considerably. These facts suggest that both sympathetic excitation and parasympathetic inhibition account for the mydriatic effect of Metrazol. Large doses of Metrazol excite the sympathetic system first and then depress it. Therefore, vagosympathectomy frequently does not affect the reflex dilatation after large doses of Metrazol. Even when the sympathetic reflex dilatation is reduced by large doses of Metrazol, the parasympathetic-inhibitory dilatation is still increased, and thus the total extent of reflex dilatation is usually increased. Occasionally when the parasympathetic dilatation is not as intensely increased by Metrazol, the total extent of reflex dilatation may be reduced, as in the case of Fig. 6.

Systemic blood pressure was raised by Metrazol, and returned to the control level after a while. The pressor response to sciatic stimulation was frequently diminished after large doses of Metrazol. This also is presumably due to depression of the excitability of the sympathetic nervous system by Metrazol.

**Chlorpromazine:** It is known that chlorpromazine exerts a depressant action on the sympathetic component of the central autonomic system [Laborit and Huguenard (30); Dasgupta and Werner (31); Spector et al. (32); and Wikler (33)]. It is conceivable that this
effect of chlorpromazine participates in the inhibition of the reflex dilatation resulting from sciatic stimulation. Moreover, chlorpromazine depresses the dilatation induced by injected adrenaline and preganglionic cervical sympathetic stimulation, whereby reduction of the response to sympathetic stimulation is more marked than that of the adrenaline-induced pupillary dilatation. This seems to be due to the fact that chlorpromazine, in addition to its adrenolytic effect, has a ganglion-blocking action. However, after injection of chlorpromazine, the response of the nictitating membrane to cervical sympathetic stimulation is not reduced as intensely as that to adrenaline. These observations suggest that the sensitivity to chlorpromazine of the adrenergic receptors of the pupil differs from that of the nictitating membrane.

In the dog [Spector et al. (32)] and rabbit [Spector et al. (32); Bogdanski and Brodie (34)], chlorpromazine contracts the pupil, whereas it dilates the pupil in the cat. Bogdanski et al. concluded that the miotic action of chlorpromazine in unanesthetized rabbits was due largely to a lowered sympathetic activity since chlorpromazine had no effect on the sympathectomized pupil.

In the cat, however, chlorpromazine dilates both the intact and the sympathectomized pupil. When both the sympathetic and parasympathetic nerves have been sectioned, the pupil remains unchanged after chlorpromazine. The mydriasis produced by chlorpromazine, therefore, appears to be due to inhibition of the parasympathetic oculomotor nucleus.

Metrazol and morphine cause the sympathectomized pupil to dilate and increase the reflex dilatation. Although chlorpromazine also produces mydriasis, it does not enhance the reflex dilatation. The mydriatic action of chlorpromazine is similar to that of atropine, but unlike the effect of atropine, it is not peripheral but central in nature.

*Morphine*: In the cat, morphine dilates the sympathectomized as well as the normal pupil. The dilatation in the sympathectomized pupil is generally weaker than that in the intact one. The morphine mydriasis in the cat, therefore, may be interpreted as follows: in addition to sympathetic excitation, there is inhibition of parasympathetic activity. Both the sympathetic-excitatory and the parasympathetic-inhibitory mechanisms appear to be concerned with the enhancement of reflex dilatation by morphine. The parasympathetic component seems to play a more important role in enhanced responses, since, after removal of the ciliary ganglion, morphine no longer caused much enhancement of pupillary reflex dilatation.

Wikler (35) reported that morphine (5 to 10 mg/kg i.v.) reduced the magnitude of the response of the nictitating membrane to sciatic stimulation in cats anesthetized by urethane; there was a slight spontaneous contraction of the nictitating membrane after morphine in some of his preparations. In the present experiments, morphine similarly caused the nictitating membrane to contract spontaneously. In some preparations, this spontaneous contraction was so intense that sciatic stimulation could not add much further contraction, so that the reflex response was reduced in amplitude even though
the total contraction was very strong. In other preparations, both the amplitude of the reflex contraction and the total level of contraction were increased. Therefore, the apparent sympathetic inhibition in Wikler’s experiments may be due to the spontaneous contraction of the nictitating membrane, evoked by sympathetic excitation after the morphine injection.

