SCIENTIFIC SECTION

FURTHER STUDIES ON THE SALVARSANS AND RELATED COMPOUNDS.

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In 1918 Myers and DuMez (1) pointed out that arsphenamine products of different manufacturers marketed in this country are not all uniform with respect to either their chemical or their physiological properties. Clinical evidence at that time corroborated the laboratory data. Many of the preparations showed the presence of sulphur compounds. Quantitative determinations of various brands of arsphenamine disclosed marked variations in the arsenic content. More than twenty specimens were examined with a deviation of approximately 0.6 per cent. These variations later led to the study of the volatile constituents of these products. Reference to these assays indicated a remarkable degree of uniformity of the original Ehrlich product. Examination of the various brands of neoarsphenamine also revealed a most unusual lack of regularity in arsenic content and in general physiological and chemical properties, a condition which to a considerable measure exists even to the present time.

Later the author pointed out the relation of physical properties to toxicity, and discussed the subject of colloidal chemistry as related to the action of the salvarsan series. This has led to a great deal of constructive research on the physical characteristics of this group of arsenicals. The interesting observation was made by Lake in our laboratory that if a solution of salvarsan in the form of the disodium salt is allowed to stand 30 minutes, there is a 20 per cent reduction in toxicity, indicating a change from the semicolloid stage to that of a pure solution. Hooper in unpublished data has shown that even in higher doses 60 per cent of the animals will survive on a balanced and regulated diet. According to their colloidal character, the various arsenicals may be arranged in the following order between the true colloids and the crystalloids: collargol (colloid), silver salvarsan, neosalvarsan, salvarsan dihydrochloride, salvarsan freshly alkalinized, salvarsan sodium, silver nitrate (crystalloid). Various impurities in salvarsan and its analogous compounds have been overemphasized as a causative factor of reactions, usually by those unfamiliar with clinical practice. A most careful statement on this subject has been made by Kolmer (2) in his treatise "Chemotherapy with Special Reference to Treatment of Syphilis," a book worthy of earnest perusal. In previous studies the author conveyed the information that in commercial salvarsan there were volatile constituents which, according to calculation, as a group corresponded to two molecules of water of crystallization. It has not been the writer's conception that a semicolloid carries water of crystallization but everyone familiar with the subject knows (as confirmed by the earlier reports of Myers and DuMez) that products have appeared on the market with an arsenic content as low as 29 per cent and as high as 32.3 per cent.

Every reasoning individual is aware that there is a cause for this deviation. Goebel (3), Raiziss (4), Gavron and Falkov (5), Kober (6), Rieger (7), Fargher and Pyman (8), and Myers (9) have expressed views on this subject, from the toxico-

logical point of view. The presence of arsenoxide is so low as to deserve little consideration. Combined sulfur derivatives are also present in minute amounts; in fact they favorably influence the therapeutic activity. Commercial salvarsan contains approximately 31.56 per cent arsenic, either by gravimetric analysis or by the official test described by the author. Various brands of arsphenamine are examined for arsenic and then each specimen is dried *in vacuo* at 110° C. at a pressure of 29.50–29.75 inches. The weight of the specimen is known, the arsenic and the arsenoxide content are found by actual determination. The toxicity studies are made simultaneously on the original material and on the dried specimen. The product is then dried for 4 hours with a trap containing a known amount of tenth-normal silver nitrate which takes out the hydrochloric acid. An additional trap is added and maintained at freezing temperature for the purpose of examining for methyl alcohol. Kolmer on page 599, states:

"*Toxicity of Methyl Alcohol and Inorganic Impurities.*—From the standpoint of toxicity, the importance of methyl alcohol has been overemphasized. In the small amounts that may be present, it cannot be held responsible for toxic reactions and is of chief interest in relation to the chemistry of arsphenamine and especially as an explanation in part for the presence of less than the theoretical amount of arsenic, as discussed above. I have found that white rats can bear without apparent injury intravenous injections of methyl alcohol in doses as high as 1 cc. of 10 per cent solutions per kilo, which is much larger than the traces to be found in arsphenamin."

After drying has taken place for 4–6 hours the specimen is reweighed under analytical precautions. Arsenic, arsenoxide and toxicity determinations are then made. The solutions give the hydrochloric acid content. From the total loss in weight the calculated arsenic content is obtained. Comparison is then made with the theoretical arsenic content which is 34.2%. In other words, arsenic found, arsenic calculated and the theoretical value are obtained at once.

