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PROLONGATION OF CHLORAL HYDRATE SLEEPING TIME BY 5-HYDROXYTRYPTAMINE AND BY CERTAIN OTHER DRUGS

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Various drugs have been tested for a capacity to prolong the hypnotic effect of chloral hydrate in mice. Amongst the compounds which, when injected subcutaneously in substantial amount shortly before the chloral hydrate (250 mg./kg. intraperitoneally), increased sleeping time significantly were adrenaline, noradrenaline, phenylephrine, methoxamine, 5-hydroxytryptamine (serotonin), histamine, ergotamine, yohimbine, and atropine. The ability of these drugs to prolong chloral hydrate sleeping time could not be related to any common circulatory property, but most of the active drugs are known to lower body temperature under comparable conditions. It was found that mice which have been pre-treated with 5-hydroxytryptamine or adrenaline suffer a much greater fall of body temperature when chloral hydrate is given subsequently than do mice which have been given chloral hydrate alone. It is suggested that some, at least, of the drugs which prolong the effects of hypnotics do so by virtue of a hypothermic action.

Mice which have received 5-hydroxytryptamine (5-HT) sleep longer than normal animals when given a fixed dose of a barbiturate (Shore, Silver, and Brodie, 1955; Fornaroli and Koller, 1955; Fastier, 1956). While some substances can prolong the effects of barbiturates by interfering with their enzymatic inactivation, the sleep-prolonging action of 5-HT seemed unlikely to be due to an action of this type because it can extend to the hypnotic action of chloral hydrate which is not metabolized in the same way as barbiturates. Experiments bearing on the mechanism of action have been discussed by Shore *et al.* (1955), Salmoiraghi, Sollero, and Page (1956), Fastier (1956), and Richards and Taylor (1956).

As the hypnotic effect of chloral hydrate in mice can be prolonged, not only by 5-HT and such nearly related compounds as tryptamine and bufotenine, but also by such agents as adrenaline and histamine, Fastier (1956) suggested that the sleep-prolonging effect of 5-HT might be due to a relatively non-specific vascular action. Like Werle and Lentzen (1938), Fastier (1956) was impressed by the fact that many of the drugs which have been shown to prolong the effects of hypnotics (under some conditions at least) are vaso-active drugs.

This paper reports the results of some experiments seeking to establish a connexion between effects on the circulatory system and hypnosis-prolonging activity. It seemed possible that some compounds might prolong effects of certain hypnotics by vascular mechanisms affecting, for example, the absorption of the drug from the site of injection, its penetration of the "blood-brain barrier," or its breakdown or excretion. Since Fuhrman (1946) has demonstrated that the lowering of body temperature by 10° C. can increase pentobarbitone sleeping time more than three-fold, experiments were also carried out to see whether pre-treatment with 5-HT or adrenaline could result in a much greater fall of body temperature than usual when a hypnotic was given.

METHODS

The dose of chloral hydrate usually employed (250 mg./kg.) was that which put mice to sleep for about 30 min. Chloral hydrate (1 g.) was dissolved in half-isotonic saline (40 ml.), whether used for intraperitoneal or intravenous injection. Other drugs were generally given subcutaneously, to prevent interference with the absorption of chloral hydrate. After the mice had fallen asleep, they were placed a short distance apart from one another, so that a waking mouse would disturb its neighbours as little as possible. The waking time was taken as that at which a mouse righted itself and began to move away from its sleeping position. The transition from sleeping to waking appeared to be very quick. Mice which

TABLE I

EFFECT OF ADRENALINE AND RELATED DRUGS, AND OF 5-HT, ON THE DURATION OF HYPNOSIS PRODUCED IN MICE BY THE INTRAPERITONEAL INJECTION OF CHLORAL HYDRATE (250 MG./KG.)

Each compound was injected either subcutaneously (s.c.) or intraperitoneally (i.p.) 10 min. before the chloral hydrate. The mice in each control group were given an injection of 0.9% NaCl by the same route 10 min. before the chloral hydrate. The sleeping times given are the means for groups of 10 mice. The numerals in brackets after each mean sleeping time give the standard deviation. The *t* test shows a significant ($P<0.01$) increase for the results marked + and a significant ($P<0.01$) decrease for the results marked −. Some earlier results of Fastier (1956) are included for comparison, these experiments being indicated by an asterisk.

