EXPERIMENTAL STUDIES

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Our experimental studies had as their objective the obtaining of pharmacological data and the verification of the chemotherapeutic activity (in relation to murine leprosy) of compounds and derivatives presumably active.

As was stated in the chemical part of these notes, various compounds were synthesised for use in the chemotherapeutic treatment of human leprosy on the basis of data obtained from the literature. Substances were synthesised only in those cases where theoretically some chemotherapeutic activity was to be expected. The immediate application to human leprosy of data collected from the literature presents certain practical difficulties, principally in that which concerns drugs active " in vitro " against various organisms and " in vivo " against other diseases than leprosy.

The multiplicity of substances synthesised, difficulties in direct application in men and difficulties in the preparation of large quantities are obstacles to the verification of chemotherapeutic activity. These can be overcome by laboratory controls. As a basis for the use of sulphones in human leprosy we used data obtained by camparison of the " in vitro " and " in vivo " chemotherapeutic action shown against the bacillus of avian tuberculosis (RIST, N., BLOCK, F. & HAMON, V.—Ann. Inst. Pasteur 64:203, 1940), against experimental tuberculosis in the guinea pig (FEIDMAN, V. H., HINSHAW, H. C. & MOSES, H. E.—Proc. Staff Meet. Mayo Clin. 16:187, 1941), against murine leprosy (COWDRY, E. V. & RUANGSIRI, C.—Arch. Path. 32:632, 1941), etc.

Experimental studies for the verification of the chemotherapeutic properties of a substance cannot be carried out in the laboratory with mycobacterium leprae because of the lack of standard cultures and susceptible animals. The use of murine leprosy might perhaps overcome this obstacle (HADLER, W. A. & MAURI, A. C.—Rev. Bras. Leprol 16:191, 1949), it being borne in mind that M.leprae muris is the organism presenting the greatest similarity to Hansen's bacillus. The similarities are morphological (SUHRLAND, L. G., BISHOP, F. & CARPENTER, C. M.—Int. J. Leprosy 16:361, 1948), biological, pathogenic, clinical, topographic, etv. Moreover, it is our experience that murine leprosy is sensitive to a few sulphone chemotherapeutic agents, inhibition of the evolution of lesions and changes in the morphological characters of the bacillus being observed after treatment has been in progress for some time.

Despite the similarities mentioned, Hansen's bacillus and Stefansky's are different and the differences may perhaps be due to biological reasons since the inoculation of Hansen's bacillus in the rat causes lesions which in practice may be superimposed upon those caused in the rat by Stefansky's bacillus, the only difference being in the evolution of the lesions. Stefansky's bacillus provokes in the rat, lesions which evolve progressively while Hansen's bacillus produces lesions that regress spontaneously. However, these facts, while on the one hand they do not permit reports related to tests with murine leprosy to be directly transposed to human leprosy, on the other hand are not strong enough to deny the possibility of transposition provided the necessary reservations be made. In principle the fact that the two diseases are different does not preclude that they might by chance behave similarly vis-a-vis a given drug, for the very reason that murine leprosy is the disease having the greater number of affinities with human leprosy.

In our laboratories it was decided to effect the verification of the chemotherapeutic activity by the use of two types of control—(1) " in vitro " by the action of the substance upon the vitality of *M.leprae muris* and other mycobacteria when in direct contact with the substances, the effect of such action being determined by inoculation of the bacilli which have been in contact with the drugs into young and healthy animals. (2) " in vivo " by the effect of the compounds upon lesions in rats experimentally inoculated with Stefansky's bacillus according to a previously described method (HADLER, W. A. & MAURI, A. C.—Rev. Brasil. Leprol. 16:139, 1948).

The " in vivo " chemotherapeutic action is observed from two standpoints: (a) action upon the evolution of lesions in rats recently inoculated; (b) action upon late and advanced lesions in murine leprosy.

The verification of the chemotherapeutic activity upon recent lesions permits the observer to see the effect at a period of active evolution of the disease and in an organism whose defences are practically entire; furthermore, the lesions are incipient and well vascularised. In these circumstances, possibly, the action of the substances may be more clearly envisaged, because the conditions are favourable for observation of the chemotherapeutic effects.

At the same time as the chemotherapeutic tests are being carried out, pharmacological control tests are made, such as determination of the lethal dose, tolerance, blood level, acute and chronic toxicity on organs and systems, etc.

We are employing a series of substances in our studies which

have been set out in the attached table. These substances were for the most part synthesised by the chemists of the Research Service, as has already been mentioned.

