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The Experimental Properties of G 30 320 (B 663)*— a New Anti-leprotic Agent†

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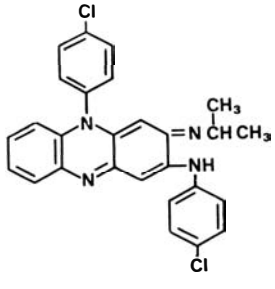
Early experimental studies, mainly on laboratory animals, of the properties of a new anti-leprotic agent, G 30 320 (B 663, Lampren), are described, with details of its absorption and metabolism. The drug is particularly active against a number of mycobacteria, including notably *Mycobacterium leprae*.

G. 30 320 was synthesized in 1954 by Barry and collaborators (Barry *et al.*, 1957; Vischer *et al.*, 1958). Chemically it is a phenazine derivative with the following structure:



G 30 320 is a deep red, crystalline powder and very stable under normal storage conditions. It is insoluble in water but soluble in organic solvents such as dimethylformamide, dioxane, dimethyl sulfoxide, and ethanol.

ANTIMICROBIAL PROPERTIES

Tests have shown G 30 320 to be especially active against mycobacteria. The *in vitro* activity has been determined by incorporation methods, using solid culture media. Under these conditions mycobacteria were inhibited by drug concentrations of between 0.03 and 10 μg per ml., depending on the species tested. The most sensitive species proved to be *Mycobacterium tuberculosis*, *Myco. bovis*, *Myco. ulcerans*, *Myco. balnei* and *Myco. johnei*. On the whole, the atypical mycobacteria strains of the Runyon Groups I, II and III were more resistant. Inhibition of the fast-growing mycobacteria strains required the highest concentration (Table 1). No cross-resistance was observed with other tuberculostatic agents or with diaminodiphenyl sulphone (DDS).

TABLE 1

In vitro activity of G 30 320 against mycobacteria in synthetic media containing serum or albumin

Organism	*Minimum inhibitory concentration in μg/ml*
Myco. tuberculosis and *Myco. bovis* (sensitive and resistant to other tuberculostatic agents)	0.05 to 0.5
Atypical mycobacteria of Runyon Groups I, II, and III	0.5 to 1
Atypical mycobacteria of Runyon Group IV (fast-growing)	1.0 to 10
Myco. ulcerans	0.03 to 0.1
Myco. johnei	1.0

In tests on animals, G 30 320 was found to inhibit the growth of mycobacteria *in vivo*, as well as *in vitro*. The majority of the tests, including the most important ones, were performed on mice which had been injected with *Myco. tuberculosis* or *Myco. bovis*. Here G 30 320 was immediately conspicuous for an outstanding effectiveness which, in certain circum-

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TABLE 2

***In vitro* activity of G 30 320 against micro-organisms other than mycobacteria**

Organism	*Minimum inhibitory concentration in μg/ml*
Staphylococcus pyogenes aureus	3
Staphylococcus epidermidis	0.01
Bacillus anthracis	3
Corynebacterium kutscheri	0.3
Corynebacterium acnes	0.1
Nocardia asteroides	1
Dermatophilus congolensis	0.1
Escherichia, Salmonella, Klebsiella	>100
Brucella except *Brucella suis* Thomsen	>100
Pasteurella tularensis	>100

stances, surpassed even that of isoniazid. At dosages as low as 1 mg per kg the survival time of mice was considerably lengthened, while the effectiveness of the drug could be further greatly improved by increasing the dose. In experiments of this kind 5 mg per kg body weight has proved to be a very effective standard dose. Unlike isoniazid, G 30 320 is effective not only when administered after infection but also when given prophylactically prior to infection. This indicates that it remains in the body for a long time.

In experiments with tuberculous infection in other animal species the effectiveness of G 30 320 varied. The impression gained was that the larger the experimental animal, the weaker was the reaction of its tuberculosis to G 30 320. Thus in rats and hamsters G 30 320 proved nearly as effective as in mice, whereas in guinea-pigs, rabbits, and monkeys very much higher doses were required. These differences can probably be ascribed to differences in the tuberculous lesions in the various species. The good results obtained with G 30 320 in animal experiments prompted us to investigate its effect in chronic pulmonary tuberculosis in man. The results were very disappointing, however, and these studies were not pursued.

In addition to its tuberculostatic effect in mice, G 30 320 is also very effective in experimental infections of mice with *Myco. ulcerans* (Lunn and Rees, 1964), *Myco. lepraemurium* (Chang, 1962), *Myco. leprae* (Shepard and Chang, 1964), and *Myco. johnei* (Gilmour, 1966). In tests on mice infected with *Myco. tuberculosis* (Vischer and Roulet, 1963) and *Myco. lepraemurium* (Chang, 1966) G 30 320 was found to be synergistic with isoniazid. These favourable results led to the testing of G 30 320 in persons suffering from leprosy and infections due to *Myco. ulcerans*. In both groups it was found that G 30 320 has a therapeutic effect (Browne and Hogerzeil, 1962; Lunn and Rees, 1964).

