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STUDIES ON BARBITURATES. XXVII. TOLERANCE AND CROSS TOLERANCE TO BARBITURATES *

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A BROAD review of the experimental and clinical literature warrants the general conclusion that some degree of tolerance in some species to some of the barbituric acid derivatives undoubtedly exists. But beyond this fact there is no unanimity of opinion.

Rabbits were reported to develop tolerance to amytal (1, 2), while other investigators could find no evidence for such a tolerance in rats or dogs (3, 4, 5). (Such examples of disagreement could be multiplied *ad infinitum* and to the point of confusion.)

It is deemed imperative, therefore, to reopen the still moot question of tolerance to barbiturates by using only one species of animal. Dogs were chosen for this purpose because in recent years striking examples of barbiturate tolerance were described in this species by Ettinger (6), Oettel and Krautwald (7) and Dallemagne (8).

Since rates of absorption from either the gastro-intestinal tract or the subcutaneous tissues may vary from time to time, particularly on frequent administration of a substance, it was decided to limit the study of tolerance development to intravenously administered barbiturates.

EXPERIMENTAL

For these studies healthy dogs weighing from 5 to 10 Kg. were used. The barbiturates in the form of an aqueous solution of their sodium salts were administered by intravenous injection. The animals were reweighed before each injection, which usually took place between 8 and 10 o'clock in the morning. Sleeping time was taken as the time elapsing

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from the moment of injection until the animal could stand and support his own weight sufficiently to take a step. The animals were fed following recovery from anesthesia.

At the end of the series of observations the animals were either used for cross-tolerance studies or were sacrificed for analyses of their peripheral responses to anesthetic doses of evipal and their respiratory movements and arterial blood pressure recorded on a smoked drum.

A. Evipal.—Evipal sodium † was administered at first in doses of 30 mg.‡ by the saphenous vein on alternate days and later, when it was established that no cumulative effects ensued, it was administered daily. The results of these experiments are shown in table 1, from which it is apparent that alternate daily injections produced some tolerance, but it was not until daily injections were instituted that a high degree of tolerance developed. In the case of two dogs, the experiment was carried beyond the twentieth day and after this period the average sleeping time was about fifteen minutes (this was about one-fourth of the original sleeping time).

TABLE 1

TOLERANCE TO EVIPAL * INJECTED INTRAVENOUSLY IN DOGS

Day of Experiment	Number of Animals	Mean Sleeping Time
		Minutes
1	4	57
3	4	46
5	4	40
7	4	39
9	4	38
11	4	41
14	4	44
16	4	28
18	4	23
19	4	36
20	4	9
22	4	25
23	4	19
24	4	18
25	3	13
26	3	15
27	3	16
29	2	13
34	2	14
36	2	13

* Evipal Sodium, 30 mg. per Kg.

The dogs rendered tolerant by repeated administration of 30 mg. of evipal also showed tolerance to a smaller dose of evipal (20 mg.) as shown in table 3. Here the mean sleeping time was reduced from nine to about three minutes.

† Unless otherwise stated all references to evipal, nostal or pentothal in this paper are intended to mean the sodium salt.

‡ Throughout the paper doses expressed in mg. are intended to mean mg./Kg.

At the end of these observations all evipal-tolerant animals were reanesthetized with 40 mg. of evipal and their carotid blood pressure and respiration recorded. Under the initial dose of evipal, the pressor effects of standard doses of epinephrine (1:100,000), carotid occlusion and the cardio-inhibitory response following faradic stimulation of the peripheral end of the cut right vagus nerve were tested. Fixed electrodes were placed on the vagus and stimulations carried out with uniform stimuli. Following 40 mg. of evipal, injection of epinephrine and carotid occlusion produced sharp pressor responses, and stimulation of the peripheral vagus caused marked slowing of the heart with a fall in blood pressure. Then at five minute intervals 10 mg. of evipal was injected intravenously, and after each injection the three above tests were carried out. Every injection of evipal resulted in a sharp fall of blood pressure, with rapid recovery to normal or slightly subnormal levels. The rate of respiration was at first accelerated and then appreciably slowed so that after the fourth or fifth of these 10 mg. doses of evipal it was usually necessary to apply artificial respiration. The pressor effects from epinephrine were not abolished or diminished even with an accumulated dose of 100 mg. of evipal. The pressor effects following the occlusion of the common carotid artery, however, gradually diminished until they were either abolished entirely or reduced to a small fraction of the original.

