TREATMENT WITH SULPHAPYRIDINE (M & B 693) OF GUINEA-PIGS IN- FECTED WITH BRUCELLA ABORTUS

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In a previous paper (Wilson and Maier, 1939) evidence was brought to show that the administration of sulphanilamide twice daily in a dosage bordering on the toxic limit to guinea-pigs infected seven weeks previously with a virulent strain of *Br. abortus* was able to bring about sterilization of the tissues after treatment for three to five weeks. The total daily dosage used corresponded to an average of 0.8 mg. per gramme of body weight. Animals given half this dosage still remained infected after two to four weeks' treatment.

These results receive confirmation from the work, in the United States, of Chinn (1939), who, starting treatment one week after inoculation with *Br. abortus*, *Br. melitensis*, or *Br. suis*, and using twice-daily doses of sulphanilamide amounting in the aggregate to 1 mg. per gramme of body weight, achieved sterilization of the tissues in twenty-one out of twenty-eight guinea-pigs treated for twenty-four days. On the other hand, they are quite incompatible with the findings of Menefee and Poston (1939), who started treatment five days after a large inoculation with *Br. melitensis*, gave a dosage of sulphanilamide of only 0.06 mg. per gramme of body weight (60 mg. per kilo) for ten days and then 0.03 mg. per gramme for a further ten days, and succeeded in sterilizing the tissues of nineteen out of twenty infected guinea-pigs. Since Chinn found that animals infected with *Br. melitensis* tended to be more refractory to treatment than those infected with *Br. abortus* or *Br. suis*, the most probable explanation of Menefee and Poston's results is that they were using a comparatively avirulent strain of *Br. melitensis*.

These results are conflicting, but on the whole it looks as if successful treatment of guinea-pigs infected with *Brucella* can be achieved by sulphanilamide only after prolonged treatment with large doses. Since sulphapyridine is said to be less toxic than sulphanilamide (Wien, 1938), it seemed desirable to make observations on this drug in the hope that cure might be obtained without the risk of poisoning the animals. The following experiments were therefore undertaken.

Experiment 1

On March 17, 1939, fourteen male and fourteen female guinea-pigs, weighing between 240 and 520 grammes, with an average weight of 340 grammes, were inoculated intramuscularly into the left thigh with 1,000,000 living organisms washed off from a two-day liver-agar culture of a virulent CO₂-sensitive strain of *Br. abortus* (Lanyon). They were then divided up into five groups, A, B, C, D, and E, of approximately equal weight. Groups A, B, and D contained each two bucks and two does, Group C contained three bucks and three does, and Group E, representing the control group, contained five bucks and five does.

Six weeks later, April 27, treatment of the first four groups was begun. It was originally intended to give Groups A, B,

and C 200-mg. doses of sulphapyridine (2-sulphanilyl-amino-pyridine) by mouth *twice a day* on six days a week for two, four, and six weeks respectively, but owing to the acute toxæmic death of one animal on the fourth day of treatment, and the partial paralysis of some of the others, the dosage was halved for a few days. It was later increased to 150 mg. twice a day, and then, on account of the death of two more animals from presumably chronic toxæmia, it was again reduced to 100 mg. twice a day. The dosage of the individual animals was varied considerably according to their condition, and sometimes had to be stopped for two or three days at a time. The sulphapyridine, which had been dispensed in tablets, was given in powdered form by means of a special glass funnel introduced into the mouth at the back of the tongue. Following the practice of Chinn (1939) it was washed down with two or three drops of 5 per cent. glucose solution.

The treatment of Group D had similarly to be altered. It was intended to give these animals subcutaneous inoculations, *twice daily* on six days a week for a fortnight, of 100 mg. of soluble sulphapyridine, made up in a 33½ per cent. solution, but owing to the acute toxæmic death of one animal on the fourth day of treatment the dosage was halved. Many of the animals in this group developed dry gangrene of the extremities, and when half of them were killed after a fortnight they were found to be suffering from large axillary abscesses containing necrotic masses of tissue. For this reason the injections were discontinued and the remaining animals were given two daily doses of 100 mg. of sulphapyridine by the mouth for another fortnight. Half the animals in Groups A, B, and C and all the animals in Group D were killed the day after their last dose; the other half were killed a week later. Two to five control animals were killed at the same time as each subgroup of infected animals.

