ADDRESSES AND ORIGINAL ARTICLES

TREATMENT OF HUMAN PUERPERAL INFECTIONS, AND OF EXPERIMENTAL INFECTIONS IN MICE, WITH PRONTOSIL*

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EARLY in 1935 a startling chemotherapeutic success was announced by Domagk [1] in Germany. Hæmolytic streptococci of human origin were injected into the peritoneum of 26 mice. An hour and a half later 12 of them received by stomach-tube a single dose of a dark red dye, the hydrochloride of 4′-sulphamido-2 : 4-diaminoazobenzol—which had been synthesised by Mietzsch and Klarer (see Hörlein [2]), and all survived, at any rate for seven days. (Their ultimate fate is not stated.) Of the remaining 14 animals which served as untreated controls 13 were dead of their streptococcal infection within three days, and the last 1 on the fourth day. It is of interest to note that some of the treated animals received only 0·02 mg. of the drug—i.e., at least 100 times less than the maximum tolerated dose. So far as we are aware this is the only animal experiment which has been reported from Germany. Domagk described the substance—which was named Prontosil—as showing an "elektive Wirkung" upon streptococcal sepsis but as having some action also on staphylococcal infections in the rabbit.

In France, Levaditi and Vaisman,[3] using a similar compound synthesised by Girard and working with a streptococcus "M" of human origin, obtained curative results in mice which were somewhat similar to those of Domagk but less completely successful. The treated animals did not as a rule survive indefinitely after a single dose of the drug but lived a few days longer than the controls. A little later Nitti and Bovet [4] showed that with hæmolytic streptococci of comparatively low mouse-virulence, freshly isolated from human infections, very little or no curative effect was obtained in mice, while with a strain of high mouse-virulence definite prolongation of life was obtained as in Levaditi and Vaisman's experiments. In their most recent paper Levaditi and Vaisman [5] have claimed that by the subcutaneous administration of a large dose (50 mg.) of prontosil in suspension, mice are frequently protected against a fatal dose of streptococcal culture injected 5–10 days later.

In addition to the reports of animal experiments, there have appeared about a dozen papers in Germany referring to the use of the drug in human infections—e.g., erysipelas, puerperal fever, and so forth (Schreus,[6] Anselm,[7] Schranz,[8] Scherber,[9] Fuge,[10] Kramer,[11] and others). These clinical reports are unanimously favourable, but their evidential value must be regarded as small since, in most cases, the recovery of patients is unhesitatingly ascribed to the treatment, and too little allowance is made for the tendency to spontaneous cure of these infections. The bacteriological and clinical data supplied are nearly always very scanty—e.g., we are not told whether the cases were all infected by hæmolytic streptococci, whether those organisms were present in the blood before the treatment was commenced, nor in how many of the cases there was present any clinical condition, such as generalised peritonitis, which habitually connotes a very high mortality. The papers do serve, however, to indicate that the drug is well tolerated by the human subject and what dosage has given apparently good results.

Laboratory Experiments

The following laboratory experiments and clinica trials have been carried out at Queen Charlotte's Hospital.

CURATIVE EXPERIMENTS ON MICE

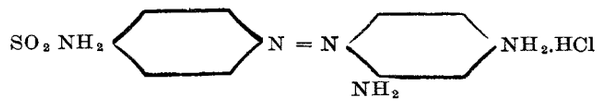
Trials were first made with strains of streptococci freshly isolated from human puerperal infections—i.e., after only two or three passages upon artificial nutrient media.

Mice were inoculated into the peritoneum with an amount of culture which preliminary experiments had shown to contain approximately 10–100 minimum lethal doses. A single dose of prontosil or the more soluble related compound (issued for a time under the name of Streptozon S) was given 1½ or 2 hours after, either by stomach-tube or by subcutaneous injection. In later experiments a series of doses was given—e.g., 1½, 5, 24, 48, 72 hours, and so forth after the injection of culture.

