

# Immunosuppressive Activity of Cyclic Imides in Laboratory Animals

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Hellmann, Duke and Tucker (2) from the Imperial Cancer Institute of London reported in September 1965 that the cyclic imide thalidomide prolongs skin homografts survival in white mice. In the same year Sheskin (4), from the Leprosary of Hadassah University in Jerusalem, demonstrated that thalidomide favorably influences the reactions of leprosy patients. Sheskin's findings were confirmed in the following years by numerous leprologists all over the world. Since then, a series of cyclic imides have been synthesized in the Grünenthal laboratories and tested with the following aims:

1. New compounds should at least have the same effects on lepra reactions as thalidomide.
2. They must be well tolerated, and
3. They should be free from teratogenicity in animal experiments (rabbits, monkeys).

## CHEMISTRY

Fig. 1 shows the different chemical variations of cyclic imides:

1. Variation of the phthalimido ring by replacing the phthalimido group ( $X = \text{CO}$ ) by *o*-sulfobenzoyl acid-imido ( $X = \text{SO}_2$ ) or phthalimidino group ( $X = \text{CH}_2$ ).
2. Introduction of the amino group in the  $\alpha$ - or  $\beta$ -position of the glutarimide ring.
3. Replacement of the hydrogen in the nitrogen atom of the glutarimide ring by Mannich-bases.

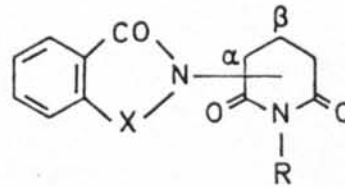
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## IMMUNOLOGIC INVESTIGATIONS

Assuming that lepra reaction is an immunologic event (3, 5) and that the effect of thalidomide in lepra reaction is an immunosuppressive one, the new cyclic imides were tested in two experimental models, having proved true for establishing immunosuppressive properties:

1. Homologous skin grafts in rats.
  2. Allergic encephalitis in guinea pigs.
- No details will be given about the trials concerned for time reasons.

Fig. 2 shows skin homograft survival in Sprague-Dawley rats treated with the cyclic imides CG 601 (Fig. 3) and CG 603 (Fig. 4). Differences from controls are significant ( $P < 0.02$ ) for CG 601 from the 10th to the 12th day and for CG 603 from the 9th to the 16th day. Fig. 5 shows the extent of encephalitic lesions in guinea pigs, and Fig. 6 their mortality rate under treatment with different cyclic imides in comparison with untreated controls. Differences between treated groups and controls are clearly significant ( $P < 0.05$ ). These



R : -H

R :  $-\text{CH}_2-\text{N}$  (Mannich - Base)

X :  $\text{CO}$ ;  $\text{SO}_2$ ;  $\text{CH}_2$

FIG. 1. Chemical variations of cyclic imides.

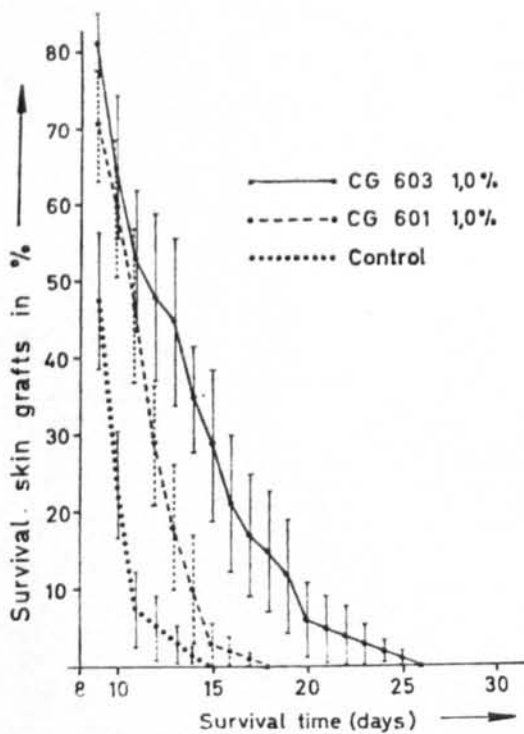
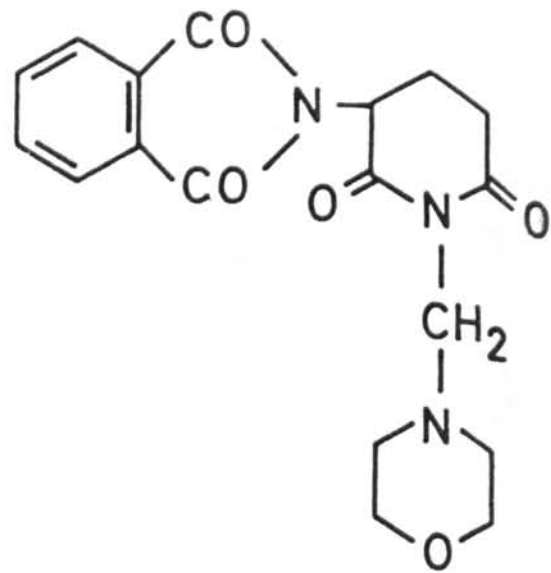
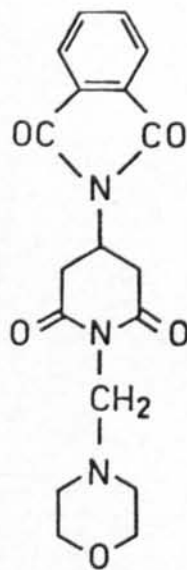


