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THE IMPORTANCE OF TESTING THE THERAPEUTIC POTENCY OF ARSPHENAMINE PREPARATIONS *

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ALTHOUGH theoretically the arsphenamine preparations employed in the treatment of syphilis are known chemical compounds, it has long been recognised that slight variations in manufacturing procedure result in products of varying quality in respect both of toxicity and therapeutic potency. Because of the first of these it has long been customary in various countries to subject each batch of an arsphenamine preparation to such tests as will guard against the sale to the public of products of undue toxicity, but much less attention appears to have been paid to the question of tests of therapeutic potency, though the importance of such tests was revealed shortly after the War in reports by Schamberg, Kolmer and Raiziss,¹ by Dale and White, with Burn, Durham, Marchal and Mills ² and by Voegtlin and Miller.³ In the experiments of Schamberg, Kolmer and Raiziss, which were primarily to compare the therapeutic potency of arsphenamine with that of neoarsphenamine, it was shown that, under the trypanocidal test in rats, the minimum trypanocidal dose varied with different makes of arsphenamine from 0.010 to 0.030 gm. per kgm. of body weight, and that of neoarsphenamine from 0.020 to 0.040. The authors found that the trypanocidal test afforded results closely similar to those which had been obtained by Castelli ⁴ in experimental spirochætal infections (*S. obermayeri*, hen spirillosis, rabbit syphilis). Voegtlin and Miller, employing the trypanocidal test, found that, while arsphenamine from different manufacturers was fairly uniform in parasitocidal power, neoarsphenamine showed great variations. The investigation by Dale and White was stimulated by the observation of certain

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members of the Salvarsan Committee of the British Medical Research Council that some of the brands of "914" then in use for the treatment of syphilis did not appear to be affording the clinical results which experience had led them to expect of such compounds. The observations of Burn, Durham and Marchal on the effect of the compounds in question on mice infected with trypanosomes and those of Mills on their effect in causing the disappearance of *Sp. pallida* from the secretion of early syphilitic lesions in man substantially supported the clinical impression as to their inferiority from the point of view of therapeutic potency. The manufacturers of the brands in question immediately took steps to remove the defects revealed by this investigation, and it can fairly be said now that their products are equal to any other in respect of power to bring about the disappearance of *Sp. pallida* from the secretion of early syphilitic lesions. This experience was thus distinctive in two respects. (1) It appeared to establish a rough parallelism between the trypanocidal activity of an arsphenamine preparation and its ability to bring about the disappearance of *Sp. pallida* from the secretion of early syphilitic lesions; it appeared to show also that a preparation that was indifferent in these two respects would afford indifferent clinical results. (2) It afforded an example of the practical value of a test of therapeutic potency, since here it at once brought about abandonment of certain manufacturing processes which were resulting in products of low toxicity, but (a fact hitherto unsuspected by the manufacturers) of comparatively low therapeutic power. The importance of a routine test of therapeutic potency does not yet, however, appear to be generally recognised in spite of the above-mentioned reports and of subsequent recommendations by various workers which include the following.

The International Conference on Biological Standards convened in Geneva by the Health Organisation of the League of Nations in September, 1925, passed the following resolutions :—

" The Conference recommends :

" I. That the internationally recognised biological standardisation of remedies of the arsenobenzene group should be made with a series of standard

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preparations, one for each of the compounds in question.

“ II. That the following are the remedies which at present should be the subject of internationally recognised standardisation :

“ (1) Dioxydiamino-arsenobenzene dihydrochloride (syn. salvarsan, arsphenamine, arsenobenzol, etc.) ; and

“ (2) Its metallic derivatives (silver-salvarsan) ; and

“ (3) Its sodium salt (sodium salvarsan) ;

“ (4) Dioxydiamino-arsenobenzene sulphonylate of sodium (syn. neosalvarsan, neoarsphenamine, novarsenobenzol, etc.) ;

“ (5) Neosilver-salvarsan ;

“ (6) Sulpharsphenamine (syn. sulfarsenol).

“ III. That Professor Kolle of the Georg-Speyer Haus, Frankfurt-on-Main, be requested to accept the responsibility for preparing, maintaining, and distributing the standard preparations (1) to (5) on behalf of the Health Organisation of the League of Nations, and that Professor Voegtlin, of the Hygienic Laboratory, Washington, be invited similarly to be responsible for the standard preparation of (6).

