MUTUAL CROSS TOLERANCE AND TOLERANCE STUDIES WITH PENTOBARBITAL AND DELVINAL SODIUM IN RATS *

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In 1930, Fitch (1) demonstrated that a tolerance was developed in rabbits following repeated doses of either noctal or amytal by stomach tube. Experiments in this laboratory have shown that a tolerance was developed in guinea pigs after repeated intraperitoneal injections of either pentobarbital sodium or delvinal sodium (2, 3, 4) and that a mutual cross tolerance between these two drugs was observed in guinea pigs (5). Development of cross tolerance to both pentobarbital sodium and delvinal sodium followed a single large intraperitoneal injection of pentothal sodium in guinea pigs (6). In 1944, Green and Koppanyi (7) found that dogs made tolerant to pentothal sodium show cross tolerance to eviptal and that eviptal-tolerant dogs show cross tolerance to pentothal sodium. Gruber and Keyser (8) demonstrated that dogs, rats and rabbits made tolerant to one barbiturate show cross tolerance to other barbiturates.

This study was made to see whether a mutual cross tolerance could be developed in the rat between pentobarbital sodium and delvinal sodium.

Normal adult rats were used throughout this series of experiments. A fresh aqueous solution of each drug was prepared before each day's injections. All injections were made intraperitoneally. After the onset of sleep, each animal was placed in an incubator at about 90 F.

To study the effects of each injection of these drugs, the periods of time required for the development of three stages in the hypnotic state were selected: (1) the time from the injection until the animal could not propel itself after stimulation by gently pinching its tail. This point was considered as the onset of sleep. (2) The time from the injection until the animal could be aroused sufficiently to propel itself forward on stimulation. This stage was considered the end of sleep. (3) The time from the injection until the animal could walk a distance of about a meter with a steady gait (without ataxia). This was con-

* The delvinal sodium was kindly furnished by Sharp and Dohme, Inc., Philadelphia, Pa.
† From the Department of Biochemistry, Medical College of Alabama, Birmingham, Alabama.
sidered as the end of hypnosis. Each animal was observed by the above scheme after being injected.

In table 1, the results are listed following the administration of 40 mg. of pentobarbital sodium per kilogram of rat, after the rats had received either repeated doses of 60 mg. of delvinal sodium per kilogram or a single dose of this drug. Eighteen control rats were given 40 mg. of pentobarbital sodium per kilogram and their average sleeping time was 111 minutes, while their average hypnotic time was 198 minutes.

**TABLE 1**

**Adult Rats Made Tolerant to Delvinal Sodium Show Cross Tolerance to 40 mg. of Pentobarbital Sodium Per Kilogram**

<table>
<thead>
<tr>
<th>No. of Rats</th>
<th>Conditioning Treatment with D. S.t per kilogram</th>
<th>Time After Treatment Before P. S.,tt days</th>
<th>Onset of Sleep, minutes</th>
<th>End of Sleep, minutes</th>
<th>Maximum Hypnotic Time, minutes</th>
</tr>
</thead>
<tbody>
<tr>
<td>18</td>
<td>Controls (40 mg. P. S.)</td>
<td>—</td>
<td>3.3</td>
<td>111</td>
<td>198</td>
</tr>
<tr>
<td>12</td>
<td>60 mg.*</td>
<td>3</td>
<td>4.4</td>
<td>108</td>
<td>167</td>
</tr>
<tr>
<td>6</td>
<td>60 mg.**</td>
<td>3</td>
<td>4</td>
<td>67</td>
<td>103</td>
</tr>
<tr>
<td>8</td>
<td>130-150 mg.*</td>
<td>3</td>
<td>3.25</td>
<td>77</td>
<td>99</td>
</tr>
<tr>
<td>13</td>
<td>110-145 mg.*</td>
<td>7</td>
<td>4</td>
<td>86</td>
<td>117</td>
</tr>
<tr>
<td>7</td>
<td>125-145 mg.*</td>
<td>21</td>
<td>4</td>
<td>34</td>
<td>89</td>
</tr>
<tr>
<td>7</td>
<td>110-120 mg.*</td>
<td>30</td>
<td>5.2</td>
<td>51</td>
<td>90</td>
</tr>
</tbody>
</table>