This being a preliminary note we shall set forth the results, in a general way, on the basis of the action of these substances when used "in vivo." These tests, which we have been carrying out for about two years, lend themselves to a few remarks which we have set out in Table No. 5. The assessment of the therapeutic results was based upon the modification of the evolution of the lesions, upon modification of the morphological characters of the bacilli, upon the longer survival of the treated animals; all these of course in relation to the respective controls.

The lesions of murine leprosy follow a course of evolution which is well-known (HADLER, W. A. & MAURI, A. C.—Rev. Brasil, Leprol. 16:139, 1948) and this can be modified by the action of substances possessing chemotherapeutic activity. The modifications of the course of evolution of the lesions have been classified as follows:— (See Table No. 5).

1. Clear and constant inhibition in some lesions = Evident action.
2. Lesser and inconstant inhibition = Slight action.
3. Vague and inconsistent retardation of the evolution of the lesions = Very slight action.
4. Absence of change in the evolution of lesions = No action.

The observations upon some substances are somewhat behind-hand; we therefore refrain from giving the respective results.

CLINICAL STUDIES (*)

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The Chemical Section of the Research Service of the Leprosy Prophylaxis Department has submitted three drugs which are at present under test with the Therapeutic Section. We cannot make any final remarks concerning any of these in the present preliminary note owing to the short time we have had them under study. A comparative study of the therapeutic activity of these sulphone derivatives in relation to those drugs which have already attained a position in routine treatment, also would be premature. We will therefore be content with some general remarks upon these substances.

(1) A M-4.4'-DIAMINO-DI-PHENYL-SULPHONE (**)

Presumed active radical on all sulphone drugs.

(*) Carried out at the Padre Bento Hospital, Copouva, S. Paulo.
(**) See Table No. 2.

Trials commenced on 11.6.48 in 46 cases (moderate and advanced Lepromatous Leprosy).

Daily dose: 0.3-gme. in 0.10-gme. tablets taken during meals in three-week courses, with one week's interval between courses.

Control of Treatment: Red cell counts and haemoglobin estimations every 2 days. Weekly examination of urine sediment.

Tolerance: Frequently causes anaemia during the first courses, but this effect tails off in the subsequent courses.

Activity: Therapeutic activity considerable so far. Observations continue and in December more detailed results will be published.

(2) A M B S 1 (***)

(a) Intravenous route—trials commenced 19.7.48 in 16 moderate and advanced Lepromatous cases not previously treated.

Maximum daily dose: 5-gmes. in 12 cc. Initial doses 1 cc. increasing gradually up to 12 cc. in 15-day courses with 7-day intervals.

Control: Fortnightly red-cell counts, haemoglobin determination and examination of urine sediment.

Tolerance: Excellent.

Activity: considerable therapeutic activity so far. Observation continues and in December more detailed results will be published.

(b) Oral route—trials commenced 5.3.49 in 9 cases (6 moderate and advanced lepromatous, 3 early lepromatous).

Daily dose: 1-gme. in sugar-coated tablets, taken during meals in 6-week courses with 2-week intervals.

Control: Fortnightly red-cell counts, haemoglobin estimation and examination of urine sediment.

Tolerance: Excellent.

Activity: Under test.

(3) Para-Amino-Salicylic Acid.

Trials commenced 1.12.48 in 10 moderate and advanced lepromatous cases, three of which have concurrent pulmonary tuberculosis.

Dose: 10 to 12 gmes. daily in tablets of 0.50 gmes., for four days of the week with three-day intervals.

Tolerance: Excellent.

Activity: So far seems to be less active than any of the sulphone drugs.

(***) See Table No. 2.

In addition to these sulphone derivatives the Therapeutic Section will commence studies on:—

1. A M F
2. A M T
3. A M G L

supplied by the Chemical Section and detailed in Table No. 2.

The following products from other sources are being studied:—

4.4′-di-amino-di-phenyl-sulphone, Sodium 2-acetyl-sulphonamide (Promacetin—Parke Davis Co.) commenced on 28.3.49 in 33 patients; 4.4′-di-amino-di-phenyl-sulphone, Sodium N,N′-bis-gamma-phenyl-propyl-di-sulphonate commenced on 29.7.48 with 53 patients (Sulphetrone) and on 19.3.49 (Sulfonazina) with 26 patients.

(Sulphetrone, Borroughs Welcome Co. and Sulfonazina, S. A. Farmaceutici.)