Pre-treatment			Sleeping Time (min.)		
Drug	Dose in mg./kg.	Route	Treated Group	Control Group	
Adrenaline* ..	5	i.p.	98 (39)	35 (17)	+
,, ..	5	s.c.	70 (58)	20 (10)	+
,, ..	3	i.p.	65 (29)	24 (6·9)	+
,, ..	3	s.c.	39 (12)	31 (15)	
Noradrenaline ..	5	,,	73 (33)	27 (19)	+
,, ..	3	,,	110 (59)	23 (8·3)	+
,, ..	2	,,	55 (21)	38 (19)	
Phenylephrine ..	50	,,	69 (32)	34 (6·9)	+
,, ..	20	,,	53 (17)	45 (11)	
Methoxamine ..	20	,,	64 (4·8)	21 (1·6)	+
,, ..	20	,,	56 (14)	18 (5·0)	+
Naphthazoline ..	20	,,	75 (27)	24 (8·3)	+
Isoprenaline ..	10	,,	77 (46)	28 (15)	+
,, ..	5	,,	50 (27)	34 (20)	
,, ..	5	,,	41 (6·6)	55 (6·8)	
Amphetamine ..	20	,,	0	29 (4·1)	−
Methylamphetamine	20	,,	0	31 (3·2)	−
5-HT*	20	i.p.	86 (36)	20 (6·9)	+
,, ...	20	s.c.	70 (18)	28 (11)	+
Tryptamine* ..	20	i.p.	37 (9·3)	22 (7·2)	+
Bufotenine* ..	20	,,	81 (31)	22 (8·4)	+

had awakened were removed from the boxes of sleeping mice to avoid their disturbing those left.

The effect of 5-HT on the mydriatic action of hexamethonium in the mouse was studied by measuring pupil size directly by means of a low-power microscope with a scalar eyepiece as described by Blackman, Fastier, Patel, and Wong (1956).

Rectal temperature in mice was measured by inserting a glue-covered constantan-copper thermocouple into the rectum to a fixed distance. Mice were cooled by placing them inside a large refrigerator for a fixed period.

RESULTS

Effects of Various Drugs on Duration of Sleep.—A number of sympathomimetic amines were tested for any obvious relationship between vasoconstrictor activity and a capacity to prolong the hypnotic effect of chloral hydrate in mice (Table I). The response to noradrenaline was found to resemble that to adrenaline. The chloral hydrate sleeping time was also substantially increased by phenylephrine (Neo-Synephrine), methoxamine (Vasoxine), and naphthazoline (Privine), sympathomimetic amines in which the vasoconstrictor and similar "excitatory" actions of adrenaline are most prominently displayed. Isoprenaline, in which the vasodilator and similar inhibitory properties of adrenaline predominate, did not affect chloral hydrate sleeping time significantly when given in the same dose as adrenaline, but it prolonged the chloral hydrate sleeping time considerably when given in the near-lethal dose of 10 mg./kg. Sympathomimetic amines like amphetamine and methylamphetamine (Methedrine), with effects predominantly on the central nervous system, antagonized the hypnotic action of chloral hydrate.

When a wider range of compounds was examined, however, no correlation between vasoconstrictor activity and sleep-prolonging activity was found (Table II). The chloral hydrate sleeping time was increased by certain drugs whose circulatory effects are quite different from those of adrenaline, such as tolazoline (Priscol), yohimbine, and physostigmine. Tolazoline and yohimbine have anti-adrenaline actions, and physostigmine is an anticholinesterase. Atropine also was found to produce a significant increase in the chloral hydrate sleeping time, while methacholine produced a significant shortening. Posterior pituitary extract (Pitibulin) did not affect sleeping time in the doses tried (0.5, 1.0 unit/kg.).

TABLE II

EFFECT OF VARIOUS DRUGS ON CHLORAL HYDRATE SLEEPING TIME IN MICE

Experiments were performed as indicated in Table I. The chloral hydrate was given intraperitoneally, all other drugs subcutaneously and in approximately isotonic solution. "S-methyl" and "S-*n*-decyl" refer to the corresponding *iso*thiouronium salts. The asterisk indicates previous results obtained by Fastier (1956). See Table I for explanation of + and − signs.