A comparison of several brands has shown the presence of comparatively large amounts of inert non-arsenic containing material which undoubtedly comes from carelessness of manufacture and materially concerns the solubility of the arsphenamines. The following table illustrates this point:

Control.	Arsenoxide. As before.	After.	Arsenic before heating.	Total volatile constitutent per cent.	Free HCl.	Arsen. anhy.	Arsen. calcd.
a	1.3	1.5	31.65	9.9	0.280	33.80	...
				9.1	0.285		
b	1.2	1.5	31.65	7.36	0.270	33.67	34.16
c	0.8	1.0	31.40	9.40	0.206	34.10	34.60
d	0.7	0.9	31.54	8.30	0.172	34.20	34.20
e	0.7	0.9	31.40	8.50	0.237	34.20	34.30
f	0.6	0.9	31.20	8.50	0.201	34.10	34.10
g	0.6	0.8	31.28	9.05	0.230	34.22	34.40
h	1.1	1.1	31.67	4.01	0.193	32.83	32.66
i	1.1	1.1	31.87	5.68	0.120	33.50	33.80
k	1.0	1.2	31.00	7.65	0.134	33.97	33.57
l	1.1	1.1	30.10	8.00	0.171	33.07	32.72
m	1.1	1.2	30.05	9.02	0.227	33.41	33.02

The foregoing table indicates that no oxidation has taken place as a result of vacuum drying when measured by the arsenoxide test and also by toxicological

examination. The hydrochloric acid content of this group of products shows a fair degree of uniformity in the percentage of mechanically held acid. As previously stated the theoretical arsenic for anhydrous salvarsan is 34.20 per cent and the last two columns reveal interesting data on this subject.

It so happens that the excess hydrochloric acid in these specimens is no serious factor in the alkalization of the preparations. However, occasionally products are found with which comparatively large excesses of alkali are required to produce the disodium salt. This factor, coupled with water that has absorbed much carbon dioxide, may be the cause of disastrous results clinically. The presence of the inert impurities and excess acid may serve as a factor influencing the formation of either fine or coarse grained particles in the process of alkalization. Officially this group of compounds is examined only for toxicity and arsenic content.

A word of warning should be added at this time against the use of either warm, hot or boiling water to which the alkali is added, for the reason that decomposition takes place with the resulting formation of arsenoxide.

It is not uncommon to find that voluminous pamphlets are distributed to clinicians that neither represent scientific accuracy nor convey helpful information to the physician or patient. After a little serious reflection, one may well ask what convincing clinical literature can be offered to a physician in behalf of a given product unless scientific evidence can be adduced that the product measures up to the Ehrlich ideals? This point was so emphatically brought to the attention of the English manufacturers and clinicians that a demand was made by the British Medical Research Committee for a uniform product with high potency. Careful study showed that to attain these ideals, a definite standard must be adopted as regards manufacture and therapeutic activity. To reach this goal the product originated by Ehrlich was selected as a standard, with which all arsenicals were made to conform. Not until better arsphenamines are made than those suggested and studied by Ehrlich will the time arrive when our standards need revision. It is the writer's opinion that the clinician should be aided to treat syphilis with the arsphenamine preparations rather than be forced to listen to a long discussion of unverified chemical theories. In this connection reference is made to a few salient statements by H. H. Dale (10) of the British Medical Research Committee. The same ideals were advocated by me several years ago and now there are a few who have tardily accepted them in principle, though not in all instances as a scientific fact.

"TWO DISTINCT CLASSES OF NEOSALVARSAN TYPE."

"A short experience with the application of this test sufficed to indicate that the preparations of the neosalvarsan type submitted for test fell into two well-marked classes. On the one hand were those which resembled the German product more or less closely in appearance, in solubility and in the rapidity with which their solutions underwent decomposition if left standing exposed to the air. On the other hand, there were others which had an advantage over the German product in their extremely free and rapid solubility, and which gave evidence of much greater stability in solution. Preparations of the former class had a border-line toxicity from the point of view of the test; many samples just passed it, and many others failed. A slight raising of the standard would have excluded nearly all. Those of the latter class—the freely and immediately soluble type—passed the routine test with an almost unbroken regularity; when, experimentally, sample batches of this type were tested on higher doses, it was found that many were tolerated in a dose of 0.5 mg., some even in a dose of 0.6 mg. per Gm.; *i. e.*, the toxicity was often only one-half of that at which they would still have passed the official control.

"No question of a control of therapeutic efficacy arose at this time. It would have been impossible, in any case, to carry it out with the staff available under war conditions. From the clinical side there was no hint of dissatisfaction; here again, in the hurry to get men through their treatment and return them to duty, no adequate control of results was possible. The practitioner appreciated the additional convenience afforded by rapid and perfect solubility, and the freedom from constitutional reactions, even when highly concentrated solutions were injected. The demand for the more soluble and less toxic type of product grew very rapidly, until manufacturers whose endeavor had been rather to copy the German product found themselves forced to modify their process, so as to produce a more soluble type. Therewith the toxicity of their products fell likewise to the lower level."

Some may raise the question as to whether this can be done and the answer is in the affirmative. Foreign preparations were studied in the following manner:

"First Comparison of British and German 914 Products.

