ON THE SENSITIVITY, RESISTANCE AND TOLERANCE OF RATS OF VARIOUS AGES TO TETANUS TOXIN

V. D. Rozanova

From the Laboratory for the Physiology and Pathology of Aging (Head – Prof. I. A. Arshavskii) of the Institute of Normal and Pathological Physiology (Director – Active Member of the Akad. Med. Nauk SSSR Prof. V. V. Parin) of the Akad. Med. Nauk SSSR, Moscow
(Presented by Active Member of the Akad. Med. Nauk SSSR V. V. Parin)
Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny,
Vol. 54, No. 12, pp. 36-41, December, 1962
Original article submitted March 5, 1962

In evaluating the differences between lethal doses of bacterial toxins for animals of different ages, many authors apply concepts of sensitivity, resistance and tolerance that differ in meaning and in physiological content.

Thus, D. F. Pletsityi [4] reported that newborn mice do not contract local or systemic tetanus, easily withstand doses of tetanus toxin which are lethal for adults, and thus, in comparison with the latter, are characterized by a higher resistance or stability. L. I. Tank [11], on the other hand, established that newborn mice do contract tetanus; the absolute lethal dose of tetanus toxin for them is almost three times greater than the analogous dose for adults. The author sometimes designates this difference as greater tolerance, and other times as less sensitivity.

In the investigations of our laboratory, attention was drawn to the necessity for differentiating the threshold doses of toxin in animals of differing ages, including the doses that first cause local tetanus, and those at which one observed the transition to systemic tetanus and death of the animals [1, 2, 5].

In analyzing the action of a series of pharmacological substances, we concluded that it was necessary to differentiate in the use of the terms, "sensitivity," "resistance," and "tolerance " for evaluating the reactions of the animals of different ages [6-9].

The purpose of this work was to determine the sensitivity, resistance, and tolerance of rats of different ages to tetanus toxin.

EXPERIMENTAL METHODS

Tetanus toxin, diluted in physiological saline, was injected subcutaneously into the left posterior extremity, using a volume of from 0.025 to 0.05 ml, and doses ranging from threshold to the absolute lethal level. Each dose, expressed in micrograms per gram of body weight, was injected into rats of known age (each group contained 5-10 animals). The experiment included newborn rats, rats that were 3-8 days old, 4-6 weeks old, and adult animals. Observations were not only carried out on the clinical symptoms of local and systemic tetanus, but also for changes in temperature, respiration, ECG and body weight.

EXPERIMENTAL RESULTS

Considering that the concept of "sensitivity," in the physiological sense, is close to that of "excitability" or "stimulation threshold," we proposed to use the minimum dose causing initial symptoms of local tetanus as the criter for sensitivity.

The investigations showed that the threshold dose of tetanus toxin for newborn rats is almost 8 times greater than for adults, and for rats 3-8 days old the dose is almost 5 times greater than for adults. Only beginning with the fourth week of age does the threshold dose of tetanus toxin approximate that for adult rats (see table).

Our data not only permitted us to evaluate the difference in threshold doses, but also to construct curves for the individual sensitivity, which reflect the variation in the threshold doses for different individuals of two age groups:

for rats 3-8 days old and for 4 week old rats (Fig. 1). The curve for the individual sensitivity of the rats in the 4 week old age group (A) was situated more to the left, in the limits of the lower doses, than the curve for the 3-8 day old rats (A_1). Thus, both newborn rats and those 3-8 days old are characterized by less sensitivity to tetanus toxin than 4 week old rats.