Bacteria in general are very much more resistant to G 30 320 than the mycobacteria; exceptions are the staphylococci, micrococci, certain corynebacteria, and organisms of the families Actinomycetaceae and Streptomycetaceae (Table 2), though in experimental infections with these organisms no therapeutic effect comparable to that found in the mycobacterial infections was observed.

TABLE 3

Anti-inflammatory action of G 30 320 in animal experiments

Test object	*Species*	*Dosage (mg per kg)*	*Reduction of inflammatory reaction compared with controls*	P *significance* (2α)
Cotton pellet granuloma	Rats	10×200	30%±10	0.00274
Cotton pellet granuloma	Rats	10×60	11%±23	0.3
Bradikinin oedema	Rats	1×200	32%±10	0.00050
Tuberculin reaction	Guinea-pigs	2×100*	46%±14.6*	0.00050
Tuberculin reaction	Guinea-pigs	2×50*	29%±10.9*	0.00465

*In each case a single dose of G 30 320 was given 30 min. before and 7½ hr after the tuberculin injection. Percentages are here based on reduction of skin thickness as compared with that of controls after 24 hr.

PHARMACOLOGICAL ACTION

G 30 320 was subjected to a variety of pharmacological tests. These revealed no action of any kind on the blood pressure or on the central nervous system, nor were any analgesic or spasmolytic effects observed. The results of clinical trials available to date reveal that in many cases G 30 320 has a favourable influence on the lepra reaction in man (Browne, 1965). Accordingly the drug was tested on a series of experimental models for its anti-inflammatory action. A significant anti-inflammatory effect was observed in cases of bradykinin oedema, cotton pellet granuloma, and the tuberculin reaction, though the doses required were above the therapeutic range (Table 3). These results may throw some light on the mechanisms underlying the favourable action of G 30 320 in the patients suffering from lepra reactions.

ABSORPTION AND METABOLISM

Methods of determining the amounts of G 30 320 in organs and body fluids have been developed. These are based on extraction and concentration of the substance and its direct colorimetric determination. Since G 30 320 is insoluble in water and the other usual non-toxic solvents, it has to be given in the form of a suspension. Following subcutaneous, intramuscular, or intraperitoneal injection it was found that the substance was absorbed extremely slowly, and that even after many months a large proportion of the dose was still present at the site of injection. In practice, therefore, G 30 320 can be given only by the oral route.

Absorption studies carried out on various laboratory animals such as mice, rats, guinea-pigs, rabbits, dogs and monkeys revealed that absorption of the compound varied widely from species to species, being good in mice, rats, and monkeys, decidedly poorer in rabbits and guinea-pigs, while in dogs it was practically nil. Tests on female mice, rats, guinea-pigs, and rabbits showed that G 30 320 is transmitted to a slight extent to the foetus via the placenta but to a considerable extent to the offspring after birth, via the mother's milk.

The mechanism of absorption remains obscure. So far our results indicate that the substance enters the bloodstream slowly and from there passes rapidly into the organs, where it is taken up especially by the cells of the reticulo-endothelial system. Initially, G 30 320 accumulates in the adipose tissue, and then in the intestinal lymph nodes, spleen and liver. High concentrations are found in these tissues after a long period of treatment. Concentrations of 1 g % and more are reached in the spleen and liver of rats after several months' administration at a dosage of 50 mg per kg per day. The concentration reached in the organs is very largely dependent on dosage. Long-term experiments in mice and rats showed that the content of G 30 320 in the organs rises progressively with daily doses of 25 mg per kg and higher. At dosages of 5 and 10 mg per kg, however, this accumulation is much less evident. After doses of 10 mg per kg and higher, the substance may be deposited in the organs in the form of crystals; these disappear, however, after discontinuation of the drug. Excretion is extremely slow, only a few mg per day being excreted by the kidney, while in addition a small amount is eliminated in the sebum and sweat. After cessation of treatment the concentration in the organs falls very slowly; for instance in rats 3 months after the last dose of G 30 320 the concentration in the organs had decreased by only about 50%.

In human subjects it is difficult to determine the degree of absorption of G 30 320, since the serum concentration is not an accurate measure of the amount absorbed. Balance studies showed, however, that when given in the form of coarse crystals G 30 320 is absorbed only to the extent of about 20%, whereas when administered in a micronized form absorption is about 50%. However, if G 30 320 is taken orally in the form of a suspension in oil about 85% is absorbed. For clinical purposes the drug is suspended in an oil-wax base and administered in a capsule; in this form an absorption rate of about 70% could be achieved. Chromatographic tests support the view that G 30 320 is stored in the body and excreted in the urine almost exclusively as the unchanged substance.

TOXICOLOGY

The acute toxicity of G 30 320 was determined in mice, rats, and rabbits, while tests of chronic toxicity were carried out over an administration period of 6 months on rats and monkeys. Tests of toxicology in regard to reproduction were carried out on mice, rats and rabbits. On the basis of these animal experiments it can so far be said that G 30 320 is fairly well tolerated.

SUMMARY

A brief description is given of the antimicrobial' pharmacological, and toxicological properties of G 30 320 and its absorption and behaviour in the organism are discussed. The substance is characterized by very high activity both *in vitro* and *in vivo* against various mycobacteria, its activity against *Myco. leprae* and *Myco. ulcerans* being of real practical importance.

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