ANAESTHESIA IN NEW-BORN ANIMALS

BY

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Pentobarbitone was more toxic to new-born than to adult rabbits and rats, produced a longer loss of righting reflex in new-born animals but did not anaesthetize them effectively in less than toxic doses. Urethane did not anaesthetize new-born animals in doses which anaesthetized adults. Ether produced loss of righting reflex at lower concentrations for new-born than for adults, but the new-born animals became anaesthetized more slowly.

The responses of new-born animals to various drugs are known to differ from those of adults of the same species (Reiss and Haurowitz, 1929; Eddy, 1939; Chesler, Labelle and Himwich, 1942; Chen and Robbins, 1944) and it is not surprising, therefore, to find that they differ from adults in their responses to anaesthetic drugs. An appreciation of these differences is of importance to those working with new-born animals.

Homburger, Etsten and Himwich (1947) found that doses of pentobarbitone (40 to 50 mg./kg.), which killed rats less than 24 hr. old, produced a loss of righting reflex for only a few hours in adult rats. The sleeping time of new-born mice (Jondorf, Maickel and Brodie, 1959) which had received hexobarbitone was much longer than that of adult mice which had received the same dose. Fazekas, Alexander and Himwich (1941) found that new-born rats took longer to die than adults when breathing nitrous oxide or cyclopropane, although the time of arrest of respiration when breathing air containing an unstated concentration of chloroform vapour did not differ between newborn mice and mice older than 18 days (Reiss and Haurowitz, 1929).

While much of the evidence quoted above refers to the lethal effects of anaesthetic drugs, this paper is concerned with a comparison of some non-lethal effects of urethane, pentobarbitone and ether in new-born and adult rats and rabbits.

METHODS

Animals were exposed to ether in a 30 l. observation chamber at a controlled environmental temperature. Air was blown through the chamber at 6 l./min. by a Starling Ideal pump (C. F. Palmer). Ether vapour was added by passing the air through a calibrated E.M.O. Inhaler (Pentland Instrument) as described

by Epstein and Macintosh (1956). The lower part of the inhaler was immersed in a water bath at 20°. With a flow of 6 l./min., the concentration of ether in the chamber could be changed by about 97% in 10 min., and this time was always allowed for equilibration after each change of ether concentration. All animals were kept in a warm chamber (30 to 35°) for not less than 30 min. before transfer to the observation chamber for exposure to ether. They were exposed to ether, either singly or in batches, and were not restrained. The chamber was closed by a perspex lid in which there were two holes 4 in. in diameter. One was covered by a soft rubber sheet with a slit, through which animals could be introduced or removed from the chamber. The rubber sheet minimized mixing of atmospheric air with the contents of the chamber. When not in use this hole was closed by a perspex shutter. The second hole had a long-sleeved rubber glove attached to its margin so that, when an animal began to stagger, the operator, with the hand inside the glove, could turn it on its back. The time was recorded at which the animal lost its righting reflex. The animal was regarded as being anaesthetized when it no longer responded to pinching of the hind paw.

Sleeping time was the interval during which the righting reflex was absent. Sleeping animals were kept at constant temperature. Deaths were recorded at 24 hr.

Sodium pentobarbitone B.P. (Abbott) was dissolved in distilled water not more than 2 hr. before use. Urethane (ethyl carbamate: M.P. 48 to 50°) solutions were prepared from B.D.H. laboratory reagent in distilled water. Injections of pentobarbitone or urethane were given intraperitoneally with the dose per kg. contained in 10 ml. fluid.

RESULTS

Pentobarbitone

Preliminary experiments are summarized in Table I, which shows that pentobarbitone was more toxic to new-born rabbits and rats than to adults. For example, a dose of 35 mg./kg. of pentobarbitone commonly given to adult rabbits to produce

light anaesthesia of about 1 hr. with subsequent recovery killed 5 of 6 rabbits less than 1 day old (Table I). These animals did not become anaesthetized until they breathed less than twice per minute and were cyanosed.

TABLE I

TOXICITY OF PENTOBARBITONE TO DAY-OLD RABBITS AND RATS

The drug was given intraperitoneally. Environmental temperature, 32°.

Dose (mg./kg.)	Deaths at 24 hr.
<i>Rabbits 1 day old</i>	
25	1/5
28	0/7
32	0/8
35	5/6
<i>Rats 1 day old</i>	
45	4/6
50	3/6
55	6/6
60	5/6
<i>Adult rats</i>	
60	3/7
100	4/7
140	6/7

The sleeping time of new-born rabbits which had received a given dose of pentobarbitone was much greater than that of adolescent or adult rabbits which had received the same dose per kg. (Table II).

TABLE II

THE SLEEPING TIMES OF RABBITS AFTER PENTOBARBITONE

The numerals in parentheses indicate the number of animals. Environmental temperature, 32°.