FIG. 2. Skin homograft survival in Sprague-Dawley rats treated with cyclic imides.



CG 601

FIG. 3. 1-(Morpholinomethyl)-3-phthalimido-piperidindione-2,6.



CG 603

FIG. 4. 1-(Morpholinomethyl)-4-phthalimido-piperidindione-2,6.

findings reveal that the tested cyclic imides possess significant immunosuppressive effects in both experimental models.

Some time ago, Coulson et al. (<sup>1</sup>), using the mixed lymphocyte culture technique, have described an immunosuppressive effect of the cyclic imides CG 601 and CG 603.

In pharmacological experiments, some of the cyclic imides, viz., those with  $\alpha$ -configuration, in different animals have a sedative effect when given orally in doses beginning with 50 mgm./kgm. or 100 mgm./kgm., respectively. Apart from this, the Mannich compounds E 298 and EM 9 lower blood pressure experimentally elevated in rats.

Toxicologically, the new cyclic imides show a relatively low acute toxicity. Oral  $LD_{50}$  of most cyclic imides is more than 2,000 mgm./kgm. in mice, rats, guinea pigs, rabbits, and dogs. In chronic toxicity tests rats and dogs tolerated 300 mgm./kgm. without pathologic changes.

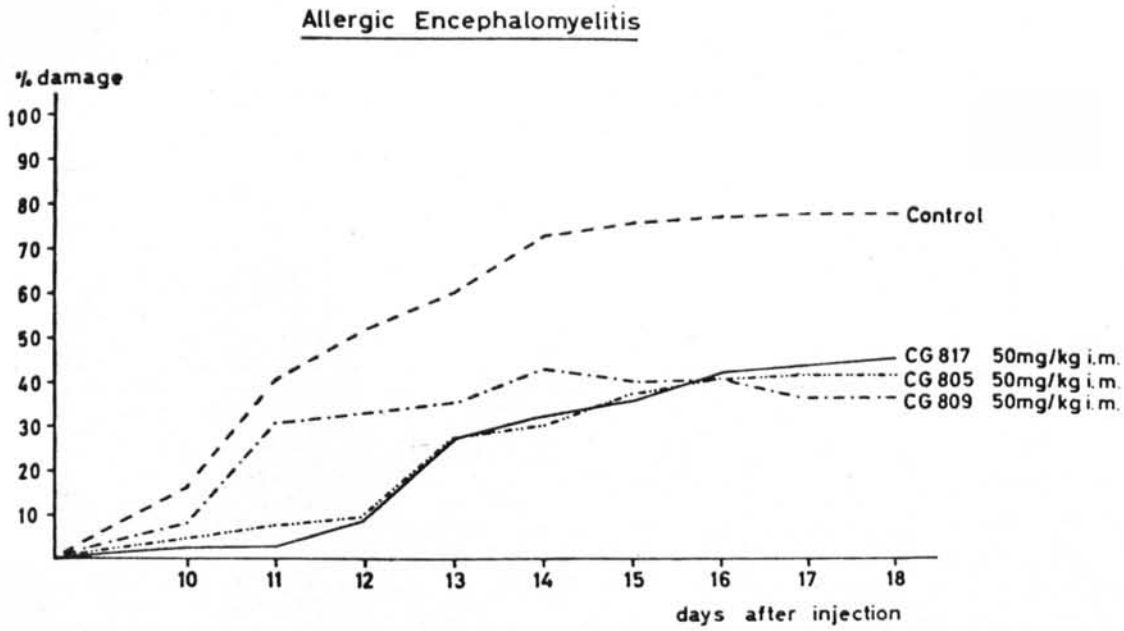


FIG. 5. Effect of cyclic imides on the clinical picture of allergic encephalomyelitis in guinea pigs.

