STUDIES OF PENTOBARBITAL DEPRESSION • † ‡

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The successful treatment of barbiturate overdosage with sodium succinate was reported by Soskin and Taubenhaus (1) who based their procedure on the fact that succinic dehydrogenase was not inhibited in vitro by barbiturate whereas the dehydrogenases of glucose, lactate and pyruvate, were inhibited (2-4). Hence it was assumed that brain tissue would utilize succinate as a source of energy until the effects of the barbiturates were dissipated and the normal metabolic cycle of the tissue restored. Barrett (5) reported that succinate was preferable to the convulsant drugs in the treatment of barbiturate depression in human beings. Beyer and Latven (6) and also Pinschmidt et al. (7) found that succinate diminished the duration of pentobarbital anesthesia, but the effect was not nearly as great as that reported by Soskin and Taubenhaus (1). Pinschmidt and co-workers (7) also reported that little additive effect was obtained when sodium succinate and picrotoxin were administered to rabbits previously given pentobarbital. Beyer and Latven (6) confirmed the statement that pentobarbital did not inhibit the in vitro oxidation of succinate. Lardy and co-workers (8) found that in the rat succinate had no effect on the duration of anesthesia induced by either pentobarbital or amytal. Schaeck and Goldbaum (9) reported that sodium succinate did not exert any effect on the duration of anesthesia in the rabbit or the blood level of either pentobarbital or thiopental (pentothal®) even when the former was administered in exceedingly large doses.

Since the information in the literature is diverse, several aspects of pentobarbital antagonism have been studied in order to gain more information concerning the value of sodium succinate and related compounds in barbiturate depression.

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Experiment I

Male albino rats of the Wistar strain, weight 150 to 350 Gm. each, were used. Their sustenance of an adequate diet was supplemented occasionally with carrots, milk, whole wheat bread, lettuce and cod liver oil. They were fasted fifteen to seventeen hours prior to the time of injection. No rats were used more often than once a month. Pentobarbital sodium was freshly prepared as a 1 per cent solution and the salts to be tested for analeptic activity were prepared as 10 per cent solutions. The pentobarbital was injected subcutaneously in a dose of 50 mg. per kilogram of body weight and the salts to be tested for analeptic activity were injected intraperitoneally immediately afterward in a dose of 1 Gm. per kilogram of body weight. To determine onset of, or recovery from anesthesia, the paw of one of the extended hind legs was stimulated by pinching with the thumb and index finger at two minute intervals; when the muscles of the thigh did not flex, anesthesia was considered to be present. The righting reflex time was also measured. Righting reflex time is defined as the period which begins when the animal loses its equilibrium or facilities for maintaining normal posture and will not return to a normal position spontaneously when placed on its back; it ends when the animal spontaneously returns to a normal stance (table 1).

The statistical procedure utilized was the determination of significant difference, “Student’s t” test (10).

TABLE 1

<table>
<thead>
<tr>
<th>Drugs Administered</th>
<th>Number of Rats</th>
<th>Average Duration of Anesthesia (Minutes)</th>
<th>Average Duration of Loss of Righting Reflex (Minutes)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pentobarbital Sodium*</td>
<td>55</td>
<td>89</td>
<td>134.4</td>
</tr>
<tr>
<td>Pentobarbital Sodium* and Sodium Succinate†</td>
<td>20</td>
<td>84.6</td>
<td>143.0</td>
</tr>
<tr>
<td>Pentobarbital Sodium* and Sodium Malonate†</td>
<td>24</td>
<td>89.8</td>
<td>166.9</td>
</tr>
<tr>
<td>Pentobarbital Sodium* and Sodium Citrate†</td>
<td>28</td>
<td>100.3</td>
<td>192.5</td>
</tr>
<tr>
<td>Pentobarbital Sodium* and Sodium Acetate†</td>
<td>28</td>
<td>74.0</td>
<td>147.8</td>
</tr>
</tbody>
</table>

* 50 mg. per kilogram.
† 1 gm. per kilogram.
Experiment II

This experiment is similar to Experiment I in design, except for the following refinements: equimolar concentrations of sodium acetate and sodium succinate were used with controls of both equimolar saline and glucose. The salts, 0.005 mol per kilogram, were administered approximately thirty minutes after the injection of the pentobarbital, in contrast to their previous simultaneous administration. Only those animals anesthetized after thirty minutes are included. A series of cross-over experiments was performed on the same rats after the lapse of a month or more.

