THE INFLUENCE OF TEMPERATURE ON THE ACTION OF STRYCHNIN IN FROGS.*

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INTRODUCTION.

There is a widespread assumption that in cold blooded animals warming increases the action of all drugs. It is repeatedly pointed out that all chemical, and therefore, presumably, all vital, processes are increased in intensity with rise of temperature, and that the reaction to all drugs is therefore also increased.

Thus Guinard (1) says: "The influence of warmth on therapeutic action is well known. Cl. Bernard stated it long since, pointing out that the sensitiveness of the frog to poisons increases in proportion as the temperature rises. It is a fact which is perfectly logical, because chemical action is the basis of all response to drugs and chemical reactions in general occur more rapidly the higher the temperature. When one administers strychnin to frogs which are each exposed to different temperatures, one notices that rise in temperature accelerates and increases the convulsions." Richet (2) uses similar language. He says: "The influence of temperature on intoxication is particularly marked in cold blooded animals, whose temperature is variable. . . . At a low temperature chemical actions are less intense, and as toxic actions are merely chemical actions, it follows that at a low temperature poisons are less active than at high temperatures. . . . The action of temperature on toxic action is evidently a very general law . . . which may be formulated thus: Toxic actions being chemical actions vary with the temperature of the animal. They are the more active the higher is the temperature of the animal."

In the text-books on pharmacology and physiology there is usually no general statement in regard to the influence of temperature, but in medical journals we meet with the same general view applied to some particular drug. Thus Luchsinger (3), speaking of picrotoxin, refers to Kunde's conclusions, which will be discussed below, and says that summer frogs show much stronger convulsions than winter frogs, and "it seems plausible (*liegt nahe*) to seek in difference of temperature the cause of the great difference in behavior of summer and winter frogs." Schlick (4) says: "Frogs react less to strychnin in the winter, when my experiments were done." The only article, however, which deals directly with the influence of temperature on the action of strychnin in frogs is that of

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Kunde (5), referred to by Luchsinger, which we shall quote at more length. Kunde's experiments were suggested by the fact that frogs react to weaker electric currents when warm, and therefore the cord is less sensitive in the cold and should be less affected by equal amounts of strychnin. He put three frogs in a 1:5,000 solution of strychnin nitrate at 16° C. for ten minutes and then transferred them to dishes containing water at respectively 31° C., 16° C., and 1° C. In ten minutes the frog at 31° C. had tetanus. After fifteen minutes the frog at 16° C. had no tetanus; it was transferred to 31° C. and had tetanus in fifteen minutes. The frog at 1° C. had no tetanus in thirty-five minutes; it was transferred to 31° C. and had tetanus in twelve minutes. The next morning all three appeared normal, but on being put between lumps of ice they developed tetanus, which disappeared when they were warmed, and returned when they were again cooled. On the basis of these experiments Kunde formulated the following law: "With a small dose of strychnin application of warmth stops the tetanus, withdrawal of warmth causes it. With a larger dose warmth brings on tetanus, withdrawal of heat prevents its occurrence." That he considers the effect of temperature on large doses of most importance is plain from his introductory remark. "If a frog is cooled, an electric current which before produced tetanus leaves the animal unaffected. . . . As it has been shown that the cord is also a reagent for strychnin, its effect on the spinal cord must change, if with a constant amount of strychnin present, the temperature of the nerve centers is changed. This hypothesis has, I believe, been confirmed."

From a series of experiments in which we have studied the influence of temperature upon the convulsant action of strychnin, it became evident that the relation of temperature to the tetanic effect of strychnin is erroneously presented when merely expressed in general statements. Neither does an observation of the animal limited to a few hours tell the entire story. In the following we shall report briefly the results which we have obtained in a more detailed study of the influence of temperature upon strychnin tetanus in frogs.

EXPERIMENTAL DATA.

The experiments were performed during the months of January and February on *Rana pipiens,* weighing between fifteen and thirty grams. After injecting in a femoral or the dorsal lymph sac a certain dose of strychnin, the frogs were put in appropriate cages where they were kept at constant, definite temperatures, ranging from 5° C. to 30° C., and observed until recovery took place or several days had passed.