In contrast to the reactions in cats, morphine invariably contracted the pupil of the dog. Henderson and Graham (36) have demonstrated that even in decorticated dogs the pupil was contracted by morphine, but this miosis changed to mydriasis after injury to or removal of the corpora quadrigemina. McCrea, Eadie and Morgan (37) reported that morphine miosis in the dog appeared to be mainly due to an exaggeration of the light reflex. Ansler (38) found that morphine produced nearly the same degree of miosis even after the cervical sympathetic nerve was cut.

The author concluded from these results in the literature and from his own investigations, that morphine miosis in the dog is probably due to a stimulation of subcortical structure, probably in the dorsal midbrain although a direct action on the oculomotor nucleus is not entirely excluded. Possibly due to the sedative effects of morphine, reduction of sympathetic activity appears to take part in the miosis to some extent.

The magnitude of reflex dilatation in the cat was found much different in individual animals. The author occasionally observed that the pressor response to sciatic stimulation was ever so weak, while the pupillary response was so marked in contrast, and vice versa. Most of the male cats experimented in January and February showed marked reflex dilatation in response to sciatic stimulation, whereas those in March and April generally showed very poor responses. Since January and February fall on the mating season of Japanese cats and March and April fall in a period of rest from their sexual activity (Shimamura (39)), the magnitude of pupillary reflex dilatation may possibly have some relation to the reproductive cycle, although it may merely have resulted from climatic or nutritional factors.

**SUMMARY**

The physiological role of the sympathetic and parasympathetic nervous systems in pupillary reflex dilatation was analyzed in unanesthetized cats and dogs which were immobilized with decamethonium. Furthermore, the responses of the pupil, nictitating membrane and systemic blood pressure to sciatic stimulation were investigated as well as the influence of some drugs upon these responses.

1. In both species pupillary reflex dilatation was always markedly diminished but not abolished by cervical sympathectomy. The sympathetic nervous system, therefore, plays a much more important role in pupillary reflex dilatation than does inhibition of the parasympathetic innervation.

2. Pupillary reflex dilatation elicited by sciatic stimulation is abolished after high cervical transection. This fact proves that the response is not mediated directly to the
peripheral sympathetic chain via Budge's ciliospinal center, but must ascend to higher brain structures before it is mediated to efferent sympathetic pathways.

3. In cats, barbiturates caused pupillary dilatation, followed by subsequent contraction, both in intact and in sympathectomized pupils of cats. Therefore, the pupillary effects of barbiturates are considered to be mostly due to the parasympathetic mechanism. The reflex dilatation via the sympathetic nerve (active sympathetic reflex dilatation) was always decreased by barbiturates, while that elicited by inhibition of the parasympathetic oculomotor nucleus was increased by smaller doses and decreased by large doses of the drug.

4. The cat's pupil dilated after injection of Metrazol and showed marked oscillations in size during the initial period. The dilatation is dependent on both sympathetic excitation and parasympathetic inhibition. In larger doses than 10 mg/kg, Metrazol excites the sympathetic nervous system first and then depresses it. Therefore, sympathetic reflex dilatation is increased first and decreased afterwards. The responses of the nictitating membrane and blood pressure to sciatic stimulation generally were also reduced after an initial increase. On the other hand, pupillary dilatation due to parasympathetic inhibition was never depressed. As the dose of Metrazol was increased, it was enhanced.

5. In cats, chlorpromazine injections produced mydriasis, both in the normal and in the sympathectomized pupil. This mydriasis is, therefore, due to parasympathetic inhibition. Furthermore, parasympathetic-inhibitory reflex dilatation appeared to be unaffected by chlorpromazine.

The sympathetic part of pupillary reflex dilatation, contraction of the nictitating membrane and elevation of blood pressure resulting from sciatic stimuli were depressed after chlorpromazine.

6. Morphone dilated the cat's pupil and contracted the pupil of the dog. In cats, morphine mydriasis in the sympathectomized pupil was generally weaker than in the normal pupil. This mydriasis is accomplished by both the sympathetic-excitatory and parasympathetic-inhibitory mechanisms. The reflex dilatation elicited by sciatic stimuli was increased after morphine. Both the sympathetic and parasympathetic mechanisms participate in this increase, but the latter appears to play a dominant role. In dogs, morphine miosis seems to depend on excitation of the midbrain.

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