In making the bacteriological examination the animal was anaesthetized with ether, the thorax opened, and blood taken for agglutination from the inferior vena cava. The great vessels were then cut and the animal was allowed to bleed to death. The spleen and sublumbar glands were excised, ground up separately in Griffith tubes, and suspended in N/4 Ringer solution; viable bacterial counts were then made on the suspensions by the roll-tube method. In addition the macroscopic appearance of the glands, spleen, and liver was carefully noted, particular attention being paid to the degree of enlargement of the organ as a whole and to the number and size of any focal lesions that might be present. The results were recorded in a series of index numbers varying from 0 to 4. The summarized results are given in Table I. (The figures in both tables represent arithmetic mean values, with the exception of agglutinin titres, for which the geometric mean has been used.)

The macroscopic appearance of the sublumbar glands did not differ greatly in the treated and control animals, but the involvement of the spleen and liver, particularly in Groups A, B, and C, was considerably less than in the corresponding controls. Bacteriologically the sublumbar glands proved sterile in all the treated animals except two belonging to Group A. The spleens of two animals in Groups B and D and of all animals in Group C were likewise sterile. The sublumbar glands of three of the control animals proved sterile, but the spleens were all heavily infected. The agglutinin titres varied greatly from animal to animal, but were lowest in Group C. Little difference was observed between the animals killed the day after and those killed a week after the cessation of treatment, suggesting that the effect of the drug was well maintained. It is worth noting that two animals in Group B and two in Group C showed no macroscopic lesions whatever and yielded no growth of *Br. abortus* on culture.

There is little doubt, from this experiment, that the treatment with sulphapyridine favourably influenced the course of the infection. The treatment with the soluble product, especially when followed by sulphapyridine by mouth, gave much the same results as treatment for the same length of time by oral administration.

TABLE I.—Experiment 1: Comparison of Treated and Control Guinea-pigs

Group	Average Daily Dosage	Treated								Control (Group E)						
		No. of Animals	Average Weight of all Animals at Start	Macroscopic Index Nos. (Maximum 4)			Colonies		Agglut. Titre	No. of Animals	Macroscopic Index Nos. (Maximum 4)			Colonies		Agglut. Titre
				Subl.	Spl.	Liver	Subl.	Spl.			Subl.	Spl.	Liver	Subl.	Spl.	
A	200 mg. sulphapyridine for 2 weeks ..	4	345 gm.	0.75	1.0	0.25	310	1,440	1/4,280	3	1.0	3.0	1.3	3,730	39,000	1/18,940
B	230 mg. sulphapyridine for 4 weeks ..	4*	335 gm.	0.5	0.25	0	0	130	1/3,200	2	1.0	3.0	1.5	500	55,000	1/1,630
C	210 mg. sulphapyridine for 6 weeks ..	5†	330 gm.	0.2	0.4	0.2	0	0	1/420	5	1.2	2.4	1.2	920	16,000	1/12,800
D (1)	90 mg. sulphapyridine soluble for 2 weeks ..	1†	300 gm.	1.0	2.0	1.0	0	1,410	1/3,200	2	1.0	3.0	1.5	370	23,000	1/14,600
D (2)	110 mg. sulphapyridine soluble for 2 weeks, 190 mg. sulphapyridine for 2 weeks ..	2	395 gm.	1.0	1.5	0	0	0	1/11,320	1	1.0	3.0	2.0	1,000	72,000	1/830

Subl.=Sublumbar glands. Spl.=Spleen.

* Including two animals that died 1 to 5 days after the cessation of treatment.

† Excluding one animal that died of acute toxæmia.

Note.—The three control animals for Group D were the same as two for Group A and one for Group B.

Experiment 2

This experiment differed from the first only in that treatment was begun three days instead of six weeks after infection. On April 24, 1939, fourteen male and fourteen female guinea-pigs, weighing between 230 and 390 grammes, with an average weight of 270 grammes, were inoculated intramuscularly into the left thigh with 1,400,000 living organisms washed off from a two-day liver-agar culture of *Br. abortus* (Lanyon). The guinea-pigs were then divided into five groups as before, the only difference being that the numbers of animals in Groups B and C were interchanged, B having three bucks and three does, and C two bucks and two does. Treatment of Groups A, B, C, and D was begun on April 27, the dosage being approximately the same as in the first experiment. The animals in Group E were left as controls.

One male in Group B died of acute toxæmia on the third day of treatment and was discarded. One female in Group B died of subacute toxæmia two days before the end of treatment, and one male in Group B died from the same cause a week after the cessation of treatment. One female in Group B died from presumably chronic toxæmia a day after the cessation of treatment. These three animals are included in the analysis of the results, which are summarized in Table II.

