III

THE VALUATION OF ANTISYPHILITIC SUBSTANCES BY PROPHYLACTIC AND THERAPEUTIC TESTS IN ANIMALS EXPERIMENTALLY INFECTED WITH SPIRILLUM MINUS

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INTRODUCTION

LAVERAN and Mesnil (1902), first gauged the therapeutic value of antisyphilitic remedies by applying them in experimental trypanosomiasis of laboratory animals. In later experimental studies on the chemotherapy of syphilis, certain other infections of laboratory animals were resorted to for test purposes. Thus infection by the relapsing fever spirochætes (Hata and Ehrlich) and *Spironema gallinarum* (Uhlenhuth, Gross and Bickel, 1907), and also experimental syphilis in rabbits (Levaditi and Yamanouchi, 1908) has been utilised for the valuation of organic arsenicals.

Experimental trypanosomiasis has probably been most widely used for the purpose. Laboratory animals can be readily infected, the resulting disease shows only slight variation, the organism is very readily demonstrable in the blood of infected animals, and the antagonism of a drug towards the infection can be observed without difficulty. While there is some correspondence between the effect of certain chemical substances *in vivo* on the syphilis spirochæte and on trypanosomes, syphilitic infection in the human subject has little pathological analogy with animal trypanosomiasis, and it would seem doubtful whether the latter constitutes a suitable experimental disease for testing antisyphilitic substances. Thus, in rats and mice, trypanosomiasis is almost entirely a blood infection, though in guinea-pigs and rabbits the fixed tissues are involved to some extent. In no case are lesions produced resembling those found in human

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syphilis. Further, arsenic-fast strains are readily developed. In favour of the use of experimental trypanosomiasis, it must be said that a ratio exists between the trypanocidal and spirochæticidal effects of salvarsan, and to a less extent of neosalvarsan (Mills and White, 1922), but the relationship fails with other products. Tryparsamide (Brown and Pearce, 1919) is highly trypanocidal, but very slightly spirochæticidal, while the reverse is the case with mercury (Browning and Mackenzie, 1924). Notwithstanding these disadvantages, experimental trypanosomiasis holds an important position as the infection of choice in standardisation tests for "606" and "914" products, and also in surveying a series of substances to ascertain which is most likely to repay closer investigation.

The spirochæte of relapsing fever, whatever the variety, produces in laboratory animals an infection of which the course is extremely variable—remissions occur, immunity develops, and natural recovery takes place. The interpretation of results, complicated by such sources of error, is extremely difficult—one might almost say impossible.

A more satisfactory experimental infection for therapeutic tests is with the *Sp. pallida* in the rabbit. This has been fully worked out by Brown and Pearce (1919), and has proved to be a very useful means of investigating the spirochæticidal power of new drugs. An emulsion of infective material may be injected into the scrotum of the rabbit, or, according to the method described by Tomaszewski (1910), a small piece of infected tissue may be inserted into a pouch made in the scrotum of a young animal. The spirochæticidal power of a drug is estimated by its effect, when administered by various routes, on the spirochætes, which are very numerous in the primary lesion. According to Brown and Pearce (1922), late lesions of a generalised character can also be produced, especially if castration is performed about a week after inoculation. For long it was thought that arsenic-resistant strains of *Sp. pallida* do not develop, but recent work has tended to show that this may occur. The results obtained with drugs in this infection have proved to be reliable as a guide to their effect in human syphilis, and, on general principles, it might be regarded as the ideal means of testing antisymphilitic remedies. Only rabbits, however, of the common laboratory animals can be successfully inoculated, and a considerable time

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(perhaps three or four months) is necessary for proof of cure. The use of this experimental infection has, therefore, been limited to the final tests of a drug already shown to possess curative properties.

Experimental infection of the lower animals with *Spirillum minus* has been little used, curiously enough, considering that Ehrlich, the originator of chemotherapeutic investigation, employed *Sp. laverani* (which is probably identical with *Sp. minus*) in his classical experiments which led to the discovery of salvarsan.

