If anything, sodium sulphocyanate showed some tendency to potentiate the effect of morphine. It undoubtedly considerably increased its toxicity, which might be expected of any substance which could potentiate morphine in all of its manifestations. Our results are not those which could be predicted from Bancroft's theory on the mechanism of the pharmacological actions of morphine, sodium sulphocyanate and mono-sodium phosphate.

We gratefully acknowledge the assistance of the Biological Research Laboratories of E. R. Squibb and Sons in which all the tests on rats and mice reported herein were carried out.

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THE TOXICITY OF BARBITAL DERIVATIVES.*,[1]

BY IVOR JONES[2] AND E. V. LYNN.

Notwithstanding the large number of derivatives of barbituric acid which have been introduced into medicine, we have as yet only meager information as to comparative toxicity and efficiency. The textbooks make certain standard statements about the older ones which are not based upon existing evidence. Whatever reports can be found in the literature have originated largely in the laboratories of manufacturers, and the few unbiased records give very little data upon which the physician can base accurate judgment. The present study was designed as a start towards making such information available.

Of the marketed compounds seven of those most often used were selected for study: Barbital U. S. P. and phenobarbital U. S. P., introduced about 1904; dial, 1912; amytal, 1924; neonal, 1926; phanodorn, 1928; pentobarbital (nembutal), 1930.

The rabbit was chosen as the experimental animal because there seems no stated objection and several disadvantages have been found for other animals.

Using oral administration for rabbits, the M. L. D. in mg. per Kg. has been determined by Fitch and Tatum (1) as follows: Barbital 275, amytal 575, pentobarbital 175, phenobarbital 150, neonal 160, phanodorn 450. Roemer (2) gave for barbital 400. By subcutaneous injection in rabbits, the only results reported have been from the laboratories of Eli Lilly & Co.: Barbital 290, amytal 110, neonal 100. Intraperitoneally, Fitch and Tatum (1) gave: Barbital 225, amytal 90, pentobarbital 65, phenobarbital 150, neonal 115, phanodorn 130. Intravenously, Herwick and Knoefel (3) reported for barbital 250, for amytal 50. Intramuscularly, Louvier (4) gave for phenobarbital 150. As far as could be determined no one else has given any results on rabbits.

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[1] Seattle, Washington, June 19, 1934.
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ORAL ADMINISTRATION.

In the present investigation, there were used only healthy animals of mixed breeds and of both sexes weighing four pounds or over, the females being in all cases non-pregnant. No variation in results because of sex was noticed in the preliminary experiments.

Oral Administration.				Intraperitoneal Injection.			
Drug.	Dose, Mg. per Kg.	Number of Animals.	Result.	Drug.	Dose, Mg. per Kg.	Number of Animals.	Result.
	300	4	S		250	2	S
Barbital	350	4	1		275	2	S
	375	4	1		300	2	S
	400	4	D	Barbital	325	2	S
					350	2	S
	100	4	S		375	2	1
Phenobarbital	125	4	1		385	2	D
	140	4	2				
	160	4	D		140	2	S
				Phenobarbital	150	2	1
	450	8	S		160	2	D
	500	4	1		170	2	D
	525	4	1				
	550	2	S		90	2	S
	600	2	S		100	2	S
Amytal	650	2	S	Amytal	110	2	D
	700	2	S		125	2	D
	800	2	S		150	2	D
	900	2	S				
	1000	1	S		90	2	S
	1500	2	S	Dial	100	2	1
					110	2	D
	100	4	S		125	2	D
Dial	125	4	2				
	140	4	3		80	2	S
					90	2	S
	100	4	S	Neonal	100	2	1
Neonal	130	4	2		110	2	1
	150	4	3		120	2	D
	160	4	D				
					40	2	S
	200	2	S		50	2	S
	250	2	S	Pentobarbital	60	2	1
	275	2	S		70	2	1
	300	2	S		80	2	D
Pentobarbital	350	2	S		90	2	D
	400	2	S				
	500	2	S		120	2	S
	600	2	S		140	2	S
	350	4	S		150	2	S
	400	4	S	Phanodorn	160	2	1
Phanodorn	425	4	2		170	2	1
	450	4	2		180	2	D
	475	4	3		190	2	D

S = All survived. Figures are used for the number that died; the letter D signifies that all died; 1, one died; 2, two died; 3, three died.