The cardio-inhibitory effect following peripheral vagus stimulation was usually abolished after an accumulated dose of 60 mg. of evipal, or more. Figure 1 shows the respiratory and circulatory response of such an evipal-tolerant animal and represents the typical responses of three other evipal-tolerant dogs used for identical experiments.

Figure 2 shows the circulatory responses of an animal under evipal anesthesia that received a barbiturate for the first time. The results obtained are almost exact duplications of those obtained in evipal-tolerant animals. The same applies to two dogs that were rendered tolerant to pentothal and their respiratory and circulatory responses registered.

Evipal failed to abolish in any of these animals the pressor effect following intravenous nicotine injections (0.05 mg. of nicotine salicylate) and the peripheral vagus effect was abolished by the larger doses of evipal which could be easily restored following administration of appropriate doses (0.05 to 0.3 mg.) of physostigmine or neostigmine (9).

In none of these cases was the fatal doses of evipal determined by this cumulative method of administration, and so the question whether the fatal dose was appreciably increased in the tolerant animals must remain unanswered.

B. Pentothal.—After a few preliminary experiments it was determined that 20 mg. of pentothal, the first administration of which results in a mean sleeping time of ninety-five minutes, is the highest dose that can be given daily to dogs without causing cumulative effects. Follow-

FIG. 1. June 10, 1941. Dog 7.3 Kg. Evipal-tolerant. Time = 5 seconds. The same line represents 0 mm. of Hg. pressure. Uppermost line respiration, lower line mean arterial blood pressure. PV = faradisation of peripheral vagus nerve. Between D and E a total of 90 mg./Kg. of evipal was injected.

FIG. 2. Oct. 3, 1941. Dog Female 9.0 Kg. Control. Time = 5 seconds. The same line represents 0 mm. Hg. pressure. Uppermost line respiration, lower line mean arterial blood pressure. PV = faradisation of peripheral vagus nerve. Between D and E a total of 90 mg./Kg. of evipal was injected.

ing the daily injection of such doses the sleeping time was gradually lowered within the first week from ninety-five to twenty-five minutes (see table 2). The results here tabulated show also that the withdrawal of pentothal from these animals for nine consecutive days caused an almost complete loss of tolerance as indicated by the fact that the sleeping time rose from thirty-one to seventy-five minutes.

TABLE 2

TOLERANCE TO PENTOTHAL AND NOSTAL INJECTED INTRAVENOUSLY IN DOGS

Day of Experiment	Barbiturate Administered			
	Pentothal Sodium*		Nostal†	
	Number of Animals	Mean Sleeping Time	Number of Animals	Mean Sleeping Time
		Minutes		Minutes
1	5	95	5	75
2	5	76	5	55
3		‡	5	38
4		‡	5	30
5	5	32		‡
6	5	31		‡
7	5	25	5	0
8	5	31	5	0
9		‡	5	0
10	5	31	5	0
11		‡	5	0
12		‡		
13		‡		
14		‡		
15		‡		
16		‡		
17		‡		
18		‡		
19		‡		
20	5	75		

* 20 mg. per Kg.
† 30 mg. per Kg.
‡ None of the animals in this group injected on this day.

C. Nostal.—Table 2 shows also the responses of dogs to daily injections of 30 mg. of nostal. These animals, which slept for seventy-five minutes after the first injection of nostal, recovered in thirty minutes following the fourth injection and on subsequent injections they failed to sleep at all and the only visible evidence of having received an anesthetic dose of a barbiturate was a slightly ataxic gait.