“ IV. That every batch of the remedies in question, before issue for therapeutic use on human patients, should be tested on normal animals for toxicity and on animals infected with a suitable strain of pathogenic trypanosomes (*T. brucei*, *T. equiperdum*, etc.) for therapeutic potency.

“ V. That samples from every batch should be tested for toxicity on at least ten mice or five rats, or on both, material from several separate ampoules of each batch being separately tested, and that only such preparations should be passed for issue as exhibit, under identical conditions of experiment, a toxicity not greater than that of the corresponding standard sample.

“ VI. That samples of each batch should be tested for therapeutic potency on mice or rats infected with a suitable strain of pathogenic trypano-

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somes (*T. brucei*, *T. equiperdum*, etc.) in accordance with the following principles :

- “(1) A series of mice or rats is to be taken, having the same degree of infection with the trypanosome employed, as determined by some method of enumeration per unit volume of blood.
- “(2) That, on such a series of animals, with a uniform degree of infection, each batch shall be tested for therapeutic action in several (*e.g.*, 2-4) doses, with at least three animals on each dose, and the result shall be evaluated by comparison with the effects of the standard preparation, administered to animals of the same species, with the same degree of infection.
- “VII. That it is further recommended that, before a batch of one of the remedies in question is certified for general issue, samples of it shall have been used on a series of human patients, under the supervision of a qualified expert.”

It is not clear if Resolution VII is to insure against undue toxicity or to test therapeutic potency, but presumably its object is the former, since no test dose is specified, and in the German tests⁵ it is laid down that each batch after being subjected to various tests on animals shall be tried clinically for certain toxic effects.

I am not closely acquainted with the actual practice in countries other than Great Britain with regard to tests applied to arsphenamine preparations before sale to the public, but believe that in a number of them, although a toxicity test may be compulsory, a test of therapeutic potency is applied far less frequently ; also that, as a rule, when a therapeutic test is applied it is only the trypanocidal power which is investigated. It might be thought that a test of *minimum* toxicity would guard against the sale of products of inferior therapeutic potency, but it has been shown that there is no reliable parallelism between toxicity and therapeutic potency. Thus Durham, Gaddum and Marchal⁶ found that a certain brand of “914” was more toxic than the standard preparation, but therapeutically much less potent.

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Schamberg and Kolmer, with Madden,⁷ in a paper advocating the imposition of a therapeutic test, showed in 18 samples of "914" from seven different makers a fairly uniform maximum tolerated dose (0.20 to 0.30 gm. per kgm.), but minimum trypanocidal doses, ranging from 0.004 to more than 0.012 gm. per kgm. of body weight. The lack of parallelism between toxicity and trypanocidal activity is also brought out in a recent paper by Rothermundt. The inevitable conclusion from the evidence so far presented is that a test of toxicity is not sufficient; it should be supplemented by one of therapeutic potency. The question then arises if a test of trypanocidal activity is a sufficient guide to therapeutic potency *vis-à-vis* *Sp. pallida*. The reports of Dale and White, of Voegtlin and Miller, and of Schamberg, Kolmer and Raiziss suggested a parallelism which was generally close enough for practical purposes, and this opinion was definitely offered in the paper by Schamberg and Kolmer, with Madden, though here the trypanocidal and spirochæticidal results were not parallel in 4 out of the 18 samples that were tested. In the paper by Rothermundt already mentioned it appears to have been assumed that brands of "914" can be compared in respect of therapeutic efficacy by the measure of their trypanocidal activity. On the other hand, Probey and McCoy⁸ found that two brands of neoarsphenamine differing strongly in trypanocidal activity (one, represented by 7 samples, had a minimum effective dose of 10 to 15 mgm. per kgm., and the other, represented by 6 samples, a M.E.D. of 25 to 35 mgm.) showed in experimental syphilis of rabbits approximately the same ability: (1) to cause the rapid disappearance of spirochætes from the chancre, (2) to cause the rapid healing of the lesion with freedom from clinical relapse, and (3) to influence the Kahn reaction in experimental rabbit syphilis over periods of sixty-seven to eighty-eight days. Probey and McCoy found, like workers already quoted, wide variations in the therapeutic potency of different brands of "914."