Minimum Convulsant Dose.—In these experiments larger doses, that is, doses which are larger than a reliable minimum, were not

used. Only the effect of minimum doses was studied. But, as will be seen presently, the minimum convulsant dose varies with the temperature at which the animal is kept. A dose which proves to be a reliable minimum at a certain temperature may prove unreliable at another. Only with doses of 0.0006 of a milligram per gram (0.015 to 0.02 of a milligram per frog of twenty-five to thirty grams) did all the frogs show, sooner or later, tetanus at all temperatures. With 0.0005 of a milligram per gram all frogs had tetanus at 5° C., 27° C., and 30° C.; four out of six had tetanus at 13° C., two out of six at 18° C., two out of six at 24° C., and none (of six) at 21° C. With 0.0003 of a milligram per gram all frogs had tetanus at 5° C., while it was inconstant at temperatures above 24° C. and was never seen between 13° and 21° C. With 0.0002 of a milligram per gram three frogs out of five showed tetanus at 5° C., and none at any higher temperature. With smaller doses tetanus was not obtained. That the tetanus occurring at 30° C. is not a heat tetanus is shown by the fact that controls kept at this temperature showed no deviation from normal.

From the above we learn that with a dose of strychnin of 0.0005 of a milligram per gram of frog, only at the low temperature of 5° C. and at the high temperatures of 27° and 30° C. was there a tetanic response in every animal tested, while at various intermediary temperatures the dose failed to be effective in some cases, and at 21° C. failed in every instance. In other words, *a certain dose may be effective at high and low temperatures and be unreliable or fail completely at an intermediary temperature.* This demonstrates the incorrectness of a general statement that strychnin tetanus is favorably or unfavorably influenced by increase of temperature.

We shall reproduce here several abbreviated protocols of experiments with doses of 0.0005 and 0.0003 of a milligram per gram of frog, which may illustrate various points to be brought out in this paper.

In the first experiments a dose of strychnin sulphate equal to 0.0005 of a milligram per gram of frog was injected into the femoral lymph sac.

Frog 102.—Female; weight, 31 gm. Temperature 5° C. January 16, 1913, 9.16 A.M. Injected 0.155 c.c. of strychnin sulphate, 0.01 per cent. 11.30 A.M. Slightly hyperesthetic. 1.00 P.M. On touch the legs fly out in extension and are immediately drawn in. 5.00 P.M. The same. January 17, 9.00 A.M. Animal found in violent continuous tetanus which lasted two days and then gradually passed off. *At 5° C. violent tetanus developed more than 8 hours after injection and lasted for two days.*

Frog 105.—Male; weight, 22 gm. Temperature 5° C. January 16, 1913, 9.54 A.M. Injected 0.11 c.c. of strychnin sulphate, 0.01 per cent. 11.30 A.M. Distinct hyperesthesia. 1.00 P.M. Momentary extension on touch. 5.00 P.M. The same. January 17, 9.00 A.M. In violent continuous tetanus. January 18. The same. January 19. The same. January 20, 9.00 A.M. Still in strong tetanus. 5.00 P.M. Tetanus somewhat less violent. January 21. Markedly spastic but no tetanus. *At 5° C. strong tetanus appeared the day after the injection and continued for about four days.*

Frog 107.—Male; weight, 22 gm. Temperature 13° C. January 16, 1913, 9.22 A.M. Injected 0.11 c.c. of strychnin sulphate, 0.01 per cent. 10.50 A.M. On touch the legs are thrown out stiffly and at once drawn in. 1.00 P.M. Tetanus of brief duration; legs then drawn in. 2.25 P.M. Attacks of longer duration. 5.00 P.M. Strong prolonged attacks of extensor tetanus spontaneously; legs drawn in after attacks. January 17, 9.00 A.M. Almost normal. *At 13° C. tetanus appeared on the day of the injection and was gone the next day.*