Doses of Tetanus Toxin for Rats of Different Ages (in micrograms per gram of body weight)

Age	Threshold	DMT	DL_{100}
Newborn	0.0066	–	–
3-8 days	0.004	0.02	0.09
4 weeks	0.00094	0.009	0.03
Adult	0.00085	–	–

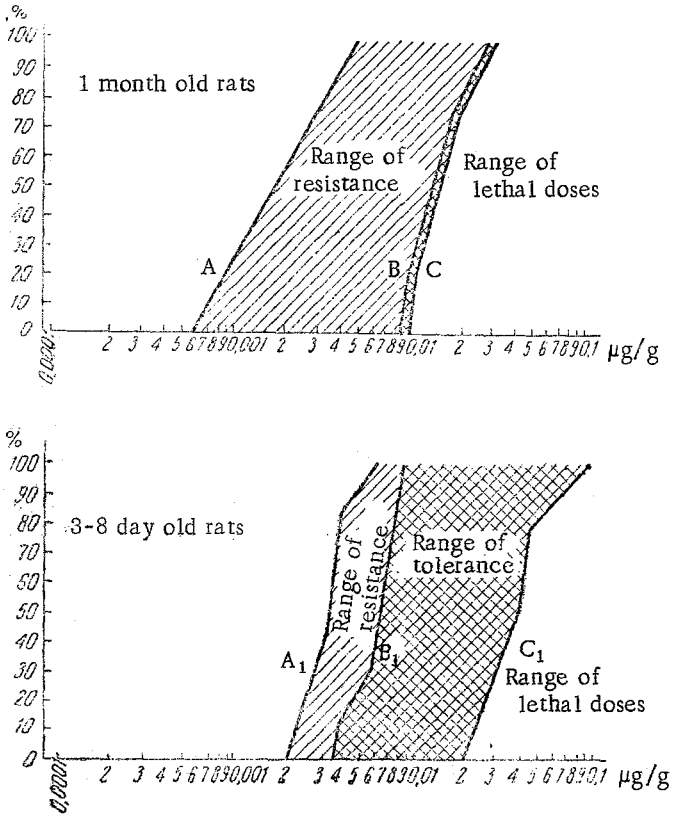

Fig. 1. Curves of the individual sensitivity (A, A_1), curves of the variation in resistance–tolerance (B, B_1), curves of the variation in tolerance–lethalness (C, C_1), in rats of different ages. Abscissa – doses of tetanus toxin (in micrograms per gram of body weight, expressed logarithmically); ordinate – reaction of the sensitivity, resistance and tolerance type (in percents).

As the criterion of resistance to tetanus toxin, we used the width of the dosage range in which the animals of differing ages maintained the homeostatic state (despite marked manifestation of local tetanus)as reflected in a series of vegetative indices and in the closeness to normal of posture and mobility. For the rats in the early age groups, the indices of normal growth and development also served as essential criteria for appraising resistance. In previous investigations it was shown that the signs of local tetanus in adult rats and rats over 3 weeks of age, when injected with tetanus toxin,

are not limited to rigidity of the extremity in which the toxin was injected, but are accompanied by reciprocal spreading of the tonus to all extremities and by a corresponding change in the indices of lability and in the summation time of the flexor and extensor centers for the ipsi- and contralateral extremity [1, 2, 5]. After injection of the tetanus toxin, the lability of the extensors on the ipsilateral side and the flexors of the contralateral side increases. At the same time, the lability of the flexors on the ipsilateral side markedly decreases.

This phenomenon was evaluated as a physiological measure against illness, which permits adult rats to maintain posture, mobility and homeostasis close to the norm in the presence of clearly manifested local tetanus, and even upon injection of doses causing the transition to systemic tetanus. Throughout almost the entire course of the intoxication the adult rats maintain a normal, or even elevated, body temperature, as well as the original indices of activity for the respiratory and cardiovascular system. The cardiac rhythm in the different rats ranged from 420-500 per minute.