Dose (mg./kg.)	Range of Sleeping Times (min.)		
	1 Day Old	30-31 Days Old	Adult
6	49-56 (4)		
12	54-143 (4)	8-23 (3)	0 (3)
18	173-265 (4)	0-31 (3)	12-27 (3)
24	300 or more (4)	5-47 (3)	8-72 (2)

Urethane

The sleeping times of both new-born rats and rabbits treated with 400 and 600 mg./kg. of urethane was longer than those of adults (Table III). However, there was a pronounced difference between the depth of anaesthesia produced in young and in old

rabbits and in rats with this drug. Only 1 of 4 rabbits, which had received 1.2 mg./kg. of urethane, less than 3 days old did not resist restraint on its back, whereas 4 of 4 adult rabbits which had received the same dose could be restrained on their backs. Young rats reacted to 1.2 g./kg. of urethane similarly to young rabbits.

TABLE III

SLEEPING TIME OF RABBITS AND RATS AFTER URETHANE INTRAPERITONEALLY

The sleeping time is given $\pm$ standard error. The numerals in parentheses indicate the number of animals used in each estimate. Environmental temperature 34°.

Dose (mg./kg.)	Sleeping Time (min.)	
	One Day Old	>90 Days
<i>Rabbits</i>		
400	68 $\pm$ 13.1 (7)	15 $\pm$ 4.5 (8)
600	90 $\pm$ 9.5 (7)	41 $\pm$ 8.5 (8)
<i>Rats</i>	Four Days Old	Adult
400	28 $\pm$ 5.4 (9)	0 (9)
600	43 $\pm$ 2.7 (9)	0 (9)

Ether

A group of 54 rabbits of three different ages (1, 3, and 30 days) were exposed to one of three concentrations of ether (4.1, 8.3 or 12.8 vol. %) at one of three temperatures (20°, 30° or 40°). Animals were allocated to experimental groups according to a latin square design and litters were randomized. The interval between exposure to ether and loss of righting reflex became less as the concentration of ether was increased. It was 9 to 11 min. at 4.1 vol. % ether, 2 to 9 min. at 8.3 vol. % and 2 to 4 min. at 12.8 vol. %. This interval was affected neither by the temperature nor by the age of the rabbits.

There was a difference in the time taken for rabbits of different ages to regain their righting reflex after removal from ether (Table IV). Two groups each of 5 rabbits (4 days old and 28 to 32 days old) were simultaneously exposed to 12.8 vol. % ether at 32° for 3.75 min. At the end of this time all rabbits had lost their righting reflex. The concentration of ether was then reduced to 1.9 vol. % and the time taken to regain the righting reflex was observed. This experiment was repeated on a different day, and, in spite of divergence of the mean times, all older rabbits

TABLE IV

TIME TAKEN BY RABBITS TO REGAIN RIGHTING REFLEX WHEN EXPOSED TO 1.9 VOL. % OF ETHER AFTER EXPOSURE FOR 3.75 MIN. TO 12.8 VOL. % OF ETHER

Environmental temperature, 32°. The times are means $\pm$ standard error.

		Time (sec.)	
		< 4 days	28-32 days
Age:			
Exp. No. 1 ..		> 60	30 $\pm$ 3.8
„ „ 2 ..		> 180	69 $\pm$ 6.0

regained their righting reflexes before any of the younger ones.

Two rats of different ages were placed simultaneously in an ether-air mixture at 30°. Five such pairs of rats were observed at each of three different concentrations of ether between 10.2 and 17.5 vol. %. Table V shows that there was little

TABLE V

TIME TAKEN BY RATS TO LOSE RIGHTING REFLEX AND TO BECOME ANAESTHETIZED WHEN EXPOSED TO ETHER.

Environmental temperature, 30°. There were 5 animals in each group. The times in min. are means $\pm$ standard error.

Ether (Vol. %)	Time to Lose Righting Reflex		Time to Lose Response to Pinch	
	5 Days	24-28 Days	5 Days	24-28 Days
10.2	65 $\pm$ 5.7	63 $\pm$ 2.0	625 $\pm$ 38	239 $\pm$ 41
14.2	52 $\pm$ 3.5	47 $\pm$ 2.2	435 $\pm$ 26	131 $\pm$ 12
17.5	42 $\pm$ 4.6	45 $\pm$ 1.6	463 $\pm$ 26	125 $\pm$ 9

difference between the times at which rats of different ages lost their righting reflex, but the younger rats took considerably longer before they became anaesthetized. The concentrations of ether used in these experiments can be regarded as high.

In other experiments, litters of rats of different ages were exposed simultaneously at 32° to lower concentrations of ether, either 1.9 or 4.1 vol. %. Table VI shows that all rats less than 1 day old had lost their righting reflex after 180 min. of exposure to 1.9 vol. % ether, whereas none of the older rats had lost their righting reflex after 240 min. of exposure to the same concentration of ether. In 4.1 vol. % ether, all the younger rats had lost their

TABLE VI

LOSS OF THE RIGHTING REFLEX IN NEW-BORN AND ADULT RATS EXPOSED TO ETHER
Environmental temperature, 32°.