D. Cross-tolerance.—Table 3 shows that the dogs at the height of their tolerance to evipal, pentothal or nostal also showed cross-tolerance to other members of the barbituric acid group. Evipal-tolerant dogs were tolerant not only to evipal but also to pentothal and nostal; pentothal-tolerant dogs were also tolerant to evipal, and nostal-tolerant dogs to evipal and pentothal. The data in this table also show that the

TABLE 3

CROSS TOLERANCE TO BARBITURATES INJECTED INTRAVENOUSLY

Group	Barbiturate Administered							
	Evipal*		Pentothal*		Nostal†		Barbital‡	
	Number of Dogs	Mean Sleeping Time	Number of Dogs	Mean Sleeping Time	Number of Dogs	Mean Sleeping Time	Number of Dogs	Mean Sleeping Time
		Minutes		Minutes		Minutes		Hours
Control	4	9	5	95	5	75	10	35
Evipal Tolerant	2	3	2	23	2	0	—	—
Pentothal Tolerant	3	4	5	31	–	—	—	—
Nostal Tolerant	2	5	5	47	5	0	5	10.5

* 20 mg. per Kg.
† 30 mg. per Kg.
‡ 225 mg. per Kg.

degree of cross-tolerance occasionally somewhat exceeded the original tolerance. The nostal-tolerant dogs were also examined for their tolerrance to the long-acting barbiturate, barbital. The mean sleeping time of thirty-five hours for a dose of 225 mg. of barbital was decreased in nostal-tolerant dogs to ten and one-half hours. The survival time of normal dogs receiving a fatal dose of barbital (500 mg.) was only one hundred fifty-two minutes, whereas nostal-tolerant animals lived nearly twice as long after the administration of the same dose. While every control dog died typically of respiratory depression, the nostal tolerant animals appeared to die from circulatory failure (see table 4).

In several of these animals, the urines were analyzed for the presence of barbital and there was no evidence that the tolerant animals excreted barbital at a faster rate or in greater percentages than the controls. The analyses were done by the lithium or micromethod described previously.

DISCUSSION

It is impossible in this discussion to review every paper regarding the tolerance to barbiturates. The mere fact that one set of authors has reported tolerance and another group has reported lack of tolerance to any given barbiturate is in itself of little significance unless we know their criteria for tolerance and methods employed by them. It is our contention that tolerance, following any route of administration, might be considered significant only providing that the author ruled out the possible variations in the absorption rates.

Drugs given by any route other than intravenously always have this variable nature of absorption to contend with, and as a consequence it is believed that only experiments involving intravenous medication can be considered valid.

Papers dealing with tolerance to barbiturates may be classified into three general groups: (1) papers reporting no tolerance; (2) papers that report a sudden drop in sleeping time between the first and second or first and third or second and third administration without further significant decrease in sleeping time following subsequent administration; and (3) papers reporting a *gradual* diminution in sleeping time reaching a minimum only after frequent administration.

Negative results (no tolerance) were reported by many authors for several barbituric acid derivatives. Masuda, Budde and Dille (1) found no decrease in sleeping time following intravenous daily administration of ortal and evipal in rabbits. With ortal, they found there was an actual increase in sleeping time, a fact which we will discuss later. At intervals of one or two days Kennedy (10) anesthetized two mice with evipal, seventeen and eighteen times, respectively, and found that neither animal exhibited tolerance. Swanson, Weaver, and Chen (5) administered about one-third of the minimum fatal dose of sodium amytal orally to dogs and intravenously to monkeys and stated definitely that no tolerance developed in any of the experimental animals during a period of administration of several months' duration. This paper, although belonging in the first category, is a connecting link with the second group of papers, for the authors state in most cases that the animals slept longer with the first, second or third doses than with subsequent doses. This fact was not attributed to tolerance but rather to an acquired ability of the animal to eliminate the barbiturate at a faster rate after it has become accustomed to the drug than at the start. This is precisely the explanation advanced by Eddy (11) to explain the same phenomena for a different body of facts. Eddy found that during the first few days of administration of barbital or phanodorn to cats a cumulative effect appeared. Later, with continuations of the same dose this effect disappeared. Eddy suggests that this was due to excretion gaining upon absorption, a cycle which was repeated when the dose was increased.

