FAILURE OF THALIDOMIDE METABOLITES TO PRODUCE MALFORMATIONS IN THE RABBIT EMBRYO

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Summary. Eight metabolites of thalidomide (as listed in Table 1) were administered to pregnant rabbits from the 6th to the 14th day of pregnancy at a dose level of 100 mg/kg/day. Unlike thalidomide in the same dosage, which is known to produce a teratogenic effect in the rabbit embryo, the metabolites did not cause any malformation in our experiments.

In order to shed light on the mechanism of the teratogenic action of thalidomide, it is useful to know whether its products of hydrolysis as they occur in the organism during the metabolization of the drug (Faigle, Keberle, Meyer-Brunot, Riess & Schmid, 1965) may also cause malformations in the rabbit embryo. These products are listed in Table 1.

pig was already found by Hay (1964) to have no teratogenic effect in the rabbit embryo when administered in daily doses of 25 to 50 mg/kg from the 1st day to the 5th day of the pre-implantation period. The experiments of Fabro, Schumacher, Smith & Williams (1964) and Fabro, Schumacher, Smith, Stagg & Williams (1964) had established that gal at a dose level of 150 mg/kg/day, given from the 7th to the 12th day of pregnancy, as well as the thalidomide analogue N-phthalyl-D,L-glutamic acid anhydride, had no teratogenic properties when administered to rabbits.

The recently published results of Fratta & Sigg (1965) show that pg and pga do not exert a teratogenic effect when administered from the 4th to the 12th day of pregnancy (150 mg/kg). In our experiments the duration of medication was fixed to comprise the pre-implantation period and embryogenesis, i.e. the 6th to 14th day. On the 28th day of pregnancy does were submitted to autopsy and foetuses were removed from the uterus for gross examination and prepared for skeletal staining with alizarine Red S.

The metabolites (in carbonic–buffer solutions) were injected subcutaneously. This route of injection was chosen to ensure that the products of hydrolysis remained as long as possible in the organism, because they are rapidly excreted following intravenous administration (Keberle, Loustalot, Maller, Faigle & Schmid, 1965). A control group received an 0-9% NaCl solution only.
As shown in Table 1, none of the foetuses in any of the groups receiving the metabolites revealed any malformation. On the other hand, it was demonstrated in an earlier investigation that thalidomide, when administered in doses of 100 mg/kg/day, gave rise to gross malformations in 29% of live foetuses (Loustalot, 1964). Similar findings with thalidomide have been reported by Somers (1962), Staples & Holtkamp (1963), Hay (1964), Loosli (1964), Giroud, Tuchmann-Duplessis & Mercier-Parot (1962a, b) and Ingalls, Curley & Zappasodi (1964).

The resorption rate following injection of the metabolites PIG, \(o\)-GCA, \(o\)-CG and \(o\)-CIG was higher than in the control group, but the difference was not significant.

Our experiments showed that the products of hydrolysis of thalidomide have no teratogenic effect, but the question now arises as to whether and in what manner these substances are able to infiltrate the blastocyst or the early embryo.

The experiments of Keberle, Faigle, Fritz, Knüsel, Loustalot & Schmid (1966) with \(^{14}\text{C}\)-labelled material show that the hydrophilic metabolites \(o\)-GCA and PGA display an infiltrative activity only during the implantation period. The preponderantly lipophilic thalidomide, however, penetrates the embryo in considerable amounts at all stages of development. Inside the blastocyst or embryo thalidomide is broken down into its metabolites which, owing to the hydrophilic nature, are prevented from leaving the embryo again. Following the administration of thalidomide the metabolites accumulate within the embryo over a long period, attaining concentrations far higher than those which can be attained by direct administration of the metabolites them-

### Table 1

<table>
<thead>
<tr>
<th>Preparation</th>
<th>No. of animals treated</th>
<th>Implantations</th>
<th>Foetuses</th>
<th>Dead or resorbed</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Total %</td>
<td>Live</td>
<td>Weight (g) Total %</td>
</tr>
<tr>
<td>Controls</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(NaCl, 0.9%)</td>
<td>11</td>
<td>10 91</td>
<td>98 97</td>
<td>36.3 3</td>
</tr>
<tr>
<td>PGA</td>
<td>9</td>
<td>6 75</td>
<td>51 96</td>
<td>39.2 2</td>
</tr>
<tr>
<td>PG</td>
<td>8</td>
<td>7 88</td>
<td>57 93</td>
<td>39.0 4</td>
</tr>
<tr>
<td>PIG</td>
<td>15</td>
<td>6 40</td>
<td>44 86</td>
<td>38.2 7</td>
</tr>
<tr>
<td>(o)-GCA</td>
<td>5</td>
<td>5 100</td>
<td>34 97</td>
<td>39.5 1</td>
</tr>
<tr>
<td>(o)-CGA</td>
<td>9</td>
<td>6 75</td>
<td>52 92</td>
<td>35.3 4</td>
</tr>
<tr>
<td>(o)-CG</td>
<td>6</td>
<td>6 100</td>
<td>48 91</td>
<td>34.5 5</td>
</tr>
<tr>
<td>(o)-CIG</td>
<td>6</td>
<td>6 100</td>
<td>54 89</td>
<td>34.5 7</td>
</tr>
<tr>
<td>GAI</td>
<td>7</td>
<td>6 86</td>
<td>54 96</td>
<td>38.0 2</td>
</tr>
</tbody>
</table>

PGA = \(N\)-phthalyl-\(D\),\(L\)-glutamic acid.  
PG = \(N\)-phthalyl-\(D\),\(L\)-glutamine.  
PAG = \(N\)-phthalyl-\(D\),\(L\)-isoglutamine.  
\(o\)-GCA = \(N\)-(\(o\)-carboxybenzoyl)-\(D\),\(L\)-glutamic acid.  
\(o\)-CGA = \(N\)-(\(o\)-carboxybenzoyl)-\(D\),\(L\)-glutamic acid acid imide.  
GAI = Glutamic acid imide \(-\text{HCl}\).
Thalidomide metabolites and rabbit embryo

selves. Hence, the possibility that the metabolites may exert a teratogenic effect following the administration of thalidomide itself cannot be excluded.

REFERENCES