During the period of experimental work the rabbits were fed twice daily with a constant diet of alfalfa hay and rolled barley. The animals were fasted for a period of sixteen hours before administering the drug. All of the rabbits were well housed and bedded on dry straw.

The dose of the drug was calculated upon body-weight to within one mg. and placed in several small gelatin capsules. These capsules were given by moistening them with water, placing them far down in the rabbit's throat, and then washing them down with a small volume of water. The time was noted as soon as an oral dose had been given and the period before partial and complete anesthesia was measured. The period of anesthesia and the various symptoms were observed and recorded. The minimum lethal dose was judged when 50 per cent of the animals died within thirty hours or during unconsciousness.

In the foregoing table are recorded the results of administration of the compounds orally to rabbits in varying doses.

INTRAPERITONEAL INJECTION.

The minimum lethal doses were determined by injecting carefully a freshly prepared solution of the drug into the peritoneal cavity of healthy rabbits. The solutions of the various compounds used were accurately prepared by dissolving a weighed sample of the product, furnished by the manufacturer, in a calculated amount of half-normal sodium hydroxide solution except, in the case of barbital, pentobarbital and phenobarbital, where the sodium salt of the hypnotic was available. Amytal, dial, neonal and phanodorn were dissolved in half-normal sodium hydroxide, the amount of solution needed for 1 Gm. of drug being 8.84 cc. for amytal, 9.60 cc. for dial, 9.42 cc. for neonal and 8.46 cc. for phanodorn. The solutions were then made up to the required volume in a calibrated glass cylinder by adding sterilized distilled water. In every case the final solution contained 100 mg. per cc. of the hypnotic as the free acid. In the case of barbital, pentobarbital and phenobarbital, the compound as the sodium salt was weighed out and dissolved in sterilized distilled water in such concentration that the final solution contained 100 mg. per cc. of the hypnotic as the free acid. With amytal, dial and neonal, complete solution was never obtained in the alkali used. Since the residue in any instance did not amount to more than a milligram or two out of two Gm., it was ignored as negligible.

The minimum lethal dose again was taken as that amount which caused death in 50 per cent of the animals within thirty hours or during anesthesia.

COMMENT.

The approximate minimum lethal doses are herewith summarized, together with those by Fitch and Tatum.

	Oral Administration.		Intraperitoneal Injection.	
	Present Report.	Fitch and Tatum.	Present Report.	Fitch and Tatum.
Barbital	385	275	375	225
Phenobarbital	140	150	150	150
Amytal	Above 1500	575	105	90
Dial	125	..	100	..
Neonal	135	160	110	115
Pentobarbital	Above 600	80	175	65
Phanodorn	450	450	170	130

The most striking differences from the former results are noted with oral doses of amytal and pentobarbital which we found practically non-lethal up to the figures given. In addition the period before appearance of complete anesthesia was always more or less uncertain. These facts would be best explained by incomplete or very slow absorption and application in human cases might be very different.

Another interesting observation is that the drugs are divided distinctly into two groups according to the degree of toxicity by oral administration. Those with a low dose (below 150) are dial, neonal and phenobarbital; the others are above 375. The ratio is, thus, 375:150 or about 2.5:1.

Comparison of the times required to produce anesthesia also furnished some interesting observations. In the table below the values given are in minutes following administration and the exact attainment of anesthesia was determined by loss of the corneal reflex.

	Orally.		Intraperitoneally.	
	Limits.	Usually.	Limits.	Usually.
Barbital	60–180	90	45–180	60
Phenobarbital	90–180	120	30–180	90
Amytal	60–240	120	10–15	10
Dial	60–240	90	20–60	45
Neonal	90–300	120	10–30	20
Pentobarbital	120–240	120	10–15	10
Phanodorn	60–240	90	10–60	30

From a study of these values, one could safely conclude that amytal and pentobarbital act at about the same rate and in the same manner. Both are the most rapidly acting of the entire group when given intraperitoneally. Barbital and phenobarbital are characterized by having essentially the same speed of action either orally or by injection. Except for barbital and phenobarbital, they all act very rapidly when injected, causing almost immediate paralysis of the limbs and flacidity of the abdominal muscles. The anesthetic action progresses until the animal is prostrated, with the eventual loss of the corneal reflex which has been taken as an indication of anesthesia. It should be noted here that, even with very large doses by mouth, amytal often paralyzed the animals for as long as thirty-six hours without giving loss of the corneal reflex.