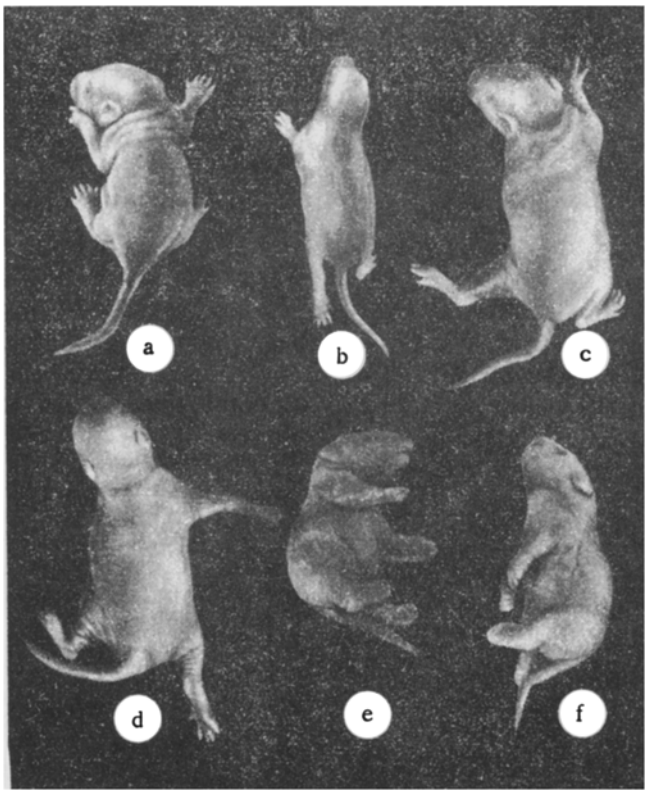

Fig. 2. Rats from 3 to 8 days old. a) Control. The others following injection of various doses of tetanus toxin (in micrograms per gram of body weight): b) 0.004; c) 0.0085; d) 0.02; e) 0.3; f) 0.09.

The young rats, beginning with the newborn period, did contract local tetanus, which was manifested by rigidity of the extremity in which the toxin was injected, and in the exclusion of this extremity from locomotion (Fig. 2b,c,d). However, the rigidity was not accompanied by reciprocal spreading of the tonus to the remaining extremities, or by a change in the state of the centers, as was observed in the adult animals.

Figure 2 shows the posture of the control rat (a), as well as the rigidity of the left posterior extremity in the rat (b) that was injected with a dose of tetanus toxin (0.004 micrograms/gram), in which the three remaining extremities retained the normal tonus and mobility. When the dose was elevated to 0.0085 micrograms/gram (c) the rigidity spread to the ipsilateral left anterior extremity; left sided flexion of the body was seen, and the extremities of the right side remained mobile. Total mobility of the rat was sharply limited. After injection of a still greater dose (0.02 micrograms/gram), which is the maximum tolerable for rats of this age, the rigidity spread to the right anterior

extremity as well (d). As could be expected with this dose, the state of rigidity or "local tetanus" involved almost the entire body of the rat, and thus left the animal almost completely deprived of mobility. This spreading of rigidity with injection of the toxin doses, without giving rise to symptoms of systemic tetanus (convulsion), was considered by us to be due to the absence of the reciprocal form of reaction of the spinal centers in rats of the early age group. This type of reaction allows adult rats to block the dissemination of a focus of excitation during the period of local tetanus, limiting it to the segments related to the affected extremity.

At higher doses (0.03-0.05 micrograms/gram), systemic tetanus developed in the rats of the early age group, manifested by tonic convulsions with orthotonic positioning of the body, which is characteristic of the early age group (e). With injection of still greater doses, there was a shift to opisthotonus, with stretching of the anterior extremities along the trunk (f).

Returning to the characteristics of the course of local tetanus in the young age group, it must be noted that the rigidity of almost the entire body, appearing in this case, markedly disrupted the posture and mobility of the rat, and led to a disturbance of homeostasis: body temperature dropped sharply (to 26-28°), and respiration and the cardiac rate were both slowed. In the rat injected with the tetanus toxin at the age of 5 days, the cardiac rate was slowed markedly (Fig. 3B), while in the 4 week old rat, the same dose of tetanus toxin did not essentially change the original cardiac rhythm (Fig. 3A).

A lag in the growth and development of the rats in the early age group was also noted along with a sharp drop in the weight gain curve. While in normally developing rats, 3-8 days of age, the weight gain for 10 days is 250-300% of the starting weight, the gain seen in the animal following injection of 0.004 micrograms/gram of tetanus toxin was 15%, and following an injection of 0.0094 micrograms/gram it was only 10%.

