TOLERANCE OF MICE AT DIFFERENT AGES TO MUSCARINE- AND NICOTINE-LIKE CHOLINOLYTICS

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Tolerance to cholinolytics at different ages has so far been studied only in respect to atropine. The tolerance to this cholinolytic shown by animals has been found to increase with age [4, 7, 9, 10, 11]. In the present investigation we studied the tolerance shown by mice of different age groups to atropine, tropacin, difacil (spasmolytin, trasentin), amisil (benactisine), amisil methylate, pachycarpine, and hexonium (hexamethonium).

EXPERIMENTAL METHOD

Experiments were carried out on albino mice from the same nursery, in the following age groups: newborn (2-3 days), after the development of vision (2 weeks), after reaching a stage of independent existence (3-4 weeks), and fully grown male mice. Aqueous solutions of the test drugs were injected subcutaneously in the dorsal region in a volume not exceeding 1.5% of the body weight. The tolerance of the animals to the cholinolytics was judged by the size of the LD_{50}, determined from lethality curves. Each curve was plotted from the results in 30-100 (usually 60-80) animals; altogether about 1800 mice of different ages were used in the experiments. The lethality curves were plotted by the method of least squares [3]. The error of the LD_{50} was calculated by the method of Miller and Tainter [8] and added to the experimental error [2]. The significance of the difference between the values of LD_{50} was determined from the criterion t in conjunction with the combined error. In the text the probability of the differences between the LD_{50} values is expressed by the term q. The differences were considered to be absolutely significant when $q \geq 0.99$ and relatively significant when $q \leq 0.95$.

EXPERIMENTAL RESULTS

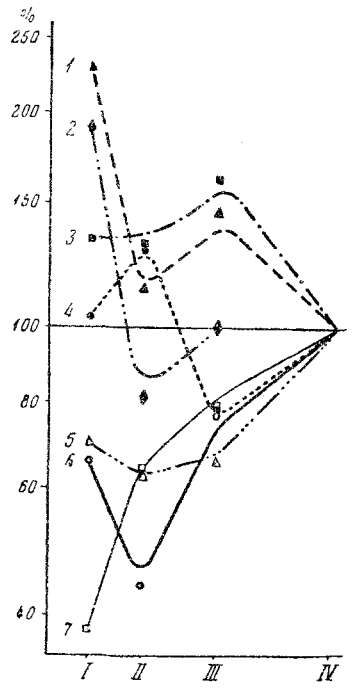
It follows from the results in Table 1 that the tolerance of mice to cholinolytics did not remain constant in the course of ontogenesis.

TABLE 1. LD_{50} of Cholinolytics for Mice of Different Age Groups when Injected Subcutaneously (in mg/kg)

Cholinolytic	Newborn	2 weeks old	3-4 weeks old	Adult
Tropacin hydrochloride	214 ± 9	102 ± 4	130 ± 5	90 ± 15
Pachycarpine	180 ± 4	72 ± 9	90 ± 1	91 ± 3
Hexonium diiodide	170 ± 16	228 ± 5	130 ± 12	166 ± 11
Amisil hydrochloride	144 ± 9	128 ± 6	136 ± 2	203 ± 3
Amisil methylate	90 ± 11	161 ± 12	192 ± 27	240 ± 8
Difacil hydrochloride	672 ± 21	694 ± 17	821 ± 21	510 ± 18
Atropine sulfate	394 ± 40	259 ± 70	441 ± 66	578 ± 24

The differences between the adult and newborn mice were particularly marked. The tolerance of the latter to tropacin, pachycarpine, and difacil was 2.4, 2.0, and 1.3 times greater than in adult mice respectively (see figure). At the time of development of sight the tolerance to tropacin and pachycarpine was lowered ($q > 0.99$); while the tolerance to tropacin moved close to that shown by the adults ($q = 0.4$), that to pachycarpine actually fell slightly below the tolerance shown by adults ($q = 0.91$). With regard to difacil, in the 2-week old mice the tolerance

remained the same as in the newborn ($q < 0.001$). At the age of 3 weeks the tolerance to all three cholinolytics increased: that to pachycarpine to the adult level ($q = 0.001$), and to tropacin and difacil to a higher level than in the adults ($q = 0.98$ and 0.99). The tolerance to atropine and amisil shown by the newborn mice was approximately 1.5, and that to amisil methylate 3.5 times smaller than in the adults ($q > 0.999$). At the age of 2 weeks, the tolerance to amisil and, in particular, to atropine fell still lower ($q = 0.85$ and 0.999), whereas that to amisil methylate, on the other hand, rose sharply ($q > 0.999$). Subsequently the tolerance to all three cholinolytics gradually increased and approached its adult level. In relation to hexonium it was the same in the newborn mice as in the adults ($q < 0.001$), rose slightly to the second week of life ($q = 0.99$), and fell again at the third week to a lower level than in adults ($q = 0.97$).



LD_{50} of cholinolytics for newborn (I), 2-week old (II), and 3-4 weeks old (III) mice as percentages of the LD_{50} for adult males (IV), taken as 100%. Along the axis of ordinates—percentages (logarithmic scale). 1) Tropacin; 2) pachycarpine; 3) difacil; 4) hexonium; 5) amisil; 6) atropine; 7) amisil methylate.

The change in the tolerance of the mice to cholinolytics in the course of ontogenesis obeyed certain general principles. The tolerance to cholinolytics blocking the N-cholinergic system (central—tropacin and difacil, and ganglion-blocking—pachycarpine) diminished with age, whereas that to cholinolytics blocking the M-cholinergic systems (atropine, amisil, and amisil methylate) increased. The tolerance to a third group of compounds (tropacin, pachycarpine, and atropine) fell during the second week and rose again during the third week of life, while the tolerance to a fourth group (the N-lytic hexonium and the M-lytic amisil methylate), on the other hand, increased during the second week of life.