Frog 108.—Male; weight, 22 gm. Temperature 13° C. January 16, 1913, 9.56 A.M. Injected 0.11 c.c. of strychnin sulphate, 0.01 per cent. 10.25 A.M. Slightly spastic. 11.05 A.M. Momentary strong extension. 2.00 P.M. The same. 4.00 P.M. The same. 5.00 P.M. The same. January 17, 9.00 A.M. Almost normal. *At 13° C. hardly any tetanus; normal the next day.*

Frog 101.—Male; weight, 25 gm. Temperature 21° C. January 15, 1913, 1.18 P.M. Injected 0.105 c.c. of strychnin sulphate, 0.01 per cent. 1.45 P.M. Slight hyperesthesia. 2.10 P.M. Markedly spastic. 3.00 P.M. The same. 4.00 P.M. The same. January 16, 9.00 A.M. Normal. *At 21° C. no definite tetanus at any time.*

Frog 100.—Male; weight, 20 gm. Temperature 24° C. January 15, 1913, 1.15 P.M. Injected 0.1 c.c. of strychnin sulphate, 0.01 per cent. 1.45 P.M. Slight hyperesthesia. 2.00 P.M. More spastic. 3.40 P.M. Markedly spastic. 5.00 P.M. Slightly spastic. January 16, 9.00 A.M. Normal. *At 24° C. no tetanus at any time.*

Frog 110.—Male; weight, 22 gm. Temperature 27° C. January 16, 1913, 9.36 A.M. Injected 0.11 c.c. of strychnin sulphate, 0.01 per cent. 9.50 A.M. Very lively and active. 10.05 A.M. The same. 10.25 A.M. Sudden strong extensor tetanus; legs not drawn in. 12.00 M. Still in strong tetanus. 1.00 P.M. Less violent. 2.25 P.M. Having attacks of extensor tetanus, but legs drawn in between attacks. 4.00 P.M. Extremely spastic. January 17, 9.00 A.M. Normal. *At 27° C. tetanus in 49 minutes; normal the next day.*

Frog 113.—Female; weight, 30 gm. Temperature 27° C. January 16, 1913, 10.50 A.M. Injected 0.15 c.c. of strychnin sulphate, 0.01 per cent. 11.15 A.M. Spastic. 11.30 A.M. Sudden attack of strong extensor tetanus; legs not drawn in. 4.30 P.M. Becoming less violent. January 17, 9.00 A.M. Almost normal. *At 27° C. tetanus after 40 minutes; normal the next day.*

Frog 128.—Female; weight, 26 gm. Temperature 30° C. January 20, 1913,

11.18 A.M. Injected 0.13 c.c. of strychnin sulphate, 0.01 per cent. 11.45 A.M. Slightly spastic. 11.53 A.M. Extensor tetanus; legs drawn in between attacks. 12.10 P.M. Strong extensor tetanus; legs not drawn in. 12.30 P.M. More violent. 1.00 P.M. Becoming less violent. 3.00 P.M. Recovering. Merely moderately spastic. *At 30° C. tetanus after 35 minutes, recovering the same day.*

In the following experiments strychnin was administered in doses of 0.0003 of a milligram per gram of frog.

Frog 31.—Female; weight, 48 gm. Temperature 5° C. January 7, 1913, 9.41 A.M. Injected 0.48 c.c. of strychnin sulphate, 0.003 per cent. 10.15 A.M. On touch the legs are thrown out stiffly and then drawn in. 4.30 P.M. Condition the same. January 8, 9.00 A.M. In strong continuous extensor tetanus. 2.00 P.M. Still in tetanus. *At 5° C. strong tetanus the next day.*

Frog 144.—Male; weight, 20 gm. Temperature 5° C. January 20, 1913, 5.11 P.M. Injected 0.2 c.c. of strychnin sulphate, 0.003 per cent. January 21, 8.00 A.M. In continuous strong extensor tetanus. 4.00 P.M. The same. 6.00 P.M. Legs drawn in at times. January 22, 8.00 A.M. On touch extensor tetanus lasting several seconds, then legs drawn in. 5.30 P.M. The same. January 23, 9.00 A.M. On touch momentary strong extension. 2.00 P.M. Markedly spastic. 5.00 P.M. Moderately spastic. January 24, 9.00 A.M. Normal. *At 5° C. onset of tetanus not later than 15 hours; lasted several days.*

