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Weekly Self-Medication of Leprosy Patients Monitored by DDS/Creatinine Ratios in Urines

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The self-administration of once-weekly doses of 300 mg Dapsone (DDS) by leprosy patients in the Mwanza region of Tanzania was monitored using the urine-test method described by Ellard *et al.* (1974*a*). DDS/creatinine ratios were determined on urine samples voided by 65 supervised leprosy patients on each of 7 successive days following the ingestion of 300 mg DDS. The method was then applied to urine samples collected by means of surprise visits to the homes of 158 out-patients 2 days after the day on which a 300 mg dose of DDS should have been taken. The extent of DDS self-administration by the out-patients was estimated by comparing the results with those obtained from controls given supervised DDS doses and from subjects not taking DDS. Significant amounts of DDS were not detected in the urine samples collected from 30% of the out-patients. Furthermore the average DDS/creatinine ratios of the urine samples of the other out-patients were significantly lower than those from the supervised controls. The implications of these findings to the treatment of leprosy in the Mwanza region and their relevance to other leprosy control schemes is discussed.

Introduction

The successful mass treatment of leprosy is largely dependent on the regularity with which out-patients take the DDS tablets they are given. Furthermore irregularity of DDS treatment is probably one of the main factors that promote the emergence of DDS-resistant strains of *Mycobacterium leprae* (Rees, 1967; Jacobson, 1973). The potential unreliability of daily self-medication of DDS has been demonstrated by Ellard and his co-workers who showed that only about a half of the prescribed DDS doses had been taken by patients in Malawi in the days immediately preceeding their attendance at the clinic (Ellard *et al.*, 1974*b*). Similar irregularity in daily self-medication was found in Ethiopia by Low and Pearson (1974). The effectiveness of costly programmes for the mass treatment of leprosy may therefore be substantially impaired if these findings are typical of the situation in the world's major endemic areas.

The quantitative determination of the ratio of DDS and its acid-labile metabolites to creatinine in the urine provides a simple and relatively sensitive method for monitoring DDS self-administration (Ellard *et al.*, 1974*a*). However,

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in contrast to the patients studied in Malawi and Ethiopia, most leprosy patients in Tanzania are prescribed weekly doses of DDS for self-administration.

This paper describes an investigation of the applicability of Ellard's method to monitor the ingestion of weekly doses of DDS, and an examination of the actual regularity of dapsone self-administration in the Mwanza region of Tanzania.

Methods

COLLECTION OF URINE SAMPLES FROM CONTROLS AND OUT-PATIENTS

Controls. DDS urine samples were collected from 18 adult in-patients (10 male, 8 female) from the leprosy ward of the hospital at Sengerema (Geita district, Mwanza region) and from 49 adult leprosy patients (23 male, 26 female) living in or around the leprosy control centre at Shirati (Tarime district, Mara region). The Sengerema patients were under treatment with weekly doses of 300 mg DDS. The Shirati patients were treated with 200 mg DDS weekly prior to this study. In the last 2 weeks before urine was collected all of these patients were treated with 300 mg DDS once a week, ingested between 09.00 and 10.00 hrs under supervision. Urine samples were collected between 08.30 and 11.00 hrs on the 7 days following the day on which the second supervised dose of 300 mg was taken. The patients were asked to empty their bladders early in the morning prior to passing urine for the required samples. The next dose of DDS was given only after the last urine sample was collected. Blank urine samples were also collected from 67 staff, patients not under treatment with DDS and visitors to the Sengerema and Shirati hospitals.

Out-patients. Urine samples ("field samples") were collected from 158 adult out-patients (80 male, 78 female) living in separate houses in the Mwanza and Kwimba districts of the Mwanza region. Patients were selected who had attended their monthly clinic regularly according to the definition of the WHO Expert Committee on Leprosy (1970). The patients were also selected so as to provide a representative sample from both the small and large clinics throughout each of the 2 districts. Urine samples were collected from these patients by means of surprise visits to their homes between 10.00 and 16.00 hrs 2 days after the day on which the fourth weekly self-administered dose of 300 mg DDS should have been taken. All urine samples mentioned were collected by the authors themselves.

ANALYSES OF URINE SAMPLES.