All the common laboratory animals are easily infected. In the white rat the experimental disease presents an apparent analogy to syphilitic infection, with the following successive phases (McDermott, 1928): "An incubation period; a primary inflammatory lesion, the organism being localised to the lesions and regional lymphatic glands at this stage; a secondary stage, during which the organisms are present in the blood; a latent stage in which the blood is free from spirilla and there are no obvious lesions; a tertiary stage, with gummatoid lesions." In mice, however, the spirillum causes almost a pure blood infection. In guinea-pigs there is a well-defined primary "sore" at the site of inoculation developing after an incubation period of about nine days, and the course of the infection is much more acute than in rats. In rabbits a primary sore occurs, but spirilla are never demonstrable in the blood by microscopic methods, although the blood is infective to mice. Late lesions develop almost constantly, such as interstitial keratitis, patchy alopecia about the head, rhinitis, circinate ulcers on the skin and generalised lymphadenitis.

It may be taken, then, that the general course of the experimental disease is similar to that of syphilis in the human subject. Some special resemblances may be emphasised.

(1) The primary ulcer (in guinea-pigs) resembles the primary chancre of syphilis very closely (Arkin, 1920; Adams, 1925).

(2) The lung lesions, especially in rats, resemble those described in a case of human syphilis by Warthin (1918).

(3) The spirilla rapidly increase in the blood post-mortem (noted in guinea-pig), as the *Sp. pallida* may do in the human infected with syphilis (Kratzeisen, 1923).

(4) The Wassermann test, which is negative in normal

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rats, may give positive results in rats infected with the spirillum. This will be discussed more fully later on.

Experimental infection with *Sp. minus* shows the following advantages over those diseases already used to test antisymphilitic drugs.

(1) The common laboratory animals, rats, mice, guinea-pigs and rabbits are easily infected.

(2) It is both a blood and a tissue infection, so that an estimation can be made of the immediate effects of the drug on the organisms in the blood stream, and of the slow penetration of the tissues by the drug to reach deep-seated areas of disease.

(3) The lesions produced in certain laboratory animals by the organism approximate those produced in man by the *Sp. pallida*, thus giving an idea as to the stage in human syphilis at which the drug might be expected to prove the most useful, *i.e.*, as a prophylactic, in neurosyphilis, etc.

(4) A ratio exists between the parasitocidal power of the drug under test in the experimental infection, and its spirochæticidal power in human syphilis.

(5) As far as is known, arsenic-resistant strains of *Sp. minus* do not develop.

The experimental infection of the lower animals with *Sp. minus* seems then to be a favourable method for chemotherapeutic investigation into the probable spirochæticidal power of a drug. For this reason the writer has used this organism for some time as a basis for the testing of standard preparations applied in the treatment of human syphilis, and has compared the results with those of some new remedies.

The strain used (Mackie and McDermott, 1926) was obtained from a case of rat-bite fever which occurred in 1926 in the Royal Hospital for Sick Children, Edinburgh.

ARSENICAL COMPOUNDS

The first preparation studied was the dioxydiamido-arsenobenzol-formaldehyde-sulphoxylate of soda or "914."*

This was chosen because its effect on all stages of syphilis in the human subject has been, more or less, fully worked out, and such data, therefore, would act as a

* Supplied by Boots Pure Drug Co., Ltd., Nottingham.

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standard of comparison for the results obtained with the drug in experimental *Sp. minus* infection.

In all the cases quoted, unless otherwise stated, white rats were the animals used, averaging 100 to 150 gm. in weight.

The drug was administered, dissolved in sterile water, by subcutaneous injection over one or other flank. By this route the minimal tolerated dose was found to be 0.75 gm. per kilogramme body-weight.