Frog 74.—Female; weight, 26 gm. Temperature 13° C. January 10, 1913, 9.36 A.M. Injected 0.26 c.c. of strychnin sulphate, 0.003 per cent. 10.00 A.M. Slight hyperesthesia. 11.00 A.M. Slightly spastic. 3.00 P.M. More spastic. 5.00 P.M. The same. January 11, 9.00 A.M. Still spastic. 5.00 P.M. Normal. *At 13° C. no tetanus.*

Frog 82.—Female; weight, 25 gm. Temperature 25° C. January 13, 1913, 11.32 A.M. Injected 0.25 c.c. of strychnin sulphate, 0.003 per cent. 12.05 P.M. Slight hyperesthesia. 12.25 P.M. Markedly spastic. 4.30 P.M. Less spastic. January 14, 9.00 A.M. Normal. *At 25° C. no tetanus.*

Frog 130.—Male; weight, 21 gm. Temperature 30° C. January 20, 1913, 12.13 P.M. Injected 0.21 c.c. of strychnin sulphate, 0.003 per cent. 12.23 P.M. Slight hyperesthesia. 12.30 P.M. The same. 1.00 P.M. In strong continuous extensor tetanus. 1.30 P.M. The same. 3.00 P.M. Legs drawn in between attacks. 4.00 P.M. Markedly spastic; no more tetanus. 4.30 P.M. Slightly spastic. *At 30° C. tetanus after 47 minutes, with full recovery on same day.*

The Length of the Interval between the Injection of Strychnin and the Onset of Tetanus.—The foregoing protocols bring out in the first place the fact already alluded to; namely, that a low temperature as well as a high one may bring on tetanus which may fail to appear at an intermediary temperature. The protocols, however, show also that the length of the interval between the injection and the onset of a definite tetanus seems to be in inverse proportion to the degree of temperature. For instance in experiments 102 and

105, in which the animals were kept at 5° C., a definite tetanus appeared the next day or at least many hours after the injection, while in experiments 110, 113, and 128, in which the animals received the same dose of strychnin (0.0005 per gram of frog), but were kept at 27° C. or 30° C., the interval was less than one hour. Such a relation may be of especial interest, since most of the earlier observations seem to have been based largely upon the early occurrence of tetanic symptoms. This may be the reason why the tetanic effect at low temperatures has been frequently overlooked. In our experiments the animals were kept under observation for several days and the late appearance of tetanic manifestations was scrutinized. Table I shows that while it is true in a general way that high temperatures tend to shorten and low temperatures to lengthen the interval, the figures in the various experiments are too irregular to make them fit into a definite general rule.

Duration of the Tetanic State.—In our experiments notes were taken also regarding the duration of the tetanic state under different temperatures. Here again it may be said, in a general way, that at lower temperatures the duration of the tetanic state is longer than when the animal is kept at higher temperatures. The figures, however, are too variable to press them into a definite rule, as will be seen on examination of the figures in table I. We may note that at 13° C. with doses of 0.0005 and 0.0006 of a milligram per gram tetanus always came on within a few hours and passed off during the following night, while with 0.0003 of a milligram per gram no tetanus was obtained, so that there are no observations on the exact duration of tetanus at this temperature.

Character of the Tetanus.—There is a marked difference in the behavior of the tetanic frog at low temperatures and that at high. In the cold each attack is of long duration, and in the interval between them the animal seems to remain in a certain state of increased tonus. The forelegs are held drawn against the chest, and if the hind legs are flexed passively they extend at once. In a frog having tetanus at a high temperature the attacks are much briefer and in the interval between the attacks there is complete relaxation. When an animal is stimulated soon after an attack has passed off,

TABLE I.