The macroscopic lesions were on the whole considerably less in the treated than in the control animals. Bacteriologically the sublumbar glands were sterile in all animals in Groups B and D, in all but one in Group C, and in one animal in

Group A. The spleen was sterile in all animals in Group C, in all but one in Group B, and in two animals in Group D. In all the control animals both the sublumbar glands and the spleen were heavily infected. The agglutinin titres varied greatly from animal to animal, but were very much lower in all the treated groups than in the control group. Little difference was observed between the animals killed a day after and those killed a week after the cessation of treatment, except in the A group, in which the infection seemed to progress somewhat after the drug had been stopped.

It may be noted that three animals in Group B, one in Group C, and one in Group D showed no macroscopic lesions whatever and yielded no growth of *Br. abortus* on culture. One of the three animals in Group B likewise gave a negative agglutination reaction, showing, therefore, no sign of infection whatever. These results are on the whole similar to those in the first experiment.

Discussion

It was both surprising and disappointing in these experiments to find that sulphapyridine was so toxic to guinea-pigs. According to Wien (1938) the toxicity of this drug for rats is only about one-quarter that of sulphanilamide. The acutely toxic dose for rats and mice is stated by Wien to be about 16 mg. per gramme of body weight; yet in our experiments two guinea-pigs died of acute toxæmia on the third and fourth days of treatment respectively after

TABLE II.—Experiment 2: Comparison of Treated and Control Guinea-pigs

Group	Average Daily Dosage	Treated								Control (Group E)						
		No. of Animals	Average Weight of all Animals at Start	Macroscopic Index Nos. (Maximum 4)			Colonies		Agglut. Titre	No. of Animals	Macroscopic Index Nos. (Maximum 4)			Colonies		Agglut. Titre
				Subl.	Spl.	Liver	Subl.	Spl.			Subl.	Spl.	Liver	Subl.	Spl.	
A	200 mg. sulphapyridine for 2 weeks ..	4	295 gm.	1.0	0	0.25	460	24,200	1/280	2	1.0	1.0	2.0	16,200	37,200	1/2,300
B	230 mg. sulphapyridine for 4 weeks ..	5*	280 gm.	0	0	0	0	19	1/16	3	1.0	2.0	2.0	8,500	116,000	1/4,000
C	220 mg. sulphapyridine for 6 weeks ..	4	285 gm.	0.25	0.5	0.75	20	0	1/55	5	1.0	2.4	0.8	910	29,500	1/7,700
D (1)	110 mg. sulphapyridine soluble for 2 weeks ..	2	305 gm.	0.5	0.5	0	0	2,750	1/350	1	1.0	1.0	3.0	15,700	12,700	1/3,200
D (2)	110 mg. sulphapyridine soluble for 2 weeks, 230 mg. sulphapyridine for 2 weeks ..	2	255 gm.	0	0	0.5	0	0	1/70	2	1.0	1.0	2.5	10,500	45,200	1/4,500

Subl.=Sublumbar glands. Spl.=Spleen.

* Excluding one animal that died of acute toxæmia, but including one animal that died two days before the end of treatment, and two that died one to seven days after cessation of treatment.

Note.—The three control animals for Group D were the same as one for Group A and two for Group B.

receiving by the mouth a total daily dose of only 1.5 mg. per gramme. It is interesting to note that one of these animals showed on post-mortem examination extensive retroperitoneal haemorrhages in the lumbar region, free blood in the bladder, and large soft friable kidneys filled in the dilated pelvic portion with greyish stony deposits. The formation of urinary calculi after oral administration of sulphapyridine has been reported by Antopol and Robinson (1939) and by Gross and Cooper (1939) in rats, rabbits, and monkeys, but this is the first time, so far as we are aware, that it has been reported in guinea-pigs.

Two other animals died of subacute toxæmia after receiving by mouth an average daily dose of 0.8 mg. per gramme for four weeks; and three others died, presumably from chronic toxæmia, after an average daily dose of 0.75 mg. per gramme for the same length of time. In some of the animals that died and in one or two others that were killed the kidneys appeared to be congested, or to be rather large, pale, and oedematous, or to contain small haemorrhages; sometimes the pelvis was dilated, though no definite calculi were found.