The intensity of the central effect of the cholinolytics evidently played no important part here: the newborn mice were very tolerant to some central cholinolytics (tropacin and difacil) and showed low tolerance to others (atropine and amisil).

By expressing the doses of the cholinolytics as gram molecules per kilogram body weight (mol/kg) we found that the tolerance shown by the mice of all age groups to difacil and atropine was significantly higher than that shown to the other compounds (Table 2). LD_{50} for difacil was 1.14-1.84 mol/kg, for atropine 0.95-1.39 mol/kg, and for the other cholinolytics it varied from 0.13 to 0.46 mol/kg.

The newborn mice were less tolerant to cholinolytics blocking the M-cholinergic systems—amisil and amisil methylate—, and more tolerant to the cholinolytics blocking the N-cholinergic systems—pachycarpine, hexonium, and tropacin (not counting atropine and difacil). At the age of 2 weeks this order was changed: tolerance to amisil and its methylate was greater than to tropacin and pachycarpine. Mice aged 3-4 weeks showed a lower tolerance to cholinolytics blocking the N-cholinergic systems and a higher tolerance to cholinolytics blocking the M-cholinergic systems. After sexual maturation this order of arrangement was not substantially changed.

TABLE 2. Distribution of Cholinolytics in Order of Diminishing Tolerance Shown to Them by Mice at Various Age Periods

Newborn	2 Weeks old	3-4 Weeks old	Adult
Difacil	Difacil	Difacil	Atropine
Atropine	Atropine	Atropine	Difacil
Pachycarpine	Hexonium	Amisil methylate	Amisil
Tropacin	Amisil methylate	Amisil	Amisil methylate
Hexonium	Amisil	Pachycarpine	Hexonium
Amisil	Pachycarpine	Hexonium	Pachycarpine
Amisil methylate	Tropacin	Tropacin	Tropacin

Hence the mice of all the younger age groups were less tolerant than the adults to cholinolytics blocking the M-cholinergic systems and more tolerant to the cholinolytics blocking the N-cholinergic systems.

Death from poisoning by cholinolytic drugs is known to result mainly from their action on the central nervous system. The variation in the tolerance shown by the animals at different age periods is apparently due to the sensitivity and functional importance of the brain systems reacting with the cholinolytic at the various stages of ontogenesis. Since these differences are associated almost exclusively with which particular cholinergic systems are blocked, it may be concluded that the toxic action of the tested drugs depends on their specific cholinolytic properties. Very probably, therefore, the lower tolerance of the newborn and 2-4-weeks old mice to M-cholinolytics is due to the earlier maturation of the M-cholinergic systems and to their greater functional importance in the young animals than in the adults for maintenance of homeostasis. The greater tolerance shown by the newborn animals to N-cholinolytics is probably determined either by the later maturation (and, possibly, differentiation) of these systems or their lesser importance for the vitally important functions in mice of this age. It is clear that the N-cholinergic systems are formed in mice not before the second week of life, whereas the M-cholinergic systems are developed at birth.

The lowering of the tolerance of the 2-week old mice to the tertiary cholinolytics may be related to the development of the hapten-forming capacity of the cholinergic systems of the brain after the stage of acquisition of sight, while the increase in the tolerance to the quaternary cholinolytics, on the other hand, may be due to stimulation of the barrier function of the blood-brain barrier [5]. This barrier is known to be less permeable to compounds containing quaternary nitrogen in their molecule [1, 6].

Peripheral effects can be of some importance only in mice poisoned with hexonium. This may be the factor responsible for the changes in the tolerance of the animals to this cholinolytic.

SUMMARY

An inquiry was made into the tolerance of mice, aged 2-3 days, 2 weeks, 3-4 weeks, and adult mice to cholinolytics, atropine, tropacine, difacil, amisil, amisil methylate, pachycarpine and hexonium. It was shown that the differences in the tolerance of mice to these compounds depended on their capacity to block the M- or N-cholinoreactive systems. Nitrogen charge in the molecule was of lesser significance. Mice of younger age groups were less tolerant to M-cholinolytics, and more so to N-cholinolytics, than adult animals.

LITERATURE CITED

1. M. Ya. Mikhel'son, In the book: The Physiological Role of Acetylcholine and the Search for New Therapeutic Substances [in Russian], p. 9 (Leningrad, 1957).
2. V. B. Prozorovskii, Zh. obshchei biol. 3, 221 (1960).
3. V. B. Prozorovskii, Farmakol. i Toksikol. 1, 115 (1962).
4. L. I. Tank, Tolerance to Pharmacological Agents in Early Stages of Postnatal Development. Doctorate dissertation (Leningrad, 1953).
5. L. Bakay, In the book: Metabolism of the Nervous System (London, 1957), p. 136.
6. A. S. V. Burgen and L. M. Chipman, Quar. J. exp. Physiol. 1952, v. 37, p. 61.
7. A. J. Clark, Quar. J. exp. Physiol., 1912, v. 5, p. 385.
8. Z. Kanda, Jap. med. Sci. Pharmacol. 1940, v. 13, p. 179.
9. L. C. Miller and M. L. Tainter, Proc. Soc. exper. Biol. (New York, 1944), v. 57, p. 261.
10. T. Obata, Jap. J. med. Sci. Pharmacol., 1931, v. 5, p. 83.
11. H. A. Schlossman, J. Pharmacol. exp. Ther., 1937, v. 60, p. 14.