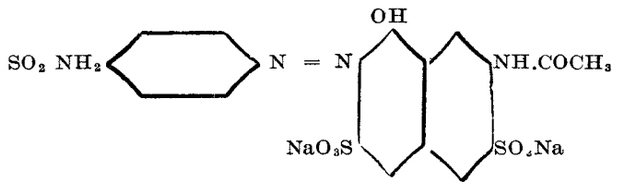
Results.—Although occasionally the treated animals survived a little longer than the untreated controls, there were practically no survivals, and the experiments were regarded as negative, failing to confirm Domagk's claim. Six different strains were employed in such tests.

It is important to note that Domagk's original paper refers to two quite distinct substances, both of which gave curative effects in infected mice. The one, prontosil, has the structural formula shown below (I.) and is only slightly soluble in water (to 0·25 per cent.); the other, issued for a time under the name Streptozon S, but now as prontosil solubile, is the disodium salt of 4′-sulphamido-phenyl-2-azo-7-acetylamino-1-hydroxynaphthalene 3 : 6-disulphonic acid and is represented by the formula II. below. This is soluble up to 4 per cent.

Formula I



Formula II



In our animal experiments we have used only prontosil solubile obtained from Germany, and *the French equivalent of prontosil* prepared by Girard. It is possible that the latter substance differed slightly from that used by Domagk in his published experiment. For the *clinical* trials reported in this paper both substances have been administered to every patient—prontosil by the mouth and prontosil solubile by injection—and both had been prepared in Germany.

* A preliminary report to the Therapeutic Trials Committee of the Medical Research Council.

TABLE I.—*Curative Effect of Prontosil Solubile*

	Deaths in each 24-hour period.									
	1	2	3	4	5	6	7	8	9	
4 mice were infected with 4000 streptococci ("Richards") intraperitoneally; and 1½ hours later received 7·5 mg. of prontosil solubile subcutaneously. Further doses were given after 1¼ hours, 5 hours, and 1, 2, 3, 4, 6 days.	0	0	0	1	0	0	0	0	0	3 remained well and were killed on 60th day.
4 mice were infected as above; and received prontosil solubile (15 mg.) by stomach-tube 1¼ hours later—and also the following day.	1	0	2	1	..	..	..	..	..	—
7 control mice (no prontosil). 4 infected with 4000 streptococci (approx.)	1	3	..	..	..	..	..	..	..	—
7 control mice (no prontosil). 3 infected with 400 streptococci	0	2	0	0	0	0	0	0	0	1 survived.

At this point we were informed by the courtesy of Dr. Buttle, of the Wellcome Physiological Research Laboratories, that he had obtained more success with a streptococcal strain "Williams" which we had formerly isolated from a puerperal fever case and sent to him. This strain differed from those we had previously employed in that it had been transmitted through a series of 23 mice and had acquired a very much higher virulence for those animals. Our next experiments were therefore carried out with a similar highly virulent passage strain "Richards" (a different serological type from "Williams"). With this strain we began at once to get striking curative results in mice, although the animals only survived indefinitely if a series of 6 or 7 doses was given over a period of several days. Typical results are given in Tables I. and II.

Comment.—The results shown in these tables—and others like them, not set out here—seem to indicate quite clearly that the administration of the drug does exert some curative effect upon infections by these hæmolytic streptococci in the mouse, which normally terminate in peritonitis and septicæmia. They can be checked in the majority of the animals if treatment is commenced within three hours of the injection of culture. If delayed much beyond that time the death of the animals may be postponed for a day or two but it is not usually avoided. It is of interest to note that one mouse survived after treatment although hæmolytic streptococci were already present in the circulating blood in considerable numbers (a small drop of blood from the tail gave colonies equivalent to 400 per c.cm.) *before the drug was administered.*

PROPHYLACTIC EXPERIMENTS ON MICE

Mice were injected subcutaneously with 50 mg. of prontosil (kindly supplied by Dr. Girard) in aqueous suspension (10 per cent.)—and four days later were given a dose of streptococcal culture into the peritoneum. The mice showed no toxic effect of the drug at any time. The results are shown in Table III. It will be seen that whereas 9 out of 12 of the animals which did not receive the drug were dead within three days, only 2 of the 12 treated mice succumbed to streptococcal infection within that period. (Two died somewhat later.)