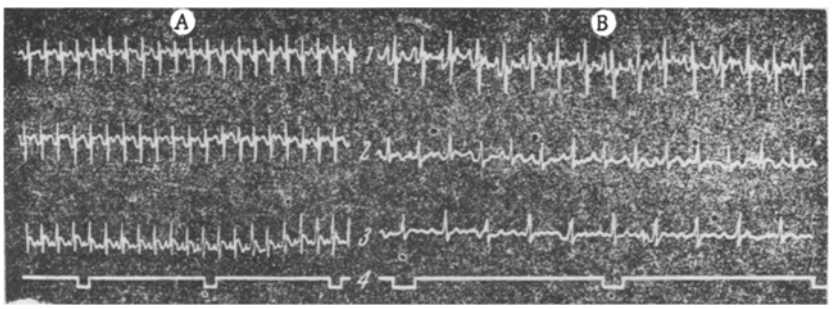

Fig. 3. Changes in the ECG of rats in the early age group following injection of tetanus toxin. A) ECG of 4 week old rat; B) ECG of 5 day old rat. 1) Original ECG; 2) 48 h after injection of tetanus toxin in a dose of 0.008 micrograms/gram; 3) 5 days after the injection; 4) time markings (in seconds).

Thus, disturbance of homeostasis in the rat of the early age group occurred even with injection of a dose of tetanus toxin causing local tetanus, i.e., starting with 0.004 micrograms/gram; in the 4 week old rat, this only occurred with a dose of 0.01 micrograms/gram. These doses must be regarded as the minimally toxic levels. For the rat of the early age group, the minimally toxic dose is markedly lower than for the 4 week old rat.

Disturbance of homeostasis in the adult rats was observed at higher doses (0.011-0.03 micrograms/gram), causing systemic tetanus. Along with the curves of individual sensitivity, these data permitted us to construct graphs which we designated as curves of the variation in incidence of resistance and tolerance (Fig. 1B, B_1). For the 3-8 day old rat (B_1) the curve was located rather close to the curve of individual sensitivity. For the 4 week old rat (B), it was located within higher dosage limits. The distance between the curves of individual sensitivity and those of resistance–tolerance were different for each age period, and were designated as the resistance range. It can be seen that the latter is markedly contracted for the 3-8 day old rats, as compared with the wide diapason for the 4 week old animals. These data permitted concluding that the resistance of rats 3-8 days old against tetanus toxin is lower than in 4 week old rats.

In contradistinction to resistance, we considered tolerance to mean a state of disrupted homeostasis, manifested by protracted collapse, which, however, was reversible. In addition to the described curves, we constructed two additional ones for the 3-8 day old and 4 week old rats (C and C_1), which represented the variation in the incidence of survival and of lethal outcomes upon injection of dosages ranging from the maximum tolerance level to the absolute lethal. In contrast to the curves for the variation in resistance–tolerance, the curve of the variation in tolerance-lethality (C_1) for rats from 3-8 days old was located in higher dose limits than was the analogous curve (C) for the 4 week old rats. The tolerance range, which was determined from the distance between the latter curve (C_1) and the curve of the variation in resistance–tolerance (B_1), was wider for the rats of the early age group than for the 4 week old animals. In the older animals it was extremely contracted, since the curve for the variation in lethalness almost bordered the curve of resistance–tolerance.

These data allowed us to conclude that despite greater resistance, i.e., the ability to maintain homeostasis at higher doses, the 4 week old rats were less tolerant toward tetanus toxin than the rats in the 3-8 day old age group. In rats 4 weeks of age and older, the collpase occurred in a shorter time interval. The absolute lethal dose for this group was one third as great as for the 3-8 day old rats.

Many authors have established the lower tolerance against convulsive drugs in animals of intermediate and adult ages as compared with animals of the earliest age group [3, 10, 12].