PROPHYLAXIS

Healthy rats were inoculated in the genitals with 0.2 c.c. citrated infective blood containing numerous spirilla. In a few series the intraperitoneal route was adopted. About half of the animals in each batch were kept as controls, while the rest received graduated doses of the drug on the third day after inoculation. This date was chosen in order to simulate as far as possible the conditions which hold in the usual case undergoing prophylactic treatment for syphilis. The smallest dose which acted as a certain prophylactic was found to be 0.1 gm. per kilogramme body-weight. (See Table I.) This gives a ratio :—

$$\frac{\text{Minimal tolerated dose}}{\text{Minimal prophylactic dose}} = \frac{0.75}{0.1} = 7\frac{1}{2} \text{ to } 1.$$

Much smaller doses were found to have a definite spirillicidal effect. Thus when an inoculated rat received a

TABLE I

Rat. No.	Weight in Grms.	Day 1.	Day 3. Drug injected (dose in grms. per kilo. body-weight).	Dark-ground examination for <i>Sp. minus</i> .	Mice infected with blood of rat.	
					Number of days of infection in rat.	Dark-ground examination of blood of inoculated mice.
82	96	Inoculated with blood containing <i>Sp. minus</i> .	0.1	Negative for 21 days.	10	Negative for 30 days.
48	180	"	0.04	Negative for 13 days.	15	Spirilla appeared in blood.
49	170	"	0.04	"	"	"
50	187	"	—	Spirilla appeared on 7th day.	—	—
51	244	"	—	"	—	—

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dose of 0.04 gm. per kilogramme, no spirilla were ever found in the animal's blood by dark-ground examination or staining methods, but, nevertheless, infection had taken place, as was shown by sub-inoculation of the rat's blood into mice which developed a *Sp. minus* infection.

SECONDARY STAGE

The "secondary" stage was considered established when spirilla could be demonstrated in the blood by dark-ground examination. This was usually four to eight days after inoculation. The drug was administered, and a week or ten days later the rat was tested for cure. This consisted of the following tests :—

(1) No spirilla were found on repeated dark-ground examination of blood.

(2) Mice inoculated with 0.4 c.c. citrated blood from the rat undergoing test did not show spirilla on dark-ground examination daily for one month.

(3) The Wassermann test was negative.

By these criteria the smallest curative dose was found to be 0.2 gm. per kilogramme body-weight. (See Table II.) This gives a chemotherapeutic index :—

$$\frac{0.75}{0.2} = 3\frac{3}{4} \text{ to } 1.$$

With very much smaller doses the blood could be cleared of spirilla within twenty-four hours. In the case of the smallest dose used (0.01 gm. per kilo. body-weight) a relapse occurred, spirilla being demonstrable in the blood by dark-ground examination within five to ten days, while with larger but still sub-curative doses, the spirilla could not be again demonstrated in the blood, although sub-inoculation into mice proved the absence of complete cure.

TABLE II

Rat. No.	Weight in grms.	Dose of drug (in grms. per kilo. body-weight).	Dark-ground examination for <i>Sp. minus</i> .	Mice inoculated with blood of rat.	
				Number of days after treatment.	Dark-ground examination of blood of inoculated mice.
68	102	0.1	Negative after 24 hours.	17	Spirilla appeared in 12 days.
63	206	0.2	"	6	Negative for 30 days.
64	135	0.3	"	4	" "

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LATENT STAGE

In this stage, which lasts usually for six to nine months, the spirilla cannot be found in the blood by dark-ground or staining methods. The animal appears well, except perhaps for some loss of hair. Cure was accepted when :

(1) The animal did not show signs of the disease alive, or on post-mortem examination.

(2) Inoculation of blood into mice did not cause infection.

(3) The blood Wassermann was negative.

The minimal dose for cure was found to be 0.4 gm. per kilogramme body-weight, given in one dose. This gives a chemotherapeutic index of $\frac{0.75}{0.4} = 1.875$ to 1.