Apart from actual death from toxæmia, many of the animals showed pareses, which recovered after the treatment was discontinued for two or three days. The animals treated for four to six weeks were mostly in poor condition, and put on only a quarter to half the weight of the corresponding control animals. Those that died late appeared emaciated. Considering that the control animals were found on post-mortem examination to be severely infected with *Brucella*, while the treated animals had nearly all freed themselves from infection, it is clear that the failure to grow normally was due to the toxicity of the drug rather than to the results of infection. In fact, the cure in this instance was worse than the disease.

Subcutaneous inoculation of soluble sulphapyridine proved very unsatisfactory. Owing presumably to the high alkalinity of this product—about pH 11—tissue necrosis rapidly occurred, leading not only to the formation of large local abscesses containing necrotic tissue but to dry gangrene of the corresponding extremities with sloughing of the toes. It seems desirable that this compound, if it is to be used, should be injected intravenously, as Marshall and Long (1939) recommend. As regards toxicity, one animal died of acute toxæmia on the fourth day of treatment after a total daily dosage of 0.55 mg. per gramme of body weight, suggesting that the soluble compound given subcutaneously is at least twice as toxic as the insoluble product given by the mouth.

A comparison of the results described in this paper with those previously reported (Wilson and Maier, 1939) shows very little difference between the therapeutic effect of sulphapyridine and sulphanilamide. In Experiment 2 with sulphanilamide, in which the animals were infected seven weeks before the beginning of treatment, sterilization of the tissues was reached after three to five weeks, using an average daily dose of 0.8 mg. per gramme of body weight. In the present Experiment 1 with sulphapyridine, in which the animals were infected six weeks before the beginning of treatment, sterilization of the tissues was nearly achieved in four weeks using an average daily dose of 0.7 mg. per gramme of body weight, and completely achieved in six weeks using an average daily dose of 0.65 mg. per gramme. As regards toxicity, however, the results were very different. None of the twelve animals treated with sulphanilamide died or showed any serious evidence of poisoning, while of the twenty-eight animals in the two experiments treated by oral administration of sulphapyridine two died of acute toxæmia, two

of subacute toxæmia, and three of presumably chronic toxæmia.

In conclusion, attention may be drawn to the fact that in the animals treated with sulphapyridine the extent and severity of the macroscopic lesions in the tissues were very much less than in the controls. That this should be so with the animals in which treatment was begun a few days after infection is not surprising, but that it should occur when treatment was not started for six to seven weeks after infection is of some interest. At such a time the sublumbar glands are enlarged; the spleen is considerably enlarged, has an irregular surface, and frequently contains necrotic areas; and the liver often shows multiple tiny necrotic foci. The fact that these lesions completely disappeared in two out of six animals treated for three to five weeks with sulphanilamide, and in four out of ten animals treated for four to six weeks with sulphapyridine, shows how rapidly healing may occur and the tissues regain their normal appearance after the infecting organisms have once been destroyed.

Summary and Conclusions

1. Two experiments are recorded in which guinea-pigs infected with *Br. abortus* received treatment with sulphapyridine by the mouth or with soluble sulphapyridine subcutaneously, and were compared with untreated control animals.

2. The original dosage of 1 to 1.5 mg. per gramme of body weight of sulphapyridine by the mouth had to be reduced after three or four days on account of acute toxæmia in some of the animals. Even with a subsequent dosage of 0.6 to 0.8 mg. per gramme by the mouth a few deaths occurred from subacute or chronic toxæmia.

3. The original dosage of 0.55 to 0.7 mg. per gramme of the soluble product subcutaneously likewise had to be reduced on account of acute toxæmia. Later this substance was abandoned altogether on account of its production of extensive local tissue necrosis, followed by dry gangrene of the extremities, due apparently to the high alkalinity of the solution in which it was dispensed. It would seem desirable that this product, if it is to be used, should be given intravenously.

4. Though the extent and severity of the lesions in the internal organs, the number of colonies growing from the sublumbar glands and spleen, and the serum agglutinin titre were favourably affected by an average dose of 0.6 to 0.8 mg. per gramme, given in two portions daily for a fortnight, complete sterility of the tissues was achieved only after maintaining this dosage for four to six weeks.

5. Compared with the results previously reported using sulphanilamide, the animals treated with sulphapyridine by the mouth in approximately equal dosage behaved very much the same. So far as the therapeutic effect is concerned in guinea-pigs there seems little to choose between the two drugs. On the other hand, sulphapyridine appears to be rather more toxic for these animals.