Urine samples were collected by filling up a small container containing an amount of 2N HCl equivalent to a third of its capacity. The hydrochloric acid served both as a preservative against bacterial growth and as an agent to hydrolyse the acid-labile metabolites of DDS. Previously it had been found that storing such acidified urine samples at room temperature for 1 week did not affect the DDS/creatinine ratios significantly Ellard *et al.*, 1974*a*). During the present study however it was shown that storing urine samples at ambient temperatures for several months influenced the estimations. Comparing aliquots of 31 urine samples stored for 90 days at $-20°C$ with aliquots of the same samples stored at room temperature ($+25°C$), the latter aliquots showed an average decrease in measurable DDS/creatinine ratios of 20%. In this study urine samples were therfore estimated as soon as possible after collection (average delay: 5 days), and only in exceptional cases were stored for a few weeks at room temperature prior to analysis. DDS/creatinine ratios were determined using the method described by Ellard *et al.* (1974*a*). The results from a few samples had to be discarded because

in the determination of acid-labile DDS, protein precipitation occurred after the addition of trichloroacetic acid (about 1% of the samples) or because the presence of sulphonamides was revealed (another 1% of the samples) (Ellard *et al.*, 1974*b*).

Results

DDS/CREATININE RATIOS IN URINES FROM 65 SUPERVISED PATIENTS, COMPARED WITH BLANK VALUES FOUND IN 62 CONTROL URINES

The values found in urines from supervised patients and controls are summarized in Table 1. A distinction was made between the ratios found in urine samples from men and those found in urine samples from women. This is based on the well established fact that the daily excretion of creatinine by women is significantly less than that in men (Documenta Geigy, 1970). The results presented in Table 1 fully justify this distinction and indicate:

1. DDS/creatinine ratios in urines collected on successive days after the ingestion of 300 mg DDS fell relatively slowly. When the ratios were corrected for the mean blank values, the DDS/creatinine ratios fell exponentially with an average half-life of 38 h in both men and women.
2. Higher ratios in urine samples from female patients than in those from male patients.
3. Significant differences between the mean blank values and the mean values of DDS/creatinine ratios in urines from patients taking 300 mg DDS, even on the seventh day after ingestion of the dose.
4. Wide ranges of individual values within each group, so that as early as the third day after ingestion of 300 mg DDS one result overlapped with those obtained from the blank urines.

The ranges, mean values and 95% confidence limits of the means are illustrated in Fig. 1.

DDS/CREATININE RATIOS IN URINES FROM 158 OUT-PATIENTS COMPARED WITH BLANK AND CONTROL VALUES

On the basis of the data summarized in Table 1 urine samples from the out-patients were classified as "positive" or "negative" according to whether the

TABLE 1

DDS/creatinine ratios in urine samples from 65 supervised patients and 62 controls

Days after 300 mg DDS	Number of urine samples		DDS/creatinine ratios (μg/mg)			
			men		women	
	men	women	range	mean ± S_m	range	mean ± S_m
1	32	32	20.0-109.3	73.1 ± 3.0	61.0-138.7	90.1 ± 3.7
2	32	33	14.8- 68.8	50.1 ± 2.0	38.9-107.6	64.9 ± 3.0
3	31	33	8.4- 51.7	33.3 ± 1.8	25.5- 67.2	42.9 ± 2.0
4	32	33	6.4- 40.8	23.1 ± 1.4	15.0- 60.3	29.5 ± 1.6
5	31	33	5.7- 33.8	17.3 ± 1.2	11.5- 57.9	24.2 ± 1.8
6	32	33	3.9- 31.4	12.7 ± 1.2	10.3- 39.4	18.1 ± 1.3
7	31	33	5.3- 19.0	10.1 ± 0.8	4.8- 27.9	12.0 ± 0.9
Blanks	32	30	1.4- 9.8	5.3 ± 0.4	0.0- 12.5	6.0 ± 0.5

Indicated are ranges of values found on each day and mean values with the standard errors of the means (S_m) for every day.