TABLE III

Rat. No.	Dose of drug (in grms. per kilo. body-weight).	Inoculation of mouse with blood of rat.	Wassermann reaction of blood.	Result.
102	0.04	Infection occurred.	Not taken.	No cure.
54	0.2	"	"	"
53	0.3	"	Weak positive.	"
52	0.4	No infection occurred.	Negative.	Cure.
45	0.4 (in two doses of 0.2)	"	"	"

GUINEA-PIGS

The same technique and tests of cure as described above were used in the experimental infection of guinea-pigs with *Sp. minus*. In these animals the infection is very acute ; so acute, indeed, that the writer was not able to cure any case which had progressed to the later stages. Death occurs within twenty-five to thirty-five days of the date of infection, in the last week of which the animal is obviously ill and shows usually conjunctivitis and keratitis.

One case is quoted illustrative of this entire failure to cure the infection. Guinea-pig No. 30 was given 0.3 gm. per kilo. body-weight of " 914 " intramuscularly on the twenty-second day after inoculation, when the blood

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showed *Sp. minus*. No spirilla were seen in the blood until the thirty-third day, when another dose, similar to the first, was given. The blood again remained negative until the thirty-eighth day, when spirilla appeared in large numbers. A third time 0.3 gm. per kilo. was given, but death occurred the next day. Similar doses had been given to healthy animals without causing death. No signs of disease other than the occurrence of spirilla in the blood were found in the guinea-pig post-mortem. It was concluded that the animal died, in spite of such doses, from the *Sp. minus* infection. Similar examples occurred, while testing other drugs on guinea-pigs, of death after the maximum tolerated dose had been given.

THE WASSERMANN TEST IN THE EXPERIMENTAL DISEASE

In the human subject with rat-bite fever the Wassermann is negative (Price, 1926). According to McDermott (1928) the Wassermann is negative also in the experimental disease in guinea-pigs, but he mentions that he obtained a positive flocculation test from an infected rat four and a half months after inoculation. The writer has obtained different results in rats.

TABLE IV

Series.	Time after inoculation.	No. of positive results, i.e., fixation of 4 or more doses of Comp.	Remarks.
VI	16 days	1 out of 3	Another was doubtful, i.e., fixation of 2 doses of complement.
	12 weeks	1 out of 1	The previous negative now fixed 2-3 doses of complement.
II	6 weeks	3 out of 4	—
	12 weeks	3 out of 3	One of the positive rats had died.
I	8 weeks	3 out of 7	—
	12 weeks	5 out of 5	Four previously negative.

The method used was that of Harrison's (1918). The normal rat gives a negative reaction. The earliest positive result obtained was sixteen days after inoculation, when the blood of the animal still showed spirilla on dark-ground examination. From sixteen days onwards, a varying proportion of infected animals gave a positive reaction, until the tenth week after inoculation, when

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every animal tested was positive. Three batches of rats in which the reactions were tested twice serve to illustrate this point.

Out of eighteen rats examined before the tenth week after inoculation, six gave negative results; that is, 66 $\frac{2}{3}$ per cent. were found to be positive; thirteen rats examined after this period all gave positive reactions, three of which were markedly positive, *i.e.*, the heated serum fixed at least eight doses of complement.

Treatment can reduce a positive Wassermann (see Table V), and, in the case of a cure, keep it permanently negative.

TABLE V

Rat No.	Treatment in grms. per kilo. body-weight.	Wassermann test.	
		Before treatment.	After treatment.
52	0.4 "914."	Weak positive.	Negative.
104	1.2 Parosan.	Weak positive.	Weak positive.
	3.0 "	"	Negative.
103	2.4 Parosan.	Strong positive.	Strong positive.
	4.8 "	"	Negative.
	Rest for 24 days.	Reverted to weak positive.	

The high percentage of positives found in the above reported cases makes it likely that in a large series similar results will be obtained. After the tenth week, or approximately so after inoculation, a negative complement fixation reaction in the rat infected with *Sp. minus* suggests that no active spirilla are left in the body, providing that treatment has not been recently applied. Before then a negative reaction does not exclude this infection.