Although Swanson *et al.* (5) did not believe that they produce a tolerance to amytal, other authors reporting similar results interpreted their findings as tolerance. Masuda *et al.* (1) reported that, following daily intravenous administration of amytal, pernoston, and pentobarbital to rabbits, tolerance developed to these drugs within two weeks and that this acquired tolerance rapidly disappeared usually within four days. Their data show that the only significant reduction of sleeping time occurred during the first three days, and after the third day there was usually no further decrease in sleeping time. Several authors reported similar results for dial and pentobarbital. Moir (12), for example, states that rats developed tolerance to pentobarbital usually in the second injection with no further reduction in the sleeping time. Fitch (2), during the course of experiments in which the chronic oral toxicity of neonal, nostal, and amytal was studied in rabbits, found that

TABLE 4

SURVIVAL TIME OF NOSTAL TOLERANT DOGS RECEIVING FATAL DOSES OF BARBITAL SODIUM*

Control Group		Tolerant Group	
Dog Number	Survival Time	Dog Number	Survival Time
	Minutes		Minutes
1	230	1	720
2	180	2	230
3	45	3	127
		4	305
		5	220
Average	152	Average	320

* 500 mg. per Kg.

the sleeping times were shortened by nearly 50 per cent after the second and third doses.

In the third group of papers, Oettel and Krautwald (7), using dogs, observed that daily administration of phanodorn produced a tolerance of such a degree that 130 mg./Kg. at the end of the experiment produced the same degree of narcosis as did 75 mg./Kg. at the beginning. Nostal, according to these authors, produced a rapid development of tolerance at the beginning (50 mg./Kg. caused sleep for from six to seven hours). After a few daily administrations of such doses, tolerance of such a magnitude developed that even doses of 75 to 90 mg. caused no sleep, and scarcely any narcotic effect. They state that in the first instance there was an increase in the excretion, and that in the second an increase in the rate of destruction of the barbiturate. Dallemagne (8) reported a gradually developing tolerance following daily intravenous administration of evipal and numal in dogs. In some cases the process was rapid and in others slower. Based on our experience, Dallemagne's initial doses were so low that they would not produce anesthesia in the average animal. The initial doses of Oettel *et al.* (7), however, were a trifle too high to be used with safety.

Ettinger (6) found that 0.3–0.5 cc./Kg. of dial-Ciba administered intravenously or intra-peritoneally to each of seven dogs produced tolerance. Two of these dogs received the barbiturate twenty and twenty-two times, respectively. The interval between the injections was between three and seven days. Where the first injection produced anesthesia of about ten hours' duration, the second anesthesia lasted only eight hours, and following the next nine injections the anesthesia varied from five to eight hours. The eighteenth injection produced an anesthesia of a minimum duration of one hour. When he used nembutal, however, he found that the duration of the second anesthesia might drop as low as one-half the initial time but thereafter as many as fifty-three successive injections of the same dose over a period of eighty-three days produced approximately the same duration of anesthesia. Carmichael

and Posey (15) also reported on a gradual development of nembutal tolerance in guinea pigs. They also found that animals that developed tolerance to one-quarter or one-third of the average fatal dose were not protected against 60 per cent of the fatal dose.

In the experiments dealing with evipal, pentothal and nostal described in this paper all drugs were administered intravenously to exclude any possible changes in absorption rates. In the case of evipal there was a sudden drop in sleeping time following second and third injections reminiscent of the results obtained in the "second group of papers." Later, however, particularly when this drug was given daily rather than alternately, the mean sleeping time decreased gradually from the original fifty-seven minutes to about fourteen minutes. The daily administration of pentothal sodium also led to tolerance (again the sleeping time dropped abruptly following the second and following the third injection). There was no significant drop after the third injection, however, and when the drug was discontinued for nine days the sleeping time returned to the level reached on the second day. With nostal, the decrease in sleeping time was rather gradual and after about one week the original dose caused no sleep whatever, but only a slight ataxia.

It was further shown that dogs developing tolerance to intravenously injected barbiturates also developed cross-tolerance to other members of this group of hypnotics. Still more interesting is the observation that the cross-tolerance was in some cases greater than the original tolerance. For example, a dose of pentothal sodium causing a sleep of thirty-one minutes' duration in pentothal-tolerant animals produced sleep of only twenty-three minutes in evipal tolerant dogs.