It appears, therefore, that although trypanocidal activity of an arsphenamine preparation may be a rough guide to its spirochæticidal power, it is safer to test this also. The spirochæticidal power could be tested on rabbit syphilis, as was done by Castelli and by Probey

and McCoy, but a method that is more practicable for the clinician is to test the effect of a given dose of the remedy in question for its power to bring about the disappearance of *Sp. pallida* from the secretion of early syphilitic lesions within a given time. This is the method which was employed in the investigation reported by Dale and White and has been applied in clinics under my control for a number of years. Whenever the therapeutic efficacy of a given arsphenamine preparation has been in question a certain number of male adult patients suffering from primary syphilitic chancres, or from secondary lesions, in the serum of which *Sp. pallida* could be demonstrated easily received each a certain dose of the preparation, and twenty-four hours later the secretion was searched again for *Sp. pallida*. If these organisms were still present, the examination was repeated twenty-four hours later, sometimes after a further dose had been given. The results of a number of tests of various preparations that have been carried out on these lines by the staff of the V.D. Department, St. Thomas's Hospital, are shown in the following tables.

In all but one (Solusalvarsan 6 c.c.) of these series the remedy was given intravenously.

The table shows that, when administered by the intravenous route, the three types, arsphenamine diglucoside, sulpharsphenamine and solusalvarsan are at any rate less rapid in action than is almost any brand of neoarsphenamine. Sulpharsphenamine is usually regarded as intended for use by the intramuscular route, and the results of these tests are included in the present series only to show its inferiority to neoarsphenamine when given intravenously. The term inferiority is used advisedly in this connection because there is no reason to suppose that after intravenous injection the slowness of action of sulpharsphenamine is compensated for by its being retained in the body longer than is neoarsphenamine. Whether or not the slowness of action of arsphenamine diglucoside and of solusalvarsan is compensated for in this way I am unable to say. The results with the former of these two types show that those who use it must reckon with the fact that *Sp. pallida* continues an active existence for longer under it than under treatment with corresponding doses of neoarsphenamine, or (as I know from similar experiments

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TABLE

The Effect of Various Arsphenamine Preparations in causing the Disappearance of Sp. pallida from the Secretion of Early Syphilitic Lesions.

Type of preparation.	Dose as stated on ampoule. gm.	No. of cases.	Sp. pallida in secretion 24 hours after first injection.		Remarks.
			Present in.	Not found in.	
NEO- Brand A	0.45	10	0	10	
ARS- " B	0.45	10	0	10	
PHEN- " C	0.45	31	3	28	
AMINE " D	0.45	16	2	14	
(Different brands " E ¹	0.45	12	0	12	
designated by " E ²	0.45	5	4	1	
different letters, " E ³	0.45	14	9	5	
different batches " F	0.45	10	3	7	
by different " F	0.60	10	1	9	
figures). " G ¹	0.45	10	6	4	
" G ¹	0.60	10	4	6	
" G ²	0.45	10	4	6	
" G ²	0.60	10	0	10	
Arsphenamine diglucoside (Stabilarsan)	0.45	10	9	1	In 2 cases Sp. pallida found until 2 x 0.45 and 1 x 0.60 had been given. * In 2 cases Sp. pallida still present 24 hours after second injection of 0.60 gm.
	0.60	10	6 *	4	
Sulpharsphenamine	0.60	5	5	0	
Solusalvarsan	4 c.c.	4	4	0	
	6 c.c.	5	3	2	
	6 c.c.	5	5	0	
	(subcutaneous)				

a number of years ago) with much smaller doses of " 606." With regard to neoarsphenamine, although most of the brands that were tested gave satisfactory results, the performance of others shows the advisability of the clinician periodically testing for himself the therapeutic efficacy of the arsphenamine preparations he is in the habit of using for the treatment of syphilis. The results of these tests may perhaps also cause some workers to reflect on the effect (or lack of such) of a smaller dose

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than 0.45 gm. neoarsphenamine (a rapidly excreted remedy) on the spirochætal population in an adult man of average weight suffering from early syphilis.

SUMMARY

(1) Investigations by numerous workers have shown that different brands, and batches of those brands, of arsphenamine preparations can differ widely not only in toxicity but in therapeutic potency as judged by trypanocidal tests.

(2) Toxicity and therapeutic potency are not parallel, so that a test of minimum toxicity is not a safeguard against inferior therapeutic efficacy.

(3) Investigations by Dale and White in human syphilis and by others in animal syphilis have shown a rough parallelism between the trypanocidal and the spirochæticidal power of arsphenamine preparations, but other work, notably that of Probey and McCoy, indicates that the trypanocidal test may not be a completely reliable guide. In any case the trypanocidal test is not practicable for the average clinician.

(4) The results of a simple test of activity against *Sp. pallida* are presented in support of the thesis that clinicians should periodically test in a similar manner the arsphenamine preparations they commonly employ in their treatment of syphilis.

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