Postictal Recovery in Normal and Diphenylhydantoin (Dilantin)-Treated Mice.* (22762)

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It is well known that supramaximal electroshock stimulation of experimental animals evokes seizures characterized by 3 components: tonic hindleg flexion, tonic hindleg extension, and terminal generalized clonus. Alteration in the severity of this seizure, by drugs or experimental procedures, may be recognized by changes in duration of the tonic components of the seizure. It was shown(1) in mice that relatively *minor* changes in seizure severity can be detected by changes in duration of tonic flexion, whereas *major* changes in seizure severity can be recognized by changes in duration of both the flexor and the extensor tonic components. These criteria for changes in seizure severity have now been employed to investigate the postictal recovery process in normal and diphenylhydantoin (Dilantin)-treated mice. The results of this investigation provide the basis for this report.

Methods. A population sample of 160 adult male albino mice obtained from the Carworth Farms (CF #1 strain) was randomly divided into 2 groups (test and control) of 80 mice each. Each animal of the test group was given orally 11.0 mg/kg of diphenylhydantoin sodium (Dilantin Sodium) suspended in 10% acacia mucilage, *i.e.*, approximately an ED_5 as previously determined by the maximal electroshock seizure (MES) test; the control group was given an equivalent volume of 10% acacia mucilage. Three hours later, at the time of peak anticonvulsant effect of Dilantin by the MES test, groups of 8-10 mice (test and control) were given an initial electroshock of 50 mA (0.2-sec. duration, 60-cycle a.c., corneal electrodes) and the pattern of the "first" seizure was recorded. At various time intervals after the initial shock, a second electroshock of the same intensity was administered and the pattern of the "second" seizure was recorded. Any one animal, however, received only 2 electroshocks. The time interval between electroshocks varied from 180 to 540 seconds for Dilantin-treated mice and from 40 to 200 seconds for control mice. The mean durations of hindleg tonic flexion and extension, terminal generalized clonus, and total seizure for both "first" and "second" convulsions were calculated as follows: the durations of the individual components were summed and the values obtained divided by the total number of mice per group. In addition, the standard deviations of the means were calculated. The endpoints for these seizure components have been described previously(1). The time (RT_{50}) required for 50% of mice to recover the ability to exhibit a second tonic-clonic seizure, regardless of duration of components, after a first maximal convulsion was determined by the graphic log-probit method of Litchfield and Wilcoxon(2).

Results. The mean durations of the various components of "first" seizures and of "second" seizures elicited at the indicated times in normal mice are shown in Table I. It may be seen from the table that the mean durations of the various components and the total duration of "first" seizures were remarkably uniform, and there was no significant difference in the values obtained in the various groups of normal mice. As might be expected, the character and the duration of the "second" seizure are crucially dependent on the time, in the postictal period, of application of the second electroshock. The longer this time is extended, the more the "second" resembles the "first" seizure. Whereas, at 200 seconds, the mean tonic flexion duration of the "second" seizure was still significantly

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TABLE I. Mean Durations of "First" and "Second" Seizure Components in Normal Mice.

Time of application of 2nd electroshock (sec.)	Mean duration of seizure components, seconds†							
	Tonic hindleg				Clonus		Total duration	
	Flexion		Extension					
	1st seizure	2nd seizure	1st seizure	2nd seizure	1st seizure	2nd seizure	1st seizure	2nd seizure
40	1.6 ± .1	6.9 ± .4	13.8 ± .7	0	6.4 ± .7	8.6 ± .7	21.8 ± .7	15.5 ± .9
60	1.6 ± .4	8.2 ± .7	13.8 ± .4	1.4 ± .9	6.4 ± .6	11.0 ± .6	21.9 ± .6	20.6 ± .6*
80	1.5 ± .2	5.9 ±1.1	14.0 ± .5	4.9 ± 1.3	7.0 ± .6	8.4 ± .6*	22.5 ± .9	19.2 ± 1.7*
100	1.8 ± .2	5.0 ± .9	14.1 ± .3	7.2 ± 1.8	6.7 ± .3	7.9 ± .7*	22.5 ± .7	20.1 ± 1.0*
120	1.4 ± .1	2.7 ± .3	15.7 ± .7	12.6 ± .8	7.8 ± .3	9.1 ± .8*	24.9 ± .8	24.4 ± 1.2*
140	1.6 ± .1	2.8 ± .2	14.2 ± .4	11.1 ± .9	7.4 ± .2	7.5 ± .8*	23.1 ± .5	21.4 ± .9*
160	1.6 ± .1	2.6 ± .2	14.1 ± .5	13.5 ± 1.0*	7.0 ± .8	8.6 ±1.0*	22.7 ±1.0	23.6 ± 1.5*
180	1.6 ± .1	2.2 ± .2	14.1 ± .8	14.6 ± .9*	7.9 ± .8	8.8 ± .9*	22.8 ± .5	24.7 ± 1.1*
200	1.5 ± .1	2.3 ± .1	14.7 ± .6	14.1 ± 1.1*	7.3 ± .7	7.2 ± .8*	23.5 ± .7	23.6 ± .9*

* Not significantly different from mean value of the first seizure ($P > .05$).
† Sum of the durations of individual components divided by total No. of mice per group ± standard deviation of the mean.

longer than that of the "first" seizure, the mean durations of tonic extension, terminal clonus and total convulsion of "second" seizures were not significantly different from those of the control values after 160, 80, and 60 seconds, respectively. Thus, the order in which the durations of the various components of the "second" seizure returned to control values was as follows: total seizure duration, terminal clonus, tonic extension, and tonic flexion.