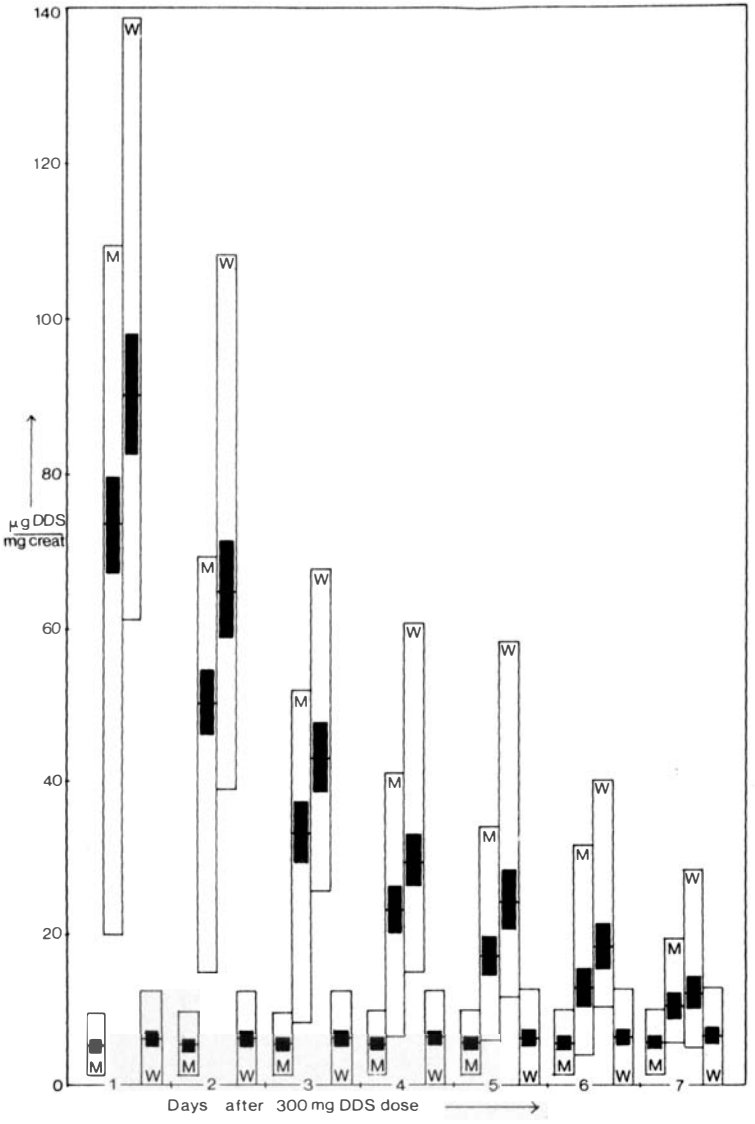

Fig. 1. DDS/creatinine ratios in urines after a 300 mg DDS dose. (□) Range of individual values, M = men. W = women. (■) Mean with its 95% confidence-area.

The ranges and means for each day repeated at the bottom of the figure concern the blank values for men and women.

TABLE 2

DDS/creatinine ratios in urine samples from 158 out-patients in comparison with blank values and second day's standard ratios

Type of urine samples	Number	%	DDS/creatinine ratios (μg/mg) Range	Mean ± S_m
From men				
Blanks	32	–	1.4- 9.8	5.3 ± 0.5
2nd day superv.	32	–	14.8- 68.8	50.1 ± 2.0
Field samples < 10 ("negative")	21	26	2.6- 9.9	6.4 ± 0.5
Field samples > 10 ("positive")	59	74	10.4- 88.3	38.8 ± 2.5
All field samples	80	100	2.6- 88.3	30.3 ± 2.4
From women				
Blanks	30		0.0- 12.5	6.0 ± 0.5
2nd day superv.	33		38.9-107.6	64.9 ± 0.3
Field samples < 12 ("negative")	27	35	0.0- 11.6	6.9 ± 0.6
Field samples > 12 ("positive")	51	65	12.5-105.8	43.4 ± 2.9
All field samples	78	100	0.0-105.8	30.8 ± 2.9

Indicated are ranges of values found in each group and mean values with the standard error of the means (S_m) for each group.

DDS/creatinine ratios (μg/mg) were greater or less than 10.0 (men) or 12.0 (women), respectively. The results obtained when samples were classified in this way are compared in Table 2 with the blank and control values. They indicate that:

1. About 30% of the urine samples were negative and among these samples the mean DDS/creatinine ratios were very similar to those of the blank urines.
2. The mean DDS/creatinine ratios of the positive urine samples were significantly lower than those of the control samples taken 2 days after dosage with 300 mg DDS ($P < 0.0005$).