When the surviving animals were subsequently killed there was a large deposit of undissolved prontosil at the site of injection, and it seems probable that the prophylactic effect shown in the table was due to slow absorption from this depôt. The urine had an orange colour during the whole 26 days after the mice received the drug.

ANIMAL EXPERIMENTS WITH *p*-AMINOBENZENE-SULPHONAMIDE

In view of the discovery by Trefouel and his collaborators [12] (since confirmed by Goissedet and others [13]) that the diazo linkage in prontosil was not essential for its therapeutic efficacy in animals and that the parent sulphonamide (a colourless compound) was equally effective, we have carried out a few experiments with this latter compound kindly prepared for us by Dr. Harold King, F.R.S., of the National Institute for Medical Research.

The curative effect upon infections by the "Richards" strain is clearly shown in Table IV. No prophylactic effect was obtained when 40 to 50 mg. of the drug was given in aqueous suspension subcutaneously four days before the injection of culture. Probably this was due to absorption of the drug from the subcutaneous depôt more rapidly than in the case of prontosil.

TABLE II.—*Curative Effect of Prontosil Solubile*

	Deaths in each 24-hour period.										
	1	2	3	4	5	6	7	8	9	10	
8 mice were infected intraperitoneally with approx. 2160 streptococci ("Richards") and 1½ hours later received 7·5 mg. of prontosil solubile subcutaneously. Further doses were given after 5 hours, 20 hours, and 2, 3, 4, 6 days.	0	0	0	0	1	2	0	0	0	0	5 remained well and were killed on 42nd day.
6 mice were infected with streptococci as above; and received the first dose (7·5 mg.) of prontosil solubile 3 hours later. Further doses were given after 7½ hours, 24 hours, and 2, 3, 4, 6 days.	0	0	0	0	0	0	0	0	0	2	4 remained well and were killed on 42nd day.
6 control mice (no prontosil). 4 infected with 2160 streptococci (approx.)	2	0	1	0	0	0	0	0	0	0	1 survived.
6 control mice (no prontosil). 2 infected with 216 streptococci (approx.)	0	2	0	0	0	0	0	0	0	0	—

TABLE III.—*Prophylactic Effect of Prontosil*

		Deaths in each 24-hour period following infection.						
		1	2	3	4	5	6	—
12 mice received 50 mg. prontosil subcutaneously.	6 were infected 4 days later with 396 streptococci ("Richards" strain) into peritoneum.	0	1	0	0	0	1	4 remained well and were killed on 22nd day after infection. (1 had a streptococcal abscess, the other 3 were normal and cultures sterile.)
	6 were infected with 3960 streptococci (same strain).	0	1	0	0	0	0	1 died on 17th and 1 on 20th day. 3 remained well and were killed on 22nd day. (Cultures sterile.)
12 control mice (no prontosil).	6 were infected with 396 streptococci as above.	0	4	1	0	0	0	1 survived.
	6 infected with 3960 streptococci.	0	4	0	0	0	0	2 survived.

Upon what Does the Antistreptococcal Influence of Prontosil Depend?

Although there can be no doubt as to the curative effects of the drug in mice its mode of action is at present obscure. The chief positive facts which emerge are that multiplication of the streptococci in the peritoneal cavity is prevented, and that this happens quickly—within a few hours. At present it appears very unlikely that the cocci are actually destroyed, either by the drug itself or by some compound formed from it in the animal body.

EXPERIMENTAL DATA

(1) Although the 2·5 per cent. solution used for treatment kills hæmolytic streptococci slowly (1–3 hours) it does not do so if diluted slightly with serum. A tenfold dilution in human serum just prevented multiplication of the cocci; a 1 in 50 dilution allowed multiplication of 57 cocci to 500,000—i.e., very much less than in control serum without prontosil.