In column 2, may be noted the conditioning treatment with delvinal sodium which consisted of a single dose of 60 mg. per kilogram of rat, 6 semiweekly doses of 60 mg. per kilogram or a large single dose of the drug. After a single dose of 60 mg. of delvinal sodium, only a slight tolerance for pentobarbital sodium developed but after 6 semiweekly doses of 60 mg. of delvinal sodium there was marked tolerance for pentobarbital sodium. The sleeping time in the 6 rats that received 6 semiweekly doses of 60 mg. of delvinal sodium was reduced from 58 minutes to 41 minutes, while the maximum hypnotic time dropped from 122 to 81 minutes, which also shows that a tolerance had been developed to delvinal sodium. The single large dose of delvinal sodium varied from 110 to 150 mg. per kilogram of rat and some tolerance to pentobarbital sodium was exhibited thirty days after the injection of the delvinal sodium.

In table 2 the results are listed which were obtained on rats that were made tolerant to pentobarbital sodium and then exhibited cross tolerance to delvinal sodium. The conditioning treatment consisted
TABLE 2

ADULT RATS MADE TOLERANT TO PENTOBARBITAL SODIUM SHOW CROSS TOLERANCE TO 60 MG. OF DELVINAL SODIUM PER KILOGRAM

<table>
<thead>
<tr>
<th>No. of Rates</th>
<th>Conditioning Treatment with P. S.† per kilogram</th>
<th>Time After Treatment Before D. S.†† days</th>
<th>Onset of Sleep, minutes</th>
<th>End of Sleep, minutes</th>
<th>Maximum Hypnotic Time, minutes</th>
</tr>
</thead>
<tbody>
<tr>
<td>20</td>
<td>Controls (60 mg. D. S.)</td>
<td>-</td>
<td>11</td>
<td>71</td>
<td>138</td>
</tr>
<tr>
<td>3</td>
<td>40 mg.*</td>
<td>3</td>
<td>14</td>
<td>50</td>
<td>102</td>
</tr>
<tr>
<td>7</td>
<td>40 mg.**</td>
<td>3</td>
<td>19</td>
<td>36</td>
<td>82</td>
</tr>
</tbody>
</table>

† P. S. = Pentobarbital sodium.  
* Three semiweekly injections of pentobarbital sodium.  
†† D. S. = Delvinal sodium.  
** Six semiweekly injections of pentobarbital sodium.

of two series of injections of 40 mg. of pentobarbital sodium per kilogram. The first series received 3 semiweekly doses. There was a considerable reduction in both the sleeping time and the hypnotic time of the first series following the administrations of delvinal sodium, but both effects were much more marked in the second series.

That a definite tolerance developed to pentobarbital sodium in the rats in the two series in table 2, is shown in table 3. Three semiweekly

TABLE 3

DEVELOPMENT OF TOLERANCE IN ADULT RATS FOLLOWING REPEATED DOSES OF 40 MG. OF PENTOBARBITAL SODIUM

<table>
<thead>
<tr>
<th>No. of Rates</th>
<th>Conditioning Treatment</th>
<th>Onset of Sleep, minutes</th>
<th>End of Sleep, minutes</th>
<th>Maximum Hypnotic Time, minutes</th>
</tr>
</thead>
<tbody>
<tr>
<td>18</td>
<td>Controls (40 mg. P. S.)</td>
<td>3.3</td>
<td>111</td>
<td>198</td>
</tr>
<tr>
<td>3</td>
<td>3 Semiweekly doses</td>
<td>5.8</td>
<td>88</td>
<td>136</td>
</tr>
<tr>
<td>7</td>
<td>6 Semiweekly doses</td>
<td>5.3</td>
<td>54</td>
<td>104</td>
</tr>
</tbody>
</table>

doses of pentobarbital sodium conferred a slight degree of tolerance, but 6 semiweekly doses of pentobarbital sodium reduced both the sleeping time and the maximum hypnotic time to about 50 per cent of what they were following the first dose of this drug.

DISCUSSION

The rats in the last 4 horizontal columns of table 1 were animals that survived a single dose of 110 to 150 mg. of delvinal sodium per kilogram
during experiments to determine the LD₉₅ for this drug (9). These large single doses confer some tolerance to pentobarbital sodium for several weeks as measured by the reduction in sleeping time and maximum hypnotic time.