Pre-treatment		Sleeping Time (min.)		
Drug	Dose in mg./kg.	Treated Group	Control Group	
2-Aminoheptane ..	20	30 (13)	30 (19)	
S-Methyl	50	36 (7·8)	22 (9·1)	+
,, ...	10	31 (5·3)	34 (2·9)	
S-*n*-Decyl	20	50 (4·4)	44 (5·8)	
Mescaline	10	17 (4·0)	16 (3·4)	
Ergotamine	10	25 (2·7)	18 (2·1)	+
Yohimbine	5	61 (7·7)	31 (2·1)	+
Tolazoline	20	46 (17)	18 (5·0)	
,, ...	10	46 (4·5)	20 (2·8)	+
Diphenhydramine ..	10	40 (6·2)	49 (6·2)	
Physostigmine ..	0·25	59 (4·8)	30 (5·0)	+
Methacholine ..	0·5	25 (4·0)	41 (3·9)	−
Atropine	20	55 (20)	41 (29)	
,, ...	10	20 (2·2)	14 (1·3)	+
Pituitrin	(0·5 units)	13 (9·5)	14 (6·0)	
,, ...	(1 unit)	34 (7·6)	27 (10)	
Histamine*	10	51 (3·9)	26 (4·2)	+
Cortisone	20	22 (2·4)	21 (2·6)	
Deoxycortone acetate	20	16 (1·9)	21 (2·7)	
Pacatal	20	46 (8·1)	30 (4·6)	
,, ...	20	27 (4·9)	20 (6·4)	
Sodium nitrite ..	40	33 (2·8)	25 (4·0)	
,, ,, ..	20	19 (2·3)	27 (4·6)	
Distilled water ..	100	34 (7·3)	31 (3·1)	

Influence of Certain Circulatory Changes.—A drug like 5-HT might influence the absorption of a drug given intraperitoneally a few minutes later by causing strong vasoconstriction in the splanchnic region. To test this idea, experiments were performed in which the hypnotic was given intravenously. As 5-HT, methoxamine and tolazoline all caused about the same increase in sleeping time whether chloral hydrate was given intravenously or intraperitoneally (Table III), the

TABLE III

EFFECT OF PREMEDICATION WITH 5-HT, METHOXAMINE, OR TOLAZOLINE ON THE DURATION OF SLEEP PRODUCED BY CHLORAL HYDRATE IN MICE

Chloral hydrate was given either intraperitoneally (i.p.) or intravenously (i.v.). The other drugs were given subcutaneously in a dose of 20 mg./kg. The numbers of mice in the treated and control groups respectively are given in column 6. Smaller numbers than usual were employed owing to the relative difficulty of giving intravenous injections of chloral hydrate quickly. The numerals given in brackets in cols. 4 and 5 are the standard deviations of the mean sleeping times.

Drug (1)	Chloral Hydrate Dose (mg./kg.) (2)	Chloral Hydrate Route (3)	Sleeping Time (min.) Treated Group (4)	Sleeping Time (min.) Control Group (5)	No. in Group (6)
5-HT	112·5	i.v.	66 (26)	21 (7·0)	4/4
,, ...	225	i.p.	70 (18)	29 (11)	9/9
Methoxamine ..	75	i.v.	20 (6·5)	8 (7·1)	7/7
,, ...	200	,,	64 (20)	22 (9·5)	5/5
,, ...	225	i.p.	66 (14)	18 (5·3)	10/10
Tolazoline ..	75	i.v.	8 (8)	8 (7)	7/7
,, ..	200	,,	46 (14)	22 (9·5)	5/5
,, ..	225	i.p.	46 (17)	18 (5·2)	10/10

prolongation of sleeping time by these drugs cannot be attributed to delayed absorption of the chloral hydrate.

The antidiuretic activity of a compound such as 5-HT might prolong the effects of certain hypnotic drugs by delaying their excretion. Experiments were therefore performed to see whether 5-HT could prolong the effects of drugs which are mainly excreted in the urine.