Ether (Vol. %)	Age	No. of Rats in Group	Number of Rats Without Righting Reflex after Exposure for (Min.)							
			10	20	30	60	90	120	180	240
1.9	< 1 day	16	0	3	1	6	10	10	16	16
	9 weeks	17	0	0	0	0	0	0	0	0
4.1	4 days	11	11	11	11	11				
	4 weeks	6	0	5	6	6				
	9 „	5	0	1	5	5				

righting reflex after 10 min., but the older ones did not lose their righting reflex until after 20 min.

As with rabbits, the older rats also regained their righting reflex more quickly after ether. Groups of rats of several ages were exposed to 12.8 vol. % ether for 2 min., by which time all animals had lost their righting reflex. The concentration of ether was then reduced to 1.9 vol. % and the rats were examined after a further 20 min. By this time all the adults and a few of the older rats had regained their righting reflex, but none of the younger rats had righted themselves. These experiments were carried out at 20°, 30° or 40°, but, as the temperature did not appear to influence either the time to lose or to regain the righting reflex, the results have been combined in Table VII.

TABLE VII

DURATION OF LOSS OF THE RIGHTING REFLEX IN RATS EXPOSED TO 12.8 VOL. % OF ETHER FOR 2 MIN. AND THEN TO 1.9 VOL. % OF ETHER FOR 20 MIN.

For further explanation, see text.

Age (Days)	Number of Rats without Righting Reflex after 20 Min.
0-5	46/46
5-10	31/31
15-20	9/9
25-30	9/9
35-40	14/20
90-95	3/20
Adult	0/7

DISCUSSION

It is evident from these results that new-born rats and rabbits differ from adults in their reactions to anaesthetic drugs, but these differences are not

consistent. After small doses of urethane (400 or 600 mg./kg.) new-born rats and rabbits lose their righting reflex for longer than adults; whereas a dose of urethane, which in both adult rats and rabbits produced relaxation sufficient for surgical manipulations, did not relax or anaesthetize new-born rats and rabbits. Similarly with ether, new-born rats did not become anaesthetized so quickly as adult rats although the new-born rats and rabbits lost their righting reflex as soon as or sooner than the adult animals and could be maintained, without their regaining a righting reflex, at lower concentrations of ether than could adult rats. Pentobarbitone, which produced longer loss of righting reflex in new-born rabbits than in adults, did not produce anaesthesia in new-born rabbits until the respiration was grossly depressed, and was more toxic to new-born rats and rabbits than to adults.

The finding that new-born rabbits were not anaesthetized by a dose of urethane sufficient to anaesthetize an adult rabbit has also been observed by Adamsons (personal communication) and by Downing (personal communication), both of whom used urethane to anaesthetize rabbits of different ages for surgical procedures (Adamsons, 1959; Downing, 1960). However, Cross, Dawes and Mott (1959) found that about 1/4 of the dose of chloralose required for an adult sheep was sufficient to produce light surgical anaesthesia in a new-born lamb.

The prolonged action of barbiturates in new-born mice has been described by Jondorf *et al.* (1959). These authors also found that all of an injected dose of hexobarbitone was present in mice less than 24 hr. old 3 hr. after injection, whereas in 21-day-old mice only 70% of the dose remained after 3 hr. The metabolism of amidopyrine and phenacetin was similarly reduced in new-born mice. The side chain oxidation of hexobarbitone by liver homogenates from rabbits 2 to 3 weeks old was only 5 to 37% of that from adult rabbits (Fouts and Adamson, 1959): while new-born guinea-pig tissues were unable to form glucuronides (Brown and Zuelzer, 1958). Indeed there is much evidence that the concentrations of various enzymes in mammals are low at birth and increase during the first days of life (Potter, Schneider and Liebl, 1945; Reem and Kretchmer, 1957; Dawkins, 1959).

It is attractive to argue that the differences between the effects of urethane, pentobarbitone and ether in new-born and adult animals are due to functional differences in the central nervous systems. However, although there are known differences in enzyme concentrations in various parts of the brain at different ages, there is not sufficient known

of the mechanism of action or of the distribution of the anaesthetic drugs compared in this paper to say how the observed differences occur. On the other hand it is probable that the prolonged action of pentobarbitone is due in part to the slow systemic breakdown of the drug by new-born animals. The same may be true of urethane, 90% of which in normal adult mice is metabolized in 24 hr. (Mitchell, Hutchison, Skipper, and Bryan, 1949). Ether is not metabolized to any appreciable extent but is eliminated through the breath and excreta (Haggard, 1924) so that slow metabolism of ether cannot explain its prolonged action in new-born animals.

The differences in the effects of these three anaesthetic drugs on new-born animals show that it is not possible to generalize about the greater "sensitivity to anaesthetics" of new-born animals as compared with adults, and with our present knowledge it is not possible to predict reliably how a new type of anaesthetic drug will act in a new-born animal.

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