Green and Koppanyi (6) observed that evipal-tolerant dogs were tolerant to pentothal sodium and nostal and that pentothal-tolerant dogs were tolerant to evipal. Since the cross tolerance was reversible, Green and Koppanyi really demonstrated mutual cross tolerance with these drugs in dogs and have confirmed my observations of mutual cross tolerance between barbiturates in guinea pigs (4). Gruber and Keys (7) also confirmed my findings of mutual cross tolerance since they demonstrated that butisol-tolerant rabbits showed cross tolerance to pentobarbital sodium and vice versa, that the same type of results were observed in the rabbit between amytal sodium and pentobarbital sodium, and that similar results were noted in the rat between butisol sodium and pentobarbital sodium.

SUMMARY AND CONCLUSIONS

After single large doses or repeated doses of delvinal sodium a tolerance is developed in adult rats to pentobarbital sodium.

After repeated doses of pentobarbital sodium in adult rats a tolerance developed to delvinal sodium.

Mutual cross tolerance has been established in the rat for pentobarbital sodium and delvinal sodium.

After 6 semiweekly doses of 60 mg. of delvinal sodium per kilogram of rat a tolerance developed to delvinal sodium. The sleeping time was reduced about 30 per cent and the hypnotic time was reduced about 35 per cent.

After repeated doses of 40 mg. of pentobarbital sodium per kilogram of adult rats a tolerance developed to pentobarbital sodium. If as many as 6 semiweekly doses of pentobarbital sodium were given, both the sleeping time and the hypnotic time were reduced almost 50 per cent.

REFERENCES

5. Carmichael, E. B.: Mutual Cross Tolerance between Pentobarbital Sodium (nembutal) and Delvinal Sodium [5-ethyl-5-(1-methyl-1-butenyl) Barbituric Acid] in Guinea Pigs, Am. J. Physiol. 133: 236 (June) 1941.
6. Carmichael, E. B.: Toxicity of Pentothal Sodium [Sodium Ethyl (1-Methyl-Butyl) Thio-
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Tolerance to Barbiturates in Experimental Animals, J. Pharmacol. & Exper. Therap.
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9. Carmichael, E. B.: The Toxicity of Delval Sodium for Both Young and Old Rats, Federa-

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FRIDAY, OCTOBER 8, 1948

9:00 A.M. to 12:00 Noon. Registration (North Garden).
9:30 A.M. to 10:45 A.M. Panel Discussion (North Garden).
Obstetrical Anesthesia. Moderator, R. J. Whitacre, E. Cleveland.
Participants: N. F. Paxson, Philadelphia; R. A. Hingson, Balti-
more; B. B. Hershenson, Boston, and W. R. Penman, Philadelphia.
10:45 A.M. to 11:00 A.M. Recess.
11:00 A.M. to 12:15 P.M. Panel Discussion.
Controlled Respiration. Moderator, P. D. Woodbridge, Reading.
Participants: S. J. Martin, Hartford; D. D. Grove, Philadelphia;
E. M. Fapper, New York; R. L. Patterson, Pittsburgh.
2:00 P.M. to 4:00 P.M. Formal Papers (North Garden). Chairman,
G. J. Thomas, Pittsburgh.
J. E. Eckenhoff, Philadelphia.
3. A New Method for Recording Arterial Blood Pressure. R. D.
Dripps, Philadelphia.
4:00 P.M. to 5:15 P.M. Combined Meeting with Anesthesia Study
Commission of the Philadelphia County Medical Society. Chair-
man, H. S. Ruth.
5:15 P.M. to 7:00 P.M. Cocktails (Oak Room).
7:00 P.M. Dinner in the Pennsylvania Manner (Rose Garden).

SATURDAY, OCTOBER 9, 1948

9:30 A.M. to 10:45 A.M. Panel Discussion (North Garden).
Participants: C. H. Robeson, Toronto; C. E. Koop, Philadelphia; M.
Van N. Deming, Philadelphia; R. M. Smith, Boston.
10:45 A.M. to 11:00 A.M. Recess.
11:00 A.M. to 12:15 P.M. Panel Discussion.
Teaching Methods. Moderator, Scott M. Smith, Salt Lake City.
Participants: (1) Undergraduate, H. S. Ruth, Philadelphia; (2)
Postgraduate, S. J. Martin, Hartford; (3) Clinical Research, R.
Charles Adams, Rochester; (4) Laboratory Research, P. R. Dunke,
Philadelphia.