The mean durations of the various components of "first" and "second" seizures elicited at the indicated times in Dilantin-treated mice are shown in Table II. As shown in the table, there was no significant difference in the "first" seizure values obtained in groups of Dilantin-treated mice. The mean durations of tonic flexion, tonic extension, terminal clonus, and total convulsion of "second" seizures were not significantly different from their appropriate control values after 540, 400, 320, and 180 seconds, respectively. Thus, the order in which the durations of the various components of the "second" seizure returned to control values was the same as observed for untreated mice, *i.e.*, total seizure duration, terminal clonus, tonic extension, and tonic flexion.

The RT_{50} and 95% confidence limits for control mice were 75 (65-86) seconds, whereas those for Dilantin-treated animals were 320 (260-394) seconds. The Dilantin-induced increase in RT_{50} was highly significant ($P < .001$), and amounted to more than 300%.

Discussion. The data presented indicate that the pattern of a "second" maximal seizure elicited after a "first" depends on the time interval between the two seizures and

TABLE II. Mean Durations of "First" and "Second" Seizure Components in Dilantin-Treated Mice.

Time of application of 2nd electroshock (sec.)	Mean duration of seizure components, seconds†							
	Tonic hindleg				Clonus		Total duration	
	Flexion		Extension					
	1st seizure	2nd seizure	1st seizure	2nd seizure	1st seizure	2nd seizure	1st seizure	2nd seizure
180	2.8 ± .2	7.8 ± .8	9.6 ± .7	1.3 ± .7	8.8 ± .8	11.3 ± 1.1	21.0 ± .9	22.8 ± .9*
280	2.9 ± .1	6.8 ±1.0	9.2 ± .9	2.6 ± 1.3	7.8 ± .8	12.3 ± 1.0	19.7 ±1.4	22.8 ± .9*
320	3.1 ± .3	6.3 ±1.0	8.7 ± .8	4.8 ± 1.5	8.6 ± .6	9.0 ± .3*	20.5 ± .4	20.1 ± .9*
360	2.4 ± .2	6.0 ±1.1	12.7 ± .8	6.9 ± 1.9	8.5 ± .9	11.1 ± 1.4*	23.6 ±1.0	24.0 ± 1.0*
400	2.9 ± .3	5.4 ± .8	10.9 ±1.0	7.2 ± 1.6*	7.5 ± .9	10.0 ± .7*	21.5 ± .6	22.6 ± .7*
480	3.2 ± .5	5.3 ± .8	10.2 ± .9	6.9 ± 1.6*	9.2 ± .7	11.6 ± 1.5*	22.3 ± .7	23.7 ± 1.2*
540	2.4 ± .3	3.5 ± .5*	12.0 ±1.2	12.3 ± 1.4*	9.5 ± .6	8.9 ± .8*	24.0 ± .9	24.7 ± 1.1*

* Not significantly different from mean value of the first seizure ($P > .05$).
† Sum of the durations of individual components divided by total No. of mice per group ± standard deviation of the mean.

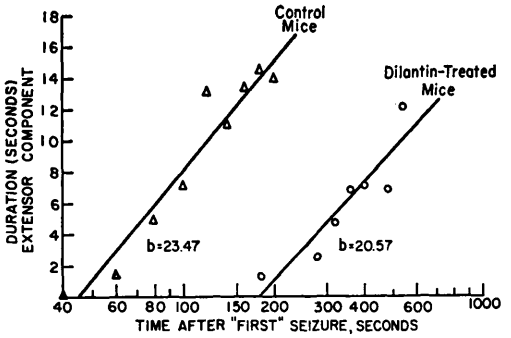

FIG. 1. Rate of recovery of hindleg tonic-extensor component in control and Dilantin-treated mice. Time interval in sec. (logarithmic scale) between "first" and "second" seizures is shown on abscissa and duration of extensor component in sec. (linear scale) is shown on ordinate. Values obtained in control mice are plotted with triangles; those obtained in Dilantin-treated mice, with circles. The continuous lines were fitted to the plotted points by the method of least square. The letter b indicates the calculated slope of the line. See text for discussion.

that the total duration of a maximal electroshock seizure is the sum of the durations of the individual seizure components. Dilantin prolonged by more than 170% the time necessary for "second" seizures to return to "first" seizure values and induced a 325% increase in RT_{50}. Previous studies from this laboratory indicate that Dilantin also increases RT_{50} in rats(3).