Hexamethonium appeared to be a suitable drug for the purpose because it is entirely excreted in the urine (Paton and Zaimis, 1952) and its mydriatic effect can be readily measured in the mouse. Fig. 1 shows that the mydriatic effect of hexamethonium in mice is much prolonged by 5-HT. The latter alone caused a slight, transient mydriasis in mice. This mydriatic effect may have been brought about indirectly, for it was observed that 5-HT slowed down the accommodation to the light of the lamp used for constant illumination in measuring pupil size. In mice treated with 5-HT the light reflex resulted in pupillary constriction when the mice were brought near the lamp only after some 10 to 45 sec., compared with 5 to 10 sec. for normal mice. Thus,

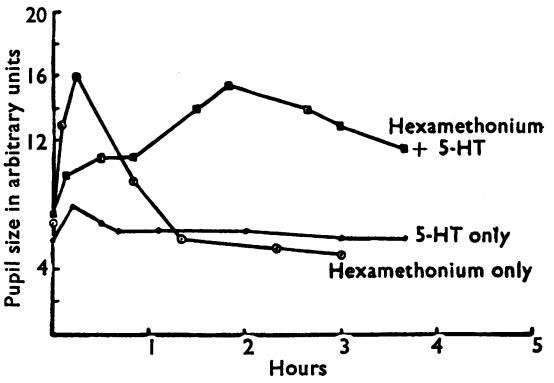

FIG. 1.—Prolongation of hexamethonium mydriasis in mice by premedication with 5-HT. Each point is the mean of 10 observations. The hexamethonium bromide (20 mg./kg. i.p.) was given 10 min. after 5-HT (20 mg./kg. s.c.). The same doses were used in control experiments with each compound alone.

the rapid measuring of pupil size could lead to the recording of a spurious mydriasis.

Analogous experiments were performed with 5-HT and Intocostrin, which is largely excreted in the urine (Marsh, 1952; Kalow, 1953). In this case, the proportion of mice dying from respiratory failure after substantial doses of Intocostrin was taken as a measure of activity. The proportion was increased by treatment with 20 mg./kg. of 5-HT (Table IV).

TABLE IV

EFFECT OF 5-HT ON THE TOXICITY OF INTOCOSTRIN

The Intocostrin dose was given in standard rabbit head drop units and the drug was administered intraperitoneally 10 min. after the 5-HT (20 mg./kg. s.c.).

Intocostrin Dose	Survivals Treated Group	Survivals Control Group	Deaths Treated Group	Deaths Control Group
4·4 units	0	4	10	6
3·3 ,, ...	1	8	9	2
2·6 ,, ...	4	10	6	0

Influence of Lowered Body Temperature.—In the preceding experiments, mice pre-treated with 5-HT always felt colder than the controls. On one cold winter day, as the room temperature dropped, the mice which had received 5-HT became more and more flaccid instead of showing signs of waking from the chloral hydrate sleep. Heat was then applied by bringing two 75-watt lamps near the mice. Within 10 min. of the application of heat the mice began to wake up. This observation suggested that 5-HT might interfere with the temperature-regulating mechanism of the mice and prolong hypnotic action by lowering body temperature.

An experiment was then performed in which two groups of mice, one of which had received

5-HT (20 mg./kg. subcutaneously) and the other control injections of saline, were exposed to a cold environment. After both groups of mice had been kept in the refrigerator for 100 min. the average fall in the oral temperature of the treated mice was 5.8° C., whereas that for the control mice was 1.2° C. A t test showed the difference between the two sets of temperature readings to be highly significant ($t_s = 8.4$). Measurement of the rectal temperature also showed a highly significant ($t_s = 9.3$) difference. Whereas the mean rectal temperature for the treated mice fell from 38.3° C. (s.e. 0.7° C.) to 28.4° C. (s.e. 2.0), a difference of 9.9° C., the mean rectal temperature for the control group fell from 38.7° C. (s.e. 0.4) to 35.5° C. (s.e. 0.7), a difference of only 3.2° C.

Next, the rectal temperatures in groups of 5 mice which had been treated respectively as shown below were compared. Group I were given a subcutaneous injection of saline followed 10 min. later by an intraperitoneal injection of chloral hydrate (200 mg./kg.). Group II received a subcutaneous injection of 5-HT (20 mg./kg.) followed 10 min. later by an intraperitoneal injection of chloral hydrate (200 mg./kg.). Group III were injected subcutaneously with 5-HT (20 mg./kg.) followed 10 min. later by an intraperitoneal injection of saline. The results obtained are shown in Fig. 2. The difference in the average drop in