6. In our previous paper we expressed the opinion that, since undulant fever in man is a self-limited disease with a very low case mortality, it is doubtful whether the ordinary patient should be exposed to the dangers accompanying intensive treatment with sulphanilamide. Considering that in the experiments reported in the present paper no fewer than seven out of twenty-eight animals receiving sulphapyridine by the mouth died of acute, subacute, or chronic toxæmia, it seems clear that the caution expressed in regard to the use of sulphanilamide for human patients is equally justified in regard to sulphapyridine.

7. Since both sulphanilamide and sulphapyridine have to be given for some weeks in a dosage bordering on the toxic limit if they are to bring about cure in guinea-pigs infected with *Brucella*, and since Colebrook (1939), among others, has drawn attention to the grave danger attending the use of these drugs for more than a few days at a time, it would seem wise to

reserve this type of therapy for cases of undulant fever that have resisted other forms of treatment.

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PLATELET-REDUCING EXTRACTS OF THE SPLEEN

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When Troland and Lee (1938a, 1938b) reported the preparation from spleens removed from patients with thrombocytopenic purpura of an extract which reproduced the condition in animals hope was felt that we might be approaching an understanding of this very puzzling disease. It subsequently appeared that Torrioli and Puddu (1938) had been working on similar lines since 1933, and they issued a warning that the results were by no means so clear-cut or so specific as Troland and Lee had claimed. More recently Pohle and Meyer (1939) were unable to duplicate Troland and Lee's results in three cases of thrombocytopenic purpura. The rarity of the disease must be our excuse for reporting results in a single case.

A married woman aged 48 presented the classical features of thrombocytopenic purpura. She had had the disease for a year before coming to us, and we kept her under observation for a further three months, until menorrhagia made splenectomy essential. Her spleen showed the negative picture described in thrombocytopenic purpura, and her platelets, which we had never found above 45,000 per c.mm. before operation, rapidly rose to over 200,000. She promptly recovered from all her symptoms.

The spleen, which weighed 250 grammes, was extracted with acetone for seventeen days and, after removing the acetone and making the extract up to 100 c.cm. with distilled water, 5 c.cm. and 20 c.cm. respectively were injected intravenously into two rabbits. Little effect was produced by 5 c.cm., but after 20 c.cm. there was a sharp fall in the number of platelets. We then injected another 45 c.cm. into the first rabbit, and the platelets fell in thirty hours from 525,000 to 109,000 per c.mm.; they had returned to normal by the fifth day. This was, of course, a relatively enormous dose, corresponding to about 112 grammes of fresh spleen. No abnormal symptoms were noted.

Our first control was an acetone extract prepared in the same way from a spleen removed post mortem from a man aged 42 who died of a fractured skull. After intravenous injection into Rabbit No. 3 of 40 c.cm., corresponding to 44 grammes of fresh spleen, the platelets fell

Acetone Extract of Thrombocytopenic Spleen

Hours	Platelets per c.mm.	
	Rabbit No. 1 (Wt. 2.62 kg.)	Rabbit No. 2. (Wt 2.71 kg.)
Before Injection		
66	424,000	553,000
48	462,000	525,000
40	398,000	486,000
24	476,000	501,000
1	484,000	576,000
After Injection	Inject 5 c.cm. I.V.	Inject 20 c.cm. I.V.
1	472,000	368,000
4	408,000	405,000
22	406,000	442,000
24	476,000	471,000
30	495,000	482,000
46	549,000	572,000
70	526,000	
94	525,000	
After Injection	Inject 45 c.cm. I.V.	
1	329,000	
3	321,000	
6	386,000	
22	167,000	
24	130,000	
30	109,000	
48	164,000	
54	214,000	
70	270,000	
72	400,000	
77	391,000	
96	390,000	
120	568,000	

Acetone Extract of Normal Spleen (Rabbit No. 3, Weight, 2.52 kg.)

Hours	Platelets per c.mm.	Hours	Platelets per c.mm.
Before Injection		After Injection	Inject 40 c.cm. I.V.
24	400,000	2	306,000
18	447,000	5	385,000
2	454,000	22	460,000

Ringer's Extract of Normal Spleen

Hours	Platelets per c.mm.	
	Rabbit No. 4. (Wt. 2.55 kg.)	Rabbit No. 5. (Wt. 2.34 kg.)
Before Injection		
42	230,000	422,000
25	254,000	421,000
19	283,000	433,000
1	219,000	351,000
After Injection	Inject 5 c.cm. I.V.	Inject 20 c.cm. I.V.
3	211,000	300,000
7	217,000	246,000
22	231,000	329,000
24	187,000	267,000
30	270,000	315,000
48	256,000	400,000