Discussion of Results.—Prophylaxis.—As in syphilis in the human, so in *Sp. minus* infection of rats, the earlier treatment is instituted the greater the certainty of cure. Prophylactic treatment is the method of choice. Comparatively small doses of the drug are required, but a distinct danger lies in giving insufficient amounts, which may mask the early diagnosis of the disease, without preventing later and much more serious lesions. The

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rats which received sub-prophylactic doses of "914" did not show spirilla in the blood by dark-ground examination and appeared well, but infection had occurred, as was proved by mouse inoculation. One series of animals illustrates this point very well. Eighteen rats were inoculated in the genitals with infected blood. Six of these were kept as controls, and twelve received small doses of various drugs.

As it happened, the doses were insufficient, and infection occurred in all cases, proved by finding spirilla in the blood, or by mouse inoculation. Four months later, without intervening treatment, it was found that the six controls were alive, and, although infected, did not appear unwell. Of the twelve partially treated animals only four survived. Eight died showing signs of the infection before death, such as loss of hair, conjunctivitis, etc., and of these the cause of death was shown in five cases by post-mortem examination, and mouse inoculation to be the spirillar infection. The other three rats exhibited clinical signs of the experimental infection, but other diseases were not excluded. This question of the effect of insufficient dosage in prophylaxis is being further investigated.

Similar cases have been reported occurring after insufficient prophylactic treatment for syphilis. If infection has occurred, the least evil following such a course is masking or absence of a primary sore, with later lesions appearing after some time. The prophylactic course given by many clinicians consists of one to three doses of "914" intravenously. Cases have been reported of infection developing after this amount, and, indeed, one is forced to the conclusion that the only certain prophylactic course consists of six to eight intravenous injections of "914."

At a meeting in Paris, where this question was fully discussed, failures were reported after various drugs, and it was repeatedly stated that (after ascertaining the patient has no latent syphilis) a full course of large doses of "914" intravenously must be given at least (Pinard, Vernier and Corbillon, 1927).

SECONDARY AND LATENT STAGES

The secondary and latent stages bear out the resemblance of the course of the experimental disease in rats to

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that of syphilis in the human subject. More active treatment is required than for prophylaxis, and the minimum amount increases as the age of the infection. The curative ratio becomes less favourable as the disease gains a firmer hold of the tissues. In both infections the subject may appear well in the latent stage, and have no signs of disease except a positive Wassermann. In both infections, too, spontaneous recovery seems unknown. Most of the rats die within nine months, but a few of the writer's have lived for twelve months, giving to the end a positive Wassermann reaction.

Thus the course, manifestations, blood serum reaction and response to "914" of the experimental infection of rats with the *Sp. minus* resembles closely that of *Sp. pallidum* infection in the human.

In order to provide a standard of comparison for the pentavalent arsenicals, stovarsol and tryparsamide were also tested.

STOVARSOL

Acetyl-oxyamino-phenyl-arsenic-acid, or stovarsol,* was first prepared by Professor Fourneau (1921). It has been used in the treatment of syphilis, yaws, tertian malaria, trypanosomiasis, amœbic dysentery and numerous other diseases with varying success. It has not, as

TABLE VI

Rat No.	Day 1.	Day 3. Dose of drug in grms. per kilo. body-weight.	Result.
56	Inoculated with <i>Sp. minus</i> .	—	Spirilla appeared in blood on 6th day.
60	"	—	Spirilla appeared in blood on 7th day.
69	"	0.2	Spirilla appeared in blood on 11th day.
68	"	0.3	Spirilla appeared in blood on 14th day.
31	"	0.4	Spirilla never found in blood. Mouse infected on inoculation with blood of rat.
81	"	0.8	Spirilla never found in blood. Mouse not infected on inoculation with blood of rat.

* Supplied by May and Baker, Battersea, London; also the Tryparsamide and Parosan used.