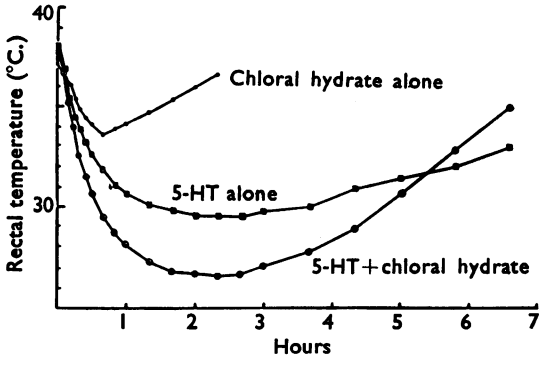

FIG. 2.—Effect of chloral hydrate and 5-HT on the rectal temperature of mice. Each point is the mean of observations made on 5 mice. Chloral hydrate (225 mg./kg. i.p.) and 5-HT (20 mg./kg. s.c.) were given either singly or in combination, in which case 5-HT was given 10 min. before chloral hydrate.

rectal temperature for the mice in Groups I and II respectively was highly significant both in extent and duration. Whereas the maximum temperature drop in Group II was 10.0° C., that in Group I was only 4.8° C.

In another series of experiments, some of the mice were kept at a temperature of 38° C. to prevent them losing heat to the surroundings, while others were kept at room temperature (20° C.). Four groups, each of 10 mice, were used. Two groups were given 5-HT (20 mg./kg.) followed by an intraperitoneal injection of chloral hydrate (225 mg./kg.). One of these groups was kept at 38° C. and the other at 20° C. The other two groups were treated similarly except that they received a control injection of saline instead of 5-HT. The average sleeping times of the four groups were as follows: 37.5 min. (s.e. 7 min.) for the mice which had received 5-HT and chloral hydrate and were kept at 38° C.; 82.5 min. (s.e. 2 min.) for the mice which had received 5-HT and chloral hydrate and were kept at 20° C.; 15 min. (s.e. 2 min.) for the mice which had received only saline before the chloral hydrate and which were kept at 38° C.; 17 min. (s.e. 3.5 min.) for the mice which had received only saline before the chloral hydrate and which were kept at 20° C.

These results show that keeping the mice at body temperature markedly reduces the prolonging effect of 5-HT on the chloral hydrate sleeping time. The difference between the average sleeping times for the first and third groups was highly significant statistically ($P<0.001$), as was the difference between the average sleeping times for the second and fourth group. The difference between the average sleeping times for the first two groups was significant at the $P<0.05$ level. Thus it seems that the hypothermic action of 5-HT may contribute largely to its hypnosis-prolonging activity.

Fig. 3 illustrates the results of an experiment in which four groups, each of 5 mice, received respectively: (*a*) adrenaline (5 mg./kg. subcutaneously) followed 10 min. later by chloral hydrate

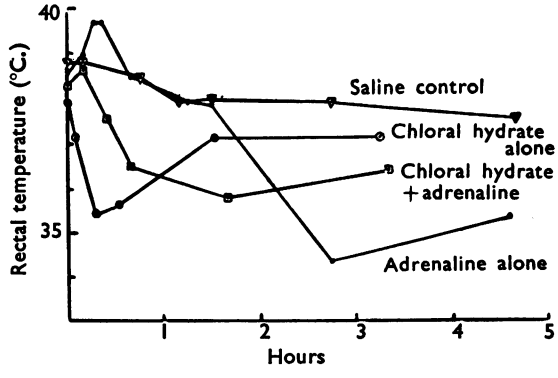

FIG. 3.—Effects of chloral hydrate and adrenaline on the recta temperature of mice. Each point is the mean of 10 observations. Chloral hydrate (250 mg./kg. i.p.) and adrenaline (5 mg./kg. s.c.) were given either singly with control injections of saline or in combination, in which case the adrenaline was given 10 min. before the chloral hydrate.

(250 mg./kg. intraperitoneally); (*b*) a control injection of saline followed 10 min. later by the same dose of chloral hydrate; (*c*) adrenaline (5 mg./kg. subcutaneously) followed 10 min. later by a control injection of saline intraperitoneally; (*d*) a control injection of saline subcutaneously followed 10 min. later by another control injection intraperitoneally. It can be seen that adrenaline alone at first increased rectal temperature by 1° C. but later caused it to drop by 4° C. Chloral hydrate alone produced a sharp, substantial, but temporary fall of rectal temperature of 2.8° C. When adrenaline was given before chloral hydrate, however, there was a sustained fall of body temperature.