The criticism might be made that, since hypnotics are being considered, the appearance of sleep should be a criterion of efficiency. However, it was found that there is no good method of determining when a rabbit has gone to sleep. The drug manifests itself by first causing paralysis of the hind legs with loss of motor reflex. This paralysis progresses to the front legs and last of all to the neck and head. At this last stage of narcosis the animal is prostated. The next stage is loss of consciousness and the animal is apparently in a deep coma with abolition of all reflexes. It was, therefore, concluded that only the minimum anesthetic doses, if they could be accurately determined, would serve as a comparison for efficiency.

The duration of narcosis also gives interesting comparisons. In the table below, the time is given in hours.

	Orally.		Intraperitoneally.	
	Limits.	Usually.	Limits.	Usually.
Barbital	10–27	18	12–24	16
Phenobarbital	12–30	17	11–27	17
Amytal	4–24	16	3–18	4
Dial	8–24	16	4–16	15
Neonal	12–36	16	4–15	12
Pentobarbital	4–18	15	1–2	1.5
Phanodorn	2–19	12	0.75–2	1.5

It is plainly evident from these figures that the average duration is about the same for all of the drugs studied when given by mouth. However, it was noted that amytal, barbital and neonal in sub-lethal doses sometimes produced narcosis lasting as long as thirty-six hours, with a subsequent general paralysis for as much

as ten hours. Pentobarbital and phanodorn by injection are characterized by producing the shortest period noted, and recovery after administration with these two drugs is more rapid than with the others.

It is thought that the period or duration of anesthesia is not a criterion of efficiency because sometimes the more rapidly acting drugs produce a deeper narcosis than the others. In addition the anesthesia was marked by shallowness of respiration and greatly diminished heart beat, although the animal recovered very quickly nevertheless.

A few other observations of interest are also herewith noted.

Excessive diuresis and constant evacuation of the colon was observed after barbital.

Two animals died two weeks after apparent recovery from dial. Autopsy of the animal revealed nothing abnormal in the intestinal organs. There was noticed a degeneration of the bones in the hind legs, which appeared as a rotting of the shaft and articulating surfaces. This condition was observed only twice with dial and not at all with the other drugs, and probably had no relation to the hypnotic.

Upon autopsy of animals that had died from lethal doses of neonal, there was observed a greatly distended bladder containing a large amount of white deposit. Microscopical examination of the deposit revealed nothing but a few epithelial cells. No barbiturate could be identified in the material, but an examination showed the presence of alkali phosphate. It was concluded that the deposit was mainly this phosphate and of no pathological importance.

By intraperitoneal injection of pentobarbital there was produced very deep anesthesia which lasted from one to two hours. Recovery was very rapid.

A short period of hyperexcitability just after injection of phanodorn was noted.

Recovery from large doses of phenobarbital is comparatively slow. Paralysis after anesthesia may persist in some cases for as long as twenty-four hours.

SUMMARY.

The toxicity in rabbits and the comparative efficiency were studied for amytal, barbital, dial, neonal, pentobarbital, phanodorn and phenobarbital, using both oral and intraperitoneal routes.

Some of the figures for the minimum lethal dose differ markedly from those found in the literature.

Apparently the general ratio of efficiency to toxicity is approximately the same for the seven compounds, except that amytal and pentobarbital seem to be extremely safe by oral administration.

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"A vigorous appeal to Japan, asking her to stamp out the wide-spread illicit opium traffic engaged in by her subjects, has been made in a resolution of the opium advisory committee of the League of Nations. This action followed the assailing of Japan's laxity toward the bootleg narcotics traffic, made by spokesmen for China, England, Canada and the United States."