By what mechanism does Dilantin prolong recovery time? Interesting information on this question is revealed by plotting the duration of the hindleg tonic extensor component of "second" seizures elicited in normal and Dilantin-treated mice against the time interval between seizures. This plot is shown in Fig. 1 from which it can be seen that recovery time, as measured in these experiments, is composed of two factors: the recovery time *per se* (indicated by the slopes of the two regression lines) and the latent period before recovery of the tonic-extensor component starts (indicated by the points of intercept of the regression lines and abscissa). Since there is no significant difference in the slopes of the recovery regression lines for control and Dilantin-treated mice and since the time before recovery starts is 45 seconds in control mice and 175 seconds in Dilantin-treated mice, it would appear that the *rate* of recovery of tonic extensor ability is unaffected by this drug and that it acts primarily to prolong the latent period before recovery of the tonic-extensor component starts. This interpretation is supported by the work of Esplin who demonstrated‡ on cat spinal cord preparations that Dilantin had little effect on the level of resting excitability, as judged by transmission of an isolated impulse, but deepens the depression following an impulse.

In recent years, we have directed considerable attention to the effects of various physiological vicissitudes, drugs, etc. on the RT_{50} for maximal electroshock seizures(1,3, 4,5); however, the data presented in Tables I and II represent our first precise measurement of the time for recovery of "second" maximal seizures as related to "first" seizure values. What percentage of CNS reactivity has been regained at the time that 50% of animals again show some detectable tonic-extensor component? The return of CNS reactivity is logarithmic; hence, a definitive answer to this question can not be derived from the data presented. Nevertheless, an interesting correlation can be revealed by comparing recovery of the tonic-extensor component and RT_{50}. It may be determined from the tables that the mean durations of the tonic-extensor component of "first" seizures are 14.27 and 10.47 seconds in normal and Dilantin-treated mice, respectively. By reference to Fig. 1, it may be seen that the times for recovery of one-half the full tonic-extensor durations are 93 and 320 seconds in normal and Dilantin-treated mice, respectively. Since the respective RT_{50}s were determined to be 75 (65-86) and 320 (260-394) seconds, it may be concluded that RT_{50} is approximately the time for recovery of 50% of the *full* tonic-extensor component. Because of the comparative ease of its determination and calculation, the RT_{50} is a more useful and accurate endpoint for the measurement of recovery of CNS reactivity than is full recovery of the tonic-extensor component.

Summary. In order to study postictal recovery, groups of normal and Dilantin-treated mice were given a supramaximal electroshock

‡ *Fed. Proc.*, 1955, v14.

and the durations of the hindleg components of the "first" seizure recorded. At various time intervals after the "first" seizure, a "second" supramaximal electroshock was given and the durations of the hindleg components of the "second" seizure recorded. This procedure was repeated, with longer time intervals between seizures, until the observed durations of the "second" seizure components were not significantly different from their "first" seizure values. In addition, the time (RT_{50}) required for 50% of animals to recover ability to exhibit a second tonic-clonic seizure after a first was determined. The data obtained may be summarized as follows: The order in which the durations of the various components and the total duration of the "second" seizure returned to their "first" seizure values were the same in both groups: total seizure duration, terminal clonus, tonic extension, and tonic flexion. The total duration of a maximal seizure is relatively independent of marked changes in duration of the individual seizure components. Dilantin prolonged the recovery time of terminal clonus, tonic extension, and tonic flexion by approximately 300%, 150%, and 170%, respectively. In addition, Dilantin prolonged RT_{50} by 325%. Evidence is presented which suggests that Dilantin acts to prolong the latent period before recovery of the tonic-extensor component starts, and that the recovery process *per se*, once initiated, is relatively unaffected by the drug.

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Dehydrogenase Activity of Rat Epiphyseal Cartilage.* (22763)

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Some years ago dehydrogenase activity was demonstrated in epiphyseal cartilage, using succinate and citrate as substrates and the decolorization of methylene blue as an indication of enzyme action(1). This technic does not allow for any precise localization of the enzymes in the cell. Hence, the application of newer technics utilizing tetrazolium compounds was undertaken, since so little is known about the metabolic activities of growing cartilage.

Materials and methods. Young (40-60 g) rats were killed by a blow on the head. After the lower extremity was dissected of muscle, free hand sections, as thin as possible, were made with a razor through the epiphyseal cartilages of the upper tibia or lower femur. Such slices were washed briefly in saline to remove any blood and were placed in depression slides containing 4 drops of .2M phosphate

TABLE I. Degree of Tetrazolium Reduction in One Hour with Different Substrates.

Control	0
Succinate	+++
Succinate and malonate	0
Citrate	++
Isocitrate	+++
Lactate	+++
Malate	+++
Pyruvate	0
Glucose	0
Glutamate	0
Oxalacetate	+
Xanthine	0

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