Table Showing for Each Dose and Each Temperature the Number of Frogs Injected, the Number Showing Distinct Tetanus, the Interval between the Injection and the Onset of Tetanus, and the Duration of Tetanus.

Dose 0.0003 mg. per gm.			Dose 0.0005 mg. per gm.			Dose 0.0006 mg. per gm.		
Temperature.	No. of experiments.	Tetanus in.	Temperature.	No. of experiments.	Tetanus in.	Temperature.	No. of experiments.	Tetanus in.
5° C.	11	11	5° C.	10	10	5° C.	10	10
No. of frog.	Before onset.	Duration.	No. of frog.	Before onset.	Duration.	No. of frog.	Before onset.	Duration.
28	144 min.	——	102	7–24 hrs.	——	148	31 min.	——
31	95 min.	——	103	7–24 hrs.	——	149	29 min.	——
32	93 min.	——	104	7–23 hrs.	——	150	34 min.	——
76	8–23 hrs.	——	105	7–23 hrs.	——	151	32 min.	——
77	8–23 hrs.	——	192	7–27 hrs.	——	164	160 min.	——
88	5–21 hrs.	——	193	25 hrs.	——	165	158 min.	——
89	5–21 hrs.	——	222	5 hrs.	80 hrs.	178	66 min.	3 dys.
144	1–15 hrs.	34 hrs.	223	6½ hrs.	73 hrs.	179	94 min.	3 dys.
145	17 hrs.	31 hrs.	224	1–16 hrs.	75 hrs.	190	3 hrs.	3 dys.
146	18 hrs.	24 hrs.	225	1–16 hrs.	80 hrs.	191	7 hrs.	3 dys.
147	1–15 hrs.	40 hrs.						
Average	10 hrs.	32 hrs.	Average	8–16 hrs.	77 hrs.	Average	2 hrs.	3 dys.

Temperature.	No. of experiments.	Tetanus in.	Temperature.	No. of experiments.	Tetanus in.	Temperature.	No. of experiments.	Tetanus in.
13° C.	10	0	13° C.	10	8	13° C.	20	20
			No. of frog.	Before onset.	Duration.	No. of frog.	Before onset.	Duration.
			80	4 hrs.	2–15 hrs.	90	130 min.	——
			98	2¼ hrs.	18 hrs.	91	130 min.	4–22 hrs.
			99	2½ hrs.	4–18 hrs.	92	123 min.	1–15 hrs.
			107	3½ hrs.	4–16 hrs.	93	67 min.	3–20 hrs.
			109	3 hrs.	4–16 hrs.	156	163 min.	4–20 hrs.
			247	3 hrs.	——	157	135 min.	4–20 hrs.
			248	3 hrs.	——	158	194 min.	4–20 hrs.
			249	3 hrs.	——	159	192 min.	4–20 hrs.
						235	136 min.	——
						236	124 min.	——
						237	132 min.	——
						265	22 min.	——
						266	40 min.	——
						267	38 min.	——
						270	89 min.	——
						271	88 min.	——
						286	70 min.	——
						287	88 min.	——
						288	197 min.	——
						289	85 min.	——
			Average	3 hrs.	4–16 hrs.	Average	105 min.	4–15 hrs.