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far as the writer knows, been used in rat-bite fever affecting man.

For administration the drug was dissolved in sterile water and delivered directly into the stomach of the animal by means of a syringe with a wide-bore needle attached, the needle having had the end filed smooth, so as to obviate injury to the œsophagus. The rats were inoculated as described above.

The minimum lethal dose was not found, as the amount of water required to dissolve large doses of the drug was too great for satisfactory oral administration. The most given was 0.8 gm. per kilo. body-weight on three successive days. After this dosage the animal did not even lose weight.

PROPHYLAXIS

The smallest prophylactic dose was found to be 0.8 gm. per kilo. body-weight (see Table VI). This gives a chemotherapeutic index of:—

$$\frac{2.4}{0.8} = \frac{3}{1} = 3 \text{ to } 1.$$

Doses up to 0.2 gm. per kilo. inhibited the appearance of spirilla in the blood for three to four days beyond the time of appearance in the controls. No spirilla were ever found by dark-ground examination of the blood in a case which received 0.4 gm. per kilo., but mice were infected on inoculations with the animal's blood.

SECONDARY AND LATENT STAGES

When the infection has become established three doses of 0.4 gm. per kilo. of stovarsol will effect a cure. This gives a ratio of over 2 to 1. Very much smaller doses (0.04 gm. per kilo.) will clear the blood of spirilla in twenty-four hours, but relapse occurs within a few days (see Table VII).

TABLE VII
Example of Relapse

Day.	1	2	3	4	5	6	7
Dark-ground examination for spirilla . . .	+	—	—	+	—	+	—
Stovarsol, grams per kilo. body-weight . . .	0.04	—	—	0.04	—	0.04	—

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No bad effects were seen with even the largest doses administered.

DISCUSSION OF THE FINDINGS

Stovarsol compares favourably with "914" preparations in the treatment of experimental rat-bite fever. The amount required for prophylaxis or cure is greater, but the chemotherapeutic index is only very slightly less.

The Japanese authors (Abe and Shimoda, 1927) inoculated fluid containing *Sp. minus* into the eyelid or scrotum of rabbits. Stovarsol was given by the mouth. They report cure with 0.2 to 0.35 gm. per kilo. body-weight in one dose, or with three doses of 0.05 gm. or six of 0.025 gm. per kilo. at intervals of two to three days. The writer has not been able to obtain cure with such doses in the rat.

It seems reasonable to suppose that stovarsol would be effective in the treatment of rat-bite fever in the human subject.

TRYPARSAMIDE

The sodium salt of *n*-phenyl glycinamide *p*-arsenic acid, or tryparsamide, was administered, dissolved in sterile water, through the subcutaneous route.

The minimum lethal dose was found to be 1.0 gm. per kilo. body-weight.

PROPHYLAXIS

Prophylaxis was unsuccessful even with large and repeated doses (see Table VIII). Even a dose of 0.5 gm. per kilo. would simply delay the time of appearance of spirilla in the blood. It was noted that administration of the drug on the day after inoculation of the animal with *Sp. minus* had a greater effect in delaying the appearance of spirilla in the blood stream than much larger doses given three to five days after inoculation. In no case did the spirilla appear in the blood during a prophylactic course of injections, but inevitably two to three days after the last injection a dark-ground examination would disclose their presence.

SECONDARY AND LATENT STAGES

Treatment of the secondary and latent stages was equally unsuccessful. Spirilla could be eliminated from

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the blood (judging by dark-ground examination), but only temporarily, except when the course was exceptionally prolonged. Then a latent stage might supervene, but the tests of cure always showed the failure of the injections to destroy all the spirilla in the body.

These results are comparable with the known weak spirochæticidal power of this preparation in the treatment of *Sp. pallida* infection in the human subject. In spirochæticidal and spirillicidal power, as exemplified in syphilis and in experimental *Sp. minus* infection respectively, these three drugs may be placed in the following order: first, the "914" series; second, stovarsol; and third, tryparsamide. It seems reasonable to conclude, therefore, that other arsenical preparations will show by their spirillicidal activity what their spirochæticidal powers may be also. Working on this basis, the writer examined a recently introduced drug named "parosan."