(P) = Preparation
(Ma) = Maximum tolerated dose
(Mi) = Minimal curative dose
(RD) = Ratio of curative dose to tolerated dose

(P.)	(Mi.)	(Ma.)	(RD.)	(P.)	(Mi.)	(Ma.)	(RD.)
German.				British.			
A1	0–02	0–3	1:15	C1	0–06	0-5	1:8
A2	0–02	0–3	1:15	C2	0–03	0-5	1:16
A3	0–02	0–3	1:15	C4	0–05	0–5	1:10
British.							
				C5	0–04	0–3	1:7-5
B1	0–04	0–4	1:10	C6	0–04	0–4	1:10
B2	0–05	0–3	1:6				

The results are obvious to one trained or even untrained in this work.

To confirm these points the same committee made a study of the action of the same products on clinical material, with complete confirmation of the experimental findings as shown in the following table:

Preparation.	Minimal curative dose of mice infected with T. Equiperdum.	Proportion of human cases in which spirochaetes were detected 18–20 hours after injection of 0.45 g.
B3	0–015	0 out of 6
A2	0–02	0 out of 4
A3	0–02	1 out of 6
C3	0–02	1 out of 6
C2	0–03	3 out of 6
B2	0–05	9 out of 10

The infection leading to syphilis is a serious one resulting in consequences which are important from clinical as well as economic points of view. It concerns the daily well-being of each and every one. Picture for yourself the many situations where decisions involving life and death must be instantaneously made. The mere failure for a moment of the engineer in obeying a signal, the lapse of mental activity on the part of the dispatcher and a host of similar conditions may plunge us precipitously into eternity. Therefore the "best" is mandatory in the treatment of this disease.

The first studies carried out in this country on the composition of the neoarsphenamines were those by Raiziss and his co-workers in which they definitely es-

tablished that there were two types of drugs. It is unnecessary to defend or criticise their mode of procedure because on a comparative basis the findings show distinct variations. The same may be said of the investigations of Macallum (11), Elvove (12), Freedman (13) and De Myttenaere (14). Of particular importance is Elvove's statement that in some brands of so-called neoarsphenamine there is a very large amount of sulpharsphenamine-like impurity. Values as high as 90% of this impurity have been found and yet the product is labeled as neoarsphenamine, a condition which should not exist. The combined researches of the above investigators have demonstrated the existence of at least three varieties of neoarsphenamine and it is difficult to understand how all can be described as superior. Furthermore, no extensive clinical literature is available for any type except the Ehrlich product. It would, therefore, seem evident that these products are largely devoid of supporting evidence. It has been my observation that syphilitics who have been treated with certain arsenicals have become progressively worse. It should be pointed out that the so-called neoarsphenamine preparations are officially controlled in respect to arsenic content and toxicity only. Some manufacturers carry on an experimental therapeutic control. During the past few weeks examination of a few types of neoarsphenamine has been made showing that some are essentially monosubstituted products, others disubstituted; another group is approximately half and half, and a fourth contained 81.6 per cent of a sulpharsphenamine-like impurity. Fordyce, Rosen and Myers (15, 16) have pointed out that disturbing late results in syphilis were associated with localization of the arsenic instead of distribution, and this in turn was related to chemical constitution.

Everyone who is familiar with the treatment of syphilis has noted the incidence of purpura, dermatitis and jaundice, as well as encephalitis and peripheral neuritis. These reactions are late and usually have some connection with the arsenical employed. On the contrary, early reactions are due to errors in technic in the preparation and administration of the drug.

Nothing has been said in regard to sulpharsphenamine. In a recent report of Stokes, it is stated that the only excuse for its existence is in the treatment of obese syphilitic patients. This is an opinion shared by a large number of clinicians for many years.

To bring to a conclusion, the ideas briefly alluded to at this time, it is evident that there is great need for uniformity of the neorasphenamine preparations so that the treatment of syphilis may be standardized and the results of one clinician be placed on a comparable basis with those of another in a different locality. Syphilis has become an economic problem and one closely related to public health. The most efficient remedy is a necessity, and thus far no one has discovered any preparations superior to those originated by Ehrlich. It is a matter of common knowledge that the League of Nations is and has been using the latter products as a standard for comparing all others. It is my opinion that there is a real necessity for the official control of the therapeutic activity of all antisyphilitic remedies as well as control of toxicity and arsenic content. The U. S. Public Health Service should willingly and fearlessly assume this responsibility for the protection of patients and clinicians. This would lead to uniformity. The present lack of efficiency of certain types of arsenicals is bound to lead to unsatisfactory results in late cases and thus give rise to unfavorable criticism about the efficacy of all treatment. If a clinician

can take the time and trouble to properly prepare solutions of salvarsan, this drug is the one of choice. For general use, neosalvarsan has been more widely adopted because of the greater convenience. In combined convenience and potency, silver salvarsan is unexcelled. It produces no systemic reaction and possesses marked efficiency in eradicating the invading organism.

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