A clue as to the probable mechanism of this tolerance is given by the cross tolerance developed in nostal-tolerant dogs to anesthetic and fatal doses of sodium barbital. With anesthetic doses of barbital the sleeping time was reduced from thirty-five to ten and one-half hours, and with fatal doses the average survival time was increased from about two and one-half hours to nearly five and one-half hours. Barbital is a drug which is little destroyed in the body and largely excreted unchanged by the kidneys. As shown by chemical analysis of the urine, there was no evidence of increased excretion in the tolerant animals over the control animals. This fact would seem to indicate that the tolerance to barbiturates may not be due to increased destruction or excretion of these drugs but rather to cellular or histogenic phenomena.

These experiments also explain at least some of the discrepancies in the literature on barbiturate tolerance. When we first studied pentothal tolerance by daily administration of 30 mg./Kg., we observed instead of a gradual decrease, an actual increase in sleeping time. When we reduced the average narcotic dose to 20 mg./Kg., however, we had no difficulty in demonstrating tolerance. Obviously then, in the first instance cumulation masked the appearance of tolerance. This may be

the explanation of the failure of Masuda *et al.* (1) to demonstrate a tolerance to ortal. It may be pointed out further that the tolerance to barbiturates is relatively short-lived. Both Masuda *et al.* (1) and the authors of this paper have demonstrated the rapid disappearance of barbiturate tolerance upon discontinuation of administration. It should be emphasized, therefore, that in any series of experiments designed to demonstrate a tolerance to barbiturates, the drug must be given frequently enough to prevent a loss of tolerance, but in doses and at time intervals so adjusted that the rate of administration should not exceed the rate of essential elimination (cumulation vs. tolerance). It should be stated also, that the criterion for tolerance is not necessarily an increase in the average fatal dose, because as far as the writers are aware this has not been demonstrated clearly even for opiates. Thus when some authors aim at the demonstration of an appreciable increase in the LD_{50} for barbiturates they may be setting too lofty a goal for themselves.

Barbiturates are not merely centrally acting drugs but possess some peripheral actions as well (9). Dallemagne (8) claimed that evipal-tolerant dogs also become tolerant to the peripheral actions of evipal, which, according to him, consist of a fall in blood pressure, suppression of the pressor effect of epinephrine, and a suppression of the pressor effect of the carotid sinus reflex elicited by occlusion of the common carotid artery. Evipal-tolerant dogs, according to Dallemagne, show none of these effects under evipal anesthesia. The results of our experiments show that evipal does not suppress the pressor effect of epinephrine but suppresses the carotid sinus pressor mechanism (in agreement with Nowak (13, 14). Furthermore, evipal produces a fall in blood pressure and, in large doses, it paralyzes the cardiac vagus. Evipal-tolerant animals when anesthetized with evipal exhibit the same peripheral effects as the control animals, namely, vasodepression, gradual diminution of the carotid-sinus pressor mechanism, and depression of the cardiac vagus activity. Thus we must disagree with Dallemagne's contention that the tolerance to evipal is not only central but also peripheral.

SUMMARY

1. Dogs, upon repeated daily injections of evipal sodium, pentothal sodium, and nostal sodium, develop a marked tolerance to these drugs characterized by a significant reduction in sleeping time. In the case of nostal, anesthetic doses eventually failed to produce sleep in tolerant animals.

2. Dogs made tolerant to evipal, pentothal, or nostal show cross-tolerance to the other two barbiturates, and also to the long-acting barbital sodium.

3. The tolerance to barbiturates is not lasting. Pentothal-tolerant animals practically lose all their tolerance in less than two weeks.

4. Dogs which developed tolerance to the narcotic action of evipal failed to show tolerance to the peripheral actions of this hypnotic,

namely: vasodepression, vagal paralysis, and prevention of the elevation of blood pressure following occlusion of the common carotid artery.

5. The tolerance to the narcotic action of these barbiturates is probably a true cellular tolerance. The development of this phenomenon can only be obtained by a careful balance between the rate of administration and the rate of essential elimination; in other words, it may be obscured either by cumulative effects or by partial loss of tolerance between administrations.

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