Experiment III

Four groups of rats were used. Each was given intraperitoneally 85 mg. pentobarbital sodium per kilogram of body weight. Twenty minutes after onset of anesthesia, animals of the various groups were given one of the following: (a) sodium succinate, 1 Gm. per kilogram; (b) pentylentetrazol (metrazol), 10 mg. per kilogram; (c) sodium succinate, 1 Gm. per kilogram, plus pentylentetrazol (metrazol), 10 mg. per kilogram, and (d) 5 cc. of isotonic sodium chloride per kilogram. The criteria for determining anesthesia and righting reflex are the same as described in Experiment I.

Discussion

The data presented in tables 1, 2, and 3, were obtained in testing the analeptic effect of succinate and other compounds against the anesthetic and other depressant effects of pentobarbital under experimental conditions. In Experiment I (table 1) the salts were administered simultaneously, whereas in Experiment II (table 2) they were administered thirty minutes after the onset of anesthesia in order to simulate the clinical picture since it is very unlikely that a depressant drug and its antagonist would be administered at the same time. In Experiment III (table 3) the additive effect and synergistic action of succinate were tested when given in conjunction with pentylentetrazol (metrazol). The slight or negative effects of succinate toward the anesthetic effect of pentobarbital which we observed are in accord with those of Lardy et al. (8) and also Schack and Goldbaum (9). Statistical analysis of the data in tables 1, 2, and 3 shows that succinate did not produce sufficient difference to warrant any positive conclusions as to its analeptic effectiveness. The data in table 3, obtained when a sublethal dose instead of an anesthetic dose of pentobarbital was administered, show that succinate not only was ineffective in decreasing duration of anesthesia but also in preventing death from over-

§ The t values are such that the probability of obtaining them by chance alone is greater than fifty times in one hundred.
dosage. Its analeptic effect, if any, did not summate with or potentiate that of a low dose of pentylentetrazol (metrazol®). Pentylentetrazol was used in conjunction with succinate because picrotoxin had already been employed with little success (7) and pentylentetrazol has been considered “the treatment of choice” (11).

Malonate was used because it is a dibasic compound of the same homologous series as succinate. Considering the results of Potter (12) and others (13) who have reported that malonate is a definite inhibitor of succinic dehydrogenase in vitro and the hypothesis of Quastel and Wheatley (2) that inhibition of oxidation causes anesthesia, an actual increase in duration of anesthesia might have been anticipated when malonate was injected simultaneously with the barbiturate. Beyer and Latven (6) have reported that malonate was without effect on the duration of pentobarbital hypnosis in mice or rats. In the present investigation, malonate had no effect on the duration of anesthesia (table 1). This lack of any in vivo effect of malonate may be due to the multiple pathways for the metabolism of essential substrates in the body. Our results substantiate Bain’s (14) view that “inhibition of respiration in vitro is not a sufficient explanation for in vivo effects.”

Acetate and citrate were tested because they are compounds that can be metabolized and represent two additional types of chemical structures, that is, monobasic and tribasic, respectively, as contrasted with the dibasic succinate. The data in table 1 show that acetate and citrate may alter the duration of the anesthesia. Acetate, according to Quastel (4), has little or no effect on the respiration of the brain, but Lee and Lifson (15) have shown that acetate can be metabolized by way of the triarboxylic cycle in the rat. Our results showing that it has a slight analeptic action are similar in some respects to

<table>
<thead>
<tr>
<th>Substance Injected 30 Minutes after Pentobarbital</th>
<th>Number of Rats</th>
<th>Average Duration of Anesthesia</th>
<th>Average Duration of Loss of Righting Reflex</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Minutes</td>
<td>t</td>
</tr>
<tr>
<td>None</td>
<td>15</td>
<td>79.0</td>
<td>—</td>
</tr>
<tr>
<td>Sodium Succinate*</td>
<td>19</td>
<td>81.0</td>
<td>0.21</td>
</tr>
<tr>
<td>Sodium Acetate*</td>
<td>18</td>
<td>83.5</td>
<td>0.32</td>
</tr>
<tr>
<td>Sodium Chloride*</td>
<td>14</td>
<td>84.3</td>
<td>0.40</td>
</tr>
<tr>
<td>Glucose*</td>
<td>15</td>
<td>89.7</td>
<td>0.82</td>
</tr>
</tbody>
</table>

* 0.005 mol per kilogram.
those of Taylor et al. (16) who demonstrated that acetate decreased the inhibition of oxygen uptake of brain produced by methadione. That acetates might have a favorable effect on decreasing the duration of anesthesia owing to their diuretic effect would seem unlikely because Koppanyi et al. (17) have shown that moderate diuresis does not affect barbiturate anesthesia. The shortening of anesthesia time by acetate in table 1 confirms our preliminary report (18). The t value, 1.92, is significant. In table 2, in our effort to simulate clinical conditions, the acetate was not administered until thirty minutes after the pentobarbital and under these conditions the acetate was not effective. Sodium citrate (table 1) did not significantly alter the duration of anesthesia.