(2) The serum of rabbits taken at intervals—e.g., ½ hour, 2 hours, 5 hours, and 24 hours after a large intravenous dose of prontosil solubile (8–10 c.cm.)—was unable to kill even a very small number of hæmolytic streptococci ("Richards" strain). These grew out as freely as in the serum taken before the drug was given.

(3) The serum of puerperal fever patients taken at intervals after doses also showed no bactericidal effect, but the outgrowth of the cocci in such sera was definitely less vigorous than in the serum taken before treatment. Example: 5 cocci of the strain isolated from Case 26 planted in her serum before treatment began grew out by the following day to 54 millions per c.cm. The same implant of cocci in serum taken one hour after the first intravenous dose (20 c.cm.) grew to 4 millions per c.cm. In serum taken two hours after the third dose (20 c.cm. intramuscular) they grew to 650,000 per c.cm. In serum taken on the third day of treatment—one hour after the fourth dose (20 c.cm. intramuscular)—they grew to 100,000 per c.cm. A similar restricted outgrowth was obtained with the sera of two other patients.

It may be noted that this growth-retarding influence of the patients' sera does not come into operation until after the first four hours of incubation.

Nor is there any evidence that administration of the drug promotes more active killing of the streptococci by the *whole blood*. This has been tested with the defibrinated blood of treated mice and rabbits—the streptococcal population in the bactericidal mixtures being sampled at different intervals—from 1 hour to 24 hours. On no occasion were we able to detect any killing of the "Richards" strain cocci and on several occasions the blood taken after treatment actually allowed more growth than that taken before.

Human *leucocytes* are but little affected by the drug. A concentration of about 1 in 10 of prontosil solubile, 2·5 per cent., in blood is necessary to diminish their bactericidal activity (as indicated by the killing of staphylococcus in slide cells); and we have failed to find satisfactory evidence that at any weaker concentrations their activity is enhanced.

The leucocyte count of patients and of animals under treatment has shown somewhat variable results.

In two patients out of three at the beginning of their treatment there occurred a rise after the first dose (5600 to 9300 in one; 15,700 to 20,000 in the other) and a subsequent fall towards the original level during the next two days. In the third patient there was a fall after the first dose (14,700 to 10,400) and the lower level was maintained. In two mice and one rabbit the count fell for some hours following relatively large doses (7·5 mg. and 250 mg. respectively).

Apart from the slight growth-retarding influence exhibited by the serum of treated cases there is therefore not much to suggest that either the blood fluids or the blood-cells play a predominant part in checking the invasion of the tissues by the streptococci. Nor have we been able to find any indication that the invasive character of the streptococcus is

TABLE IV.—*Curative Effect of p-Aminobenzenesulphonamide in Mice*

	Deaths in each 24-hour period.							
	1	2	3	4	5	6	7	—
3 mice were infected intraperitoneally with 30,000 streptococci ("Richards"); and 3 hours later were given 5·25 mg. of sulphonamide subcutaneously. Further doses were given after 9 hours, 24 hours, and 2, 3, 4, 5 days.	0	0	0	0	0	0	0	1 died of streptococcal infection on 32nd day. The other 2 were killed on 35th day and showed no infection. (Cultures sterile.)
3 mice were infected with 30,000 streptococci and received 9 mg. of sulphonamide subcutaneously at intervals stated above.	0	0	0	0	0	0	0	All remained well for 34 days and were killed. (Cultures sterile.)
9 control mice (no sulphonamide). 3 infected with 30,000 streptococci ..	0	2	0	0	0	0	0	1 survived.
2 ,, ,, 3,000 ,, ..	0	2	0	0	0	0	0	—
2 ,, ,, 300 ,, ..	0	2	0	0	0	0	0	—
2 ,, ,, 36 ,, ..	1	1	0	0	0	0	0	—