Discussion

The results obtained with sympathomimetic amines (Table I) suggested that there might be a connexion between vasoconstrictor activity and hypnosis-prolonging activity. However, the fact that such drugs as yohimbine, tolazoline, histamine, physostigmine, and atropine can also increase chloral hydrate sleeping time under comparable conditions (Table II) forces us to conclude either that (*a*) several quite distinct mechanisms of action are involved, or (*b*) the mechanism of action, if there be only one, is not directly related to the circulatory activity of the compounds.

Although the compounds known to possess hypnotic-potentiating activity differ greatly in their chief effects on the circulatory system, there is one property which the majority of them possess and which could explain their ability to potentiate the effects of hypnotics—namely, the ability to lower body temperature, especially in the presence of an hypnotic. Figs. 2 and 3 show the magnitude of the changes in body temperatures produced by doses of 5-HT and adrenaline which substantially increased the chloral hydrate sleeping time. These observations naturally raised the question, Do the other drugs which prolong chloral hydrate sleeping time have a similar effect on body temperature?

Numerous reports in the literature indicate that most of the compounds shown to have hypnotic-prolonging activity (Tables I and II) can lower body temperature to a noteworthy extent—for example, histamine (Fabinyi-Szebehely and Szebehely, 1952; Kind, 1954), ergot alkaloids (Githens, 1917; Flacke *et al.*, 1953), yohimbine (Dimitrijević, 1938), tolazoline (Horwitz, Montgomery, Longaker and Sayen, 1949), and atropine (Burn and Dutta, 1948).

As many investigators have reported that adrenaline has a calorigenic action, resulting in an increase in body temperature and in oxygen consumption, we thought it important to see how adrenaline affected the body temperature of mice under the experimental conditions of this study. The results illustrated in Fig. 3 show that mice pre-treated with adrenaline have a much more prolonged fall in body temperature when given chloral hydrate than control mice. Gyermek (1950) found in rats that the effect of adrenaline on oxygen consumption and body temperature depended on the environmental temperature: at 20° C., adrenaline (0.5 mg./kg. s.c.) decreased both oxygen consumption and body temperature, whereas at 30° C. it increased them. From such findings, many of which have been reviewed by Griffith (1951), it appears that the variety of effects which adrenaline has been reported to produce (according to the dose, animal species, and other experimental conditions) can be explained best by assuming that adrenaline can affect body temperature by several distinct actions which may be opposed. Thus an action on the central nervous system tending to lower body temperature may be opposed by a peripheral action, such as the liberation of glucose, which tends to raise it.

Some of the drugs (colchicine, certain "tranquillizers," and antihistamines) which have been found by other investigators to prolong the effects of hypnotic drugs have also been shown to have a hypothermic action. When Burn and Dutta (1948) observed that procaine shares its body temperature lowering action in mice with such drugs as atropine, diphenhydramine, pethidine, and quinidine, all of which produce a number of pharmacological effects attributable to antagonism of particular actions of acetylcholine, they suggested "that the maintenance of body temperature depends on a mechanism in which acetylcholine plays a part." This does not necessarily imply that the action is a central one. However, it seems likely that the chief site of the hypothermic action of compounds like atropine and quinidine is in the central nervous system, possibly in the hypothalamus.

Even if many of the compounds which prolong the effects of hypnotics do lower body temperature under comparable conditions, there may be no causal relationship between the two effects; both effects might be the result of an action affecting chiefly the hypothalamic region of the brain. Against this interpretation is the fact that some of the compounds which prolonged chloral hydrate sleeping time are ones like histamine,

whose effects on body temperature are likely to be brought about principally by a direct action on peripheral blood vessels. Moreover, it has been shown in the case of 5-HT that the sleep-prolonging effect is much reduced when the environmental temperature is raised to make heat loss minimal.

One finding which was disconcerting at first was the great variation in the mean sleeping times for the control groups of mice. We now attribute this mainly to the fact that the laboratory temperature varied considerably from day to day. The importance of temperature control was realized subsequently. Because of the variation in mean sleeping times, the effect of a compound on sleeping time was usually tested more than once and by different observers. The results of duplicated experiments were usually concordant.

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