Variations in the duration of anesthesia in the two control groups of tables 1 and 2 may be accounted for by the following factors: in-

<table>
<thead>
<tr>
<th>TABLE 3</th>
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</thead>
<tbody>
<tr>
<td><strong>Succinate and Pentylenetetrazol (Metrazol) in Pentobarbital Depression</strong></td>
</tr>
<tr>
<td>---------------------------------</td>
</tr>
<tr>
<td>Substance Injected 20 Minutes after Pentobarbital</td>
</tr>
<tr>
<td>---------------------------------</td>
</tr>
<tr>
<td>Sodium Chloride (Control)</td>
</tr>
<tr>
<td>Sodium Succinate</td>
</tr>
<tr>
<td>Pentylenetetrazol (Metrazol)</td>
</tr>
<tr>
<td>Sodium Succinate and Pentylenetetrazol (Metrazol)</td>
</tr>
</tbody>
</table>

increased weight and age may have been an influence as the rats in table 2 were used in a cross-over procedure; some tolerance may have developed (although a month or more elapsed between the repetition of the doses) as Carmichael (19) has reported that large single doses confer some tolerance for several weeks; environmental temperatures of the animals varied, which influences the action of drugs (20).

The duration of the loss of righting reflex was increased by all of the injected salts used in Experiment I (table 1), with the effect of malonate, citrate and acetate significant statistically. Some of the factors have been discussed by Richards et al. (21) and Lamson et al. (22 and 23). This increase in hypnotic effect, that is, reinduction of sleep, has been observed by Richards et al. (21) chiefly in guinea pigs, but also in rabbits, whereas Lamson et al. did not observe this action

‖ This result could have been attributable to chance only five times in one hundred.
in rats. Several explanations of these observations have been made. Richards et al. (21) believed there is a change of permeability of the blood-brain barrier to the barbiturate. Further possibilities may be: diffusion of the sodium ions of the original salt and the residual sodium ions after the combustion of the organic radical, into the extracellular fluid, the latter causing an increase in the alkaline reserve of the tissue. These factors would cause hydration of tissue and therefore depression.

The duration of the righting reflex time was not significantly altered by succinate in either Experiment I or II. Lamson et al. (23) reported that animals other than the rat show the "glucose reaction," that is, a depressant effect from succinate and acetate. Acetate in Experiment I significantly increased the righting reflex time, but in Experiment II there was no real change. This can be accounted for by two factors. In Experiment I the amounts of acetate were greater and also were administered simultaneously.

In Experiment III (table 3) sodium succinate again proved ineffective in decreasing the righting reflex time. Pentylenetetrazol when given with succinate showed neither additive nor synergistic action.

The statements of Lamson et al. (22) that in the rat anesthesia is not reinduced by the injection of glucose upon awakening from barbiturate anesthesia are in accord with our results (table 2). Glucose was used for two reasons: (1) the early reports of Lamson and (2) to serve as a control.

SUMMARY

Sodium succinate was ineffective in shortening either the duration of anesthesia or the righting reflex time in male white rats given anesthetic or sublethal doses of pentobarbital sodium. Sodium succinate and pentylenetetrazol (metrazol) injected simultaneously showed neither summation nor synergistic analeptic action against sublethal doses of pentobarbital sodium on either the duration of anesthesia or the righting reflex time. Sodium malonate or citrate showed no definite analeptic effect on the anesthetic action of pentobarbital sodium; they significantly increased the duration of the loss of righting reflex. Sodium acetate when injected simultaneously with pentobarbital sodium decreased slightly the duration of anesthesia and increased the righting reflex time. When injected thirty minutes after the pentobarbital, however, it exhibited no analeptic action on either the duration of anesthesia or the righting reflex time. Glucose was without effect on either the duration of anesthesia or on the loss of the righting reflex induced by pentobarbital sodium.

REFERENCES

11. Jones, A. W.; Dooley, J., and Murphy, J. R.: Treatment of Choice in Barbiturate Poisoning; Series of 29 Cases of Barbiturate Poisoning Treated with Pentylenetetrazole (Metrazol) and Supportive Therapy, J.A.M.A., 143: 884-888 (July 8) 1950.