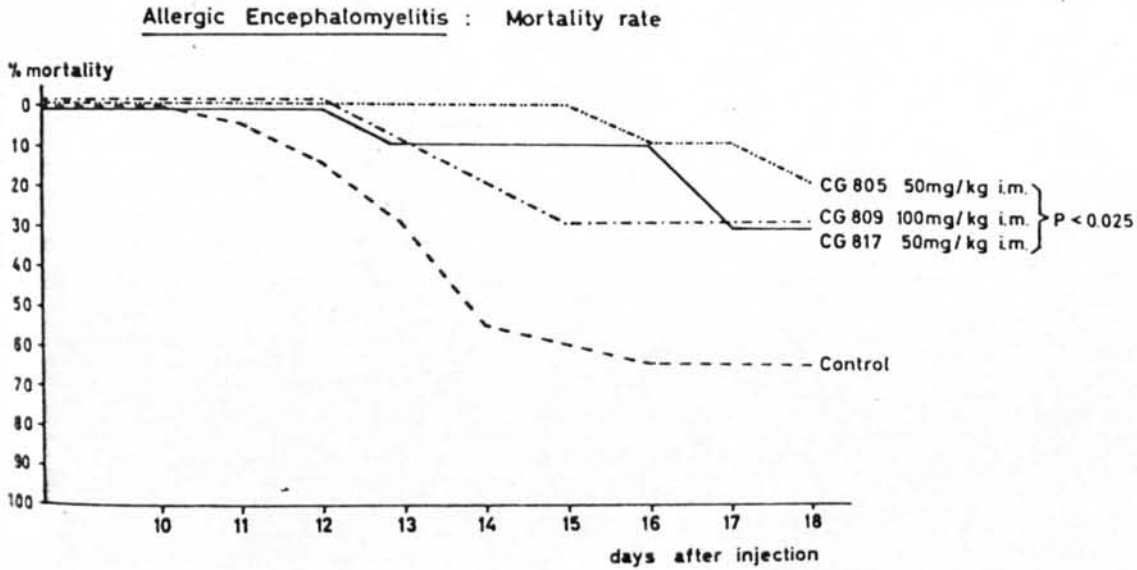


FIG. 6. Effect of cyclic imides on the mortality of guinea pigs with allergic encephalomyelitis.

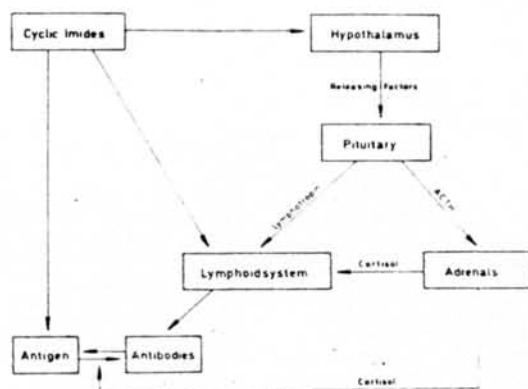
Of special interest is the **teratologic** behavior of the substances. Up to now no teratogenic effects have been observed in rats. In white New Zealand rabbits the cyclic imides with  $\beta$ -configuration, as well as the *o*-sulfobenzoic acid-imido compounds, showed no teratogenicity in preliminary investigations, whereas the compounds having phthalimido- or phthalimidino radicals in the  $\alpha$ -position were teratogenic. In monkeys only limited trials have been possible hitherto. It seems, however, that the compounds, which did not prove teratogenic in rabbits, likewise do not have teratogenic effects in monkeys in doses up to 100 mgm./kgm. We know that these find-

tors in the sense of influencing corresponding functions of the pituitary gland, which in turn cause immunosuppressive effects on the reacting organ.

For informative clinical trials the most important compounds have been selected from the various chemical variations. Dr. Jäger will report the results of these investigations.

### SUMMARY

Stimulated by the successful results achieved by the employment of thalidomide in lepra reactions in man, we synthesized in the Grüenthal research laboratories a number of cyclic imides in recent years. These substances were developed with the objective of influencing favorably the above mentioned reactions, avoiding the teratogenic risks if possible. The new substances showed immunosuppressive effects in skin transplantation tests of the rat and in allergic encephalitis of the guinea pig. In both acute and chronic tests they were of low toxicity. Some of the cyclic imides of certain chemical constitution showed no teratogenic effects in rats, rabbits, and monkeys in preliminary teratogenic investigations. Speculations about the mode of action are being discussed.



Cyclic imides and immunosuppression

FIG. 7. Mechanism of action of cyclic imides.

ings in rats, rabbits, and monkeys do not allow binding conclusions concerning man, but we think them to be of importance for our attempts to develop a cyclic imide without teratologic risk for the treatment of human lepra reactions.

Concerning the mechanism of action of cyclic imides, we can only give speculations. Fig. 7 indicates that cyclic imides possibly mask the antigen, or influence the lymphoid system directly or indirectly, or modify some hypothalamic releasing fac-

### REFERENCES

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