TABLE VIII

Rat No.	Days after inoculation with <i>Sp. minus</i> .										Result.
	1	2	3	4	5	6	7	8	9	10	
	Tryparsamide in grms. per kilo. body-weight.										Spirilla appeared in blood on— 11th day. 10th " 9th " 5th " 7th " 14th " 14th " 19th " 10th " 11th "
9											
10											
11											
3	0.3										
4	0.4										
5	0.5										
6	0.6										
7	0.7										
29	—	—	0.4	—	0.4	—	0.4				
34	—	—	—	—	0.8	0.8	0.8				

PAROSAN

The structural formula of this preparation is shown by the following diagrams:—

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The formula is thus 8-acetyl-amino-3-hydroxy 1:4-benzisoxazine-6-arsenic acid. Parosan was tested in a similar manner to the above described drugs. All doses were given by the mouth.

The minimum lethal dose for guinea-pigs was found to be 0.8 gm. per kilo. Survival in rats was noted after as much as 4.0 gm. per kilo, but doses of this size were extremely difficult to administer orally as the amount of water required to dissolve the parosan was rather large; 3.0 gm. per kilo. body-weight was taken as the minimum lethal dose for rats.

PROPHYLAXIS

Prophylaxis was unsuccessful, even with large and repeated doses, but even small doses had some effect, as evidenced by delay in the appearance of spirilla in the blood.

SECONDARY AND LATENT STAGES

Doses of 0.4 gm. per kilo. cleared the blood of spirilla (as judged by dark-ground examination) within twenty-four hours. In four to five days the organisms were again present. Large amounts, in single or repeated doses, might clear the blood permanently, a "latent" stage then supervening. No cure was obtained in any case.

The toxicity of this drug is very low, both as regards single and repeated doses. The writer has given as many as seventeen doses of 0.5 gm. per kilo. orally within a month to a rat without causing any apparent poisoning. Indeed, the weight increased during such treatment in almost every case. In a few cases it was found that a previously positive Wassermann was abolished during an intensive course, but the reaction always reappeared later.

The administration of parosan orally seems to be at least as potent in the experimental infection with *Sp. minus* as tryparsamide intramuscularly. Ewins and Everett (1928) tested this preparation orally in *Tr. equiperdum* infection. The minimum tolerated dose by this route was 16 gm. per kilo. for mice, and 0.5 gm. per kilo. for rabbits. In cases of human primary syphilis as much as 1.0 to 1.5 gm. were given orally per day without untoward results, but equally without curative action.

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Ewins and Everett further state that the product has been tried in disseminated sclerosis with encouraging results, and in a "few cases of yaws (in which disease it appears to be only feebly active) and also in sleeping sickness. Preliminary reports suggest that its effects in the treatment of the latter disease on oral administration are not pronounced, but that the product appears to be trypanocidal when injected intravenously as the sodium salt." They point out the "comparative freedom from the possibility of the production of amblyopia or other neurotropic symptoms."

The above results make the preparation seem worth an extended trial in the later stages of human syphilis. This is now being carried on by Mr. Lees, who is also using it in the treatment of disseminated sclerosis.

SUMMARY

(1) The writer has examined the similarity between experimental infection of laboratory animals with *Sp. minus* and *Sp. pallida* infection in the human subject.

(2) The value of this experimental disease for testing drugs with a view to their use in human syphilis is discussed.

(3) The "914" series of drugs and stovarsol exercise a similar effect on both infections.

(4) Tryparsamide is found to be ineffective as a prophylactic and therapeutic agent, but displays some spirillicidal action. Similarly, its spirochæticidal action is weak.

(5) The results of treatment with "parosan" are noted, and the trial of this drug is suggested in certain cases of neurosyphilis.

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