We obtained data which not only permitted us to establish the age differences for the lethal doses of strychnine, but also the differences in the threshold doses and the diapasons of resistance and tolerance. The tolerance diapason in rabbits of the early age group, associated with the action of strychnine, is analogous to the action of tetanus toxin, also being wider than in the adult rabbits [9]. It may be postulated that the reasons for the greater tolerance in the early age group to convulsive drugs, regardless of their type, are related to the great tolerance to oxygen hunger seen at this age.

Thus, in order to characterize more completely the reaction of animals of different ages to bacterial toxins, it is necessary to separately evaluate sensitivity, resistance, and tolerance, and to use adequate criteria for recording those states in correspondence with the physiological implications of these concepts.

SUMMARY

Tetanus toxin sensitivity, resistance, and tolerance of 3 to 8 day ratlings and of one month old rats were determined separately. The smallest dose to cause local rigidity was accepted as the minimal dose. The threshold tetanus toxin dose for 3-8 day rats was 5 times greater than for adult rats or for 4 week old rats. The range of tetanus toxin doses within which (notwithstanding the increasing manifestations of local tetanus) the state of homeostasis was maintained and also the nearness of posture and mobility to normal were accepted as resistance criteria. The range of resistance to tetanus toxin in 3-8 day ratlings was much narrower than in the 1 month old rats. The range of tetanus toxin doses, within which the body homeostasis disturbances, although present, were still reversible, was taken as a tolerance criterion.

Notwithstanding the lesser resistance to tetanus toxin the range of tolerance in the 3 to 8 day ratlings is wider than in the 4 week old rats.

Absolutely lethal doses for 3 to 8 day ratlings was three times greater than for one month old rats.

For more complete description of reactions of animals of different ages to bacterial toxins differential evaluation of the sensitivity, resistance, and tolerance should be done; adequate criterion may be employed for this purpose by adherence to the physiological implications of these concepts.

LITERATURE CITED

1. I. A. Arshavskii, in the book: Contemporary Questions in General Pathology and Medicine [in Russian] (Moscow, 1950), p. 128.
2. S. I. Enikeeva and V. D. Rozanova, in the book: The Problem of Reactivity in Pathology [in Russian] (Moscow, 1954), p. 84.
3. T. L. Nevskaya, On the Question of Age Tolerance of the Organism Toward Certain Therapeutic Drugs and Venoms. Avtoref. fiss. kand. (Chernovtsy, 1959).
4. D. F. Pletsityi, Experimental Study of the Pathogenesis of Tetanus Intoxication [in Russian] (Moscow, 1958).

5. V. D. Rozanova, Theses and References from the Reports of the Symposium on the Pathogenesis of Tetanus [in Russian] (Moscow, 1959), p. 42.
6. V. D. Rozanova, Works of the 3rd Scientific Conference of the Institute of Physical Education and School Hygiene on the Morphology, Physiology, and Biochemistry of Aging [in Russian] (Moscow, 1959), p. 314.
7. V. D. Rozanova, Uspekhi sovr. biol., No. 1 (1960), p. 86.
8. V. D. Rozanova, Byull. eksper. biol., No. 5 (1960), p. 87.
9. V. D. Rozanova, Theses from the Reports of the 2nd Conference of the Physiologists, Biochemists, and Pharmacologists of Central Asia and Kazakhstan [in Russian] (Frunze, 1960), p. 282.
10. A. M. Rusanov, Farmakol. i toksikol., No. 4 (1949), p. 40 and 42.
11. L. I. Tank, Works of the All-Union Association of Physiologists, Biochemists and Pharmacologists [in Russian] Vol. 4 (1958), p. 187.
12. G. I. Tsobkallo, Works of the I. P. Pavlov Institute of Evolutional Physiology and Pathology of Higher Nervous Activity [in Russian] Vol. 1 (Leningrad, 1947), p. 369.

All abbreviations of periodicals in the above bibliography are letter-by-letter transliterations of the abbreviations as given in the original Russian journal. *Some or all of this periodical literature may well be available in English translation.* A complete list of the cover-to-cover English translations appears at the back of this issue.