TABLE I.—*Concluded.*

Dose 0.0003 mg. per gm.			Dose 0.0005 mg. per gm.			Dose 0.0006 mg. per gm.		
			Temperature.	No. of experiments.	Tetanus in.	Temperature.	No. of experiments.	Tetanus in.
			18° C.	6	2	16°–18° C.	7	7
			No. of frog.	Before onset.	Duration.	No. of frog.	Before onset.	Duration.
			118	50 min.	2½ hrs.	176	160 min.	——
			119	124 min.	1½ hrs.	177	180 min.	——
						186	85 min.	3 hrs.
						187	148 min.	2 hrs.
						241	54 min.	——
						242	85 min.	——
						243	80 min.	——
			Average	87 min.	2 hrs.	Average	113 min.	2½ hrs.
Temperature.	No. of experiments.	Tetanus in.	Temperature.	No. of experiments.	Tetanus in.	Temperature.	No. of experiments.	Tetanus in.
21°–24° C.	11	2	21°–24° C.	10	2	21°–24° C.	8	8
No. of frog.	Before onset.	Duration.	No. of frog.	Before onset.	Duration.	No. of frog.	Before onset.	Duration.
72	16 min.	6 hrs.	194	120 min.	½ hr.	94	35 min.	4 hrs.
73	14 min.	5½ hrs.	195	96 min.	1 hr.	95	110 min.	5 hrs.
						96	23 min.	2 hrs.
						97	31 min.	2 hrs.
						174	34 min.	3 hrs.
						175	32 min.	4½ hrs.
						212	48 min.	1 hr.
						213	57 min.	3 hrs.
Average	15 min.	5¾ hrs.	Average	108 min.	¾ hr.	Average	46 min.	3 hrs.
Temperature.	No. of experiments.	Tetanus in.	Temperature.	No. of experiments.	Tetanus in.	Temperature.	No. of experiments.	Tetanus in.
27°–30° C.	12	4	27°–30° C.	16	16	27°–30° C.	4	4
No. of frog.	Before onset.	Duration.	No. of frog.	Before onset.	Duration.	No. of frog.	Before onset.	Duration.
78	22 min.	4 hrs.	110	51 min.	5½ hrs.	218	24 min.	3½ hrs.
79	35 min.	4 hrs.	111	57 min.	1¼ hrs.	219	53 min.	3 hrs.
130	45 min.	½ hr.	112	42 min.	3½ hrs.	220	62 min.	2½ hrs.
131	40 min.	2 hrs.	113	40 min.	2½ hrs.	221	60 min.	3½ hrs.
			116	21 min.	1¼ hrs.			
			117	17 min.	——			
			120	25 min.	——			
			121	36 min.	——			
			128	35 min.	2 hrs.			
			129	29 min.	½ hr.			
			138	30 min.	2¼ hrs.			
			139	58 min.	1½ hrs.			
			140	23 min.	1 hr.			
			141	50 min.	½ hr.			
			142	33 min.	2 hrs.			
			143	27 min.	2 hrs.			
Average	35 min.	2½ hrs.	Average	36 min.	2 hrs.	Average	69 min.	3 hrs.

there is no response and the legs if passively flexed are not extended until another attack of tetanus occurs.

DISCUSSION AND SUMMARY.

In order to establish the influence of temperature upon the effect of varying doses of strychnin injected into frogs, the animals must be kept under observation for several days and at various definite degrees of temperature. Statements that the animal was kept "cold," "at room temperature," or "warm" are insufficient. With a certain dose tetanus may result constantly at 30° C. yet never appear at 21° C., and either of these temperatures might be described as warm, when compared to a room temperature of 15° C. Furthermore an animal may apparently fail to respond in the cold to an injection of certain doses of strychnin and yet be found in tetanic convulsions the next day. That an animal may have late, long lasting, or strong tetanus while kept at such a low temperature as 5° C. after an injection of a dose of strychnin smaller than 0.01 of a milligram per frog emphasizes the fact that great caution must be exercised in formulating laws as to the influence of temperature on drug action.

The main results of this investigation may be summarized as follows: Doses of strychnin amounting to 0.0006 of a milligram per gram of frog will cause tetanus at all temperatures between 5° C. and 30° C., although at low temperatures the tetanus may appear late. A dose of 0.0003 of a milligram per gram of frog will frequently produce tetanus at 5° C. as well as at 30° or 27° C., but may nevertheless fail to produce any reaction at such an intermediary temperature as 21° C. Smaller doses, 0.0002 of a milligram per gram, will cause tetanus in the cold but not at high temperatures.

It may be stated in general that in frogs kept at low temperatures the tetanic state sets in later, continues longer, and each tetanic attack is of longer duration, while in the interval between the attacks the state of tonus is higher and the animals are more irritable than when they are kept at higher temperatures.

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