The frequency distributions of the DDS/creatinine ratios are illustrated in Fig. 2.

Discussion

The organization of the field study was based on the results obtained from the supervised patients and controls. The easiest method of obtaining urine samples from the out-patients would have been to have collected them when the patients attended at the monthly clinic to collect their supply of DDS tablets for the following 4 weeks. However, because of the large overlap between the DDS/creatinine ratios determined among supervised controls 7 days after dosage with 300 mg DDS and the values for untreated subjects (Table 1, Fig. 1), for a high proportion of subjects it would have been impossible to judge with certainty from such urines whether or not the previous dose had been taken. Furthermore it was found that a number of the patients often did not come to the clinics themselves but sent a relative or neighbour to collect their DDS tablets for them. As a

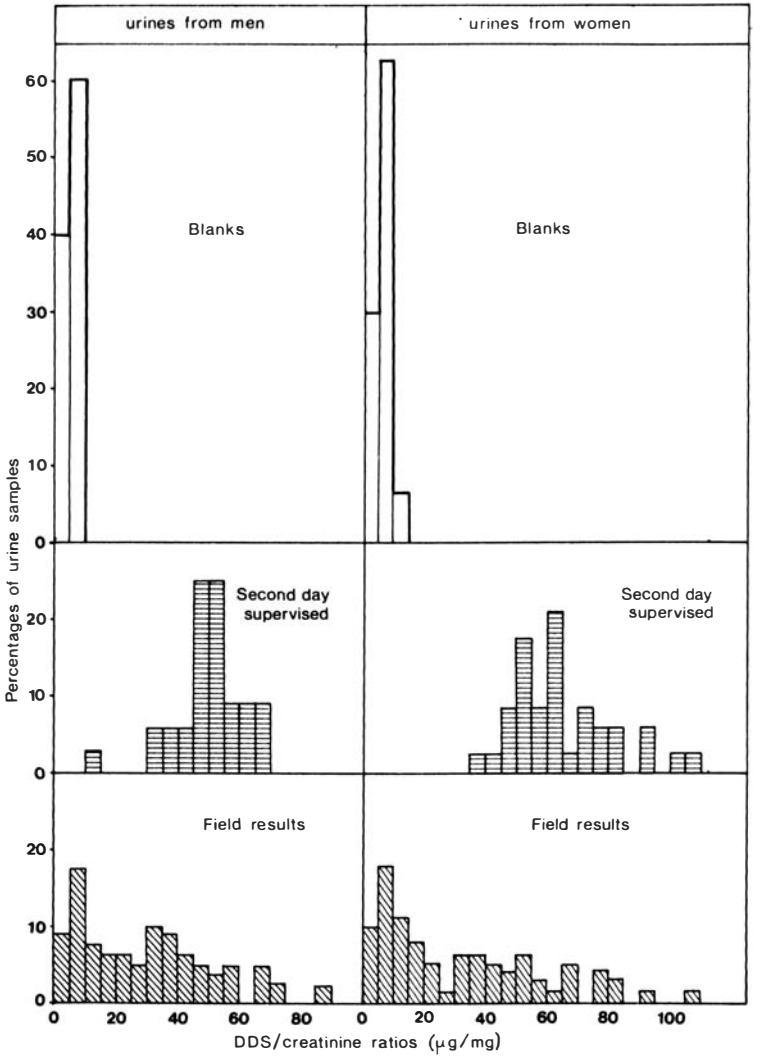

Fig. 2. Frequency distributions of DDS/creatinine ratios.

consequence the more laborious approach of collecting urine samples by means of home visits had to be followed.

End-points for classifying urine samples as positive and negative had to be chosen so that they would misclassify the least numbers of genuinely positive and negative urine samples. The end-points chosen for men and women were 10 and 12 μg DDS/mg creatinine, respectively. If such criteria had been applied to the urine samples collected for up to 4 days from the controls given supervised 300 mg doses of DDS. only 2 of the 258 samples would have been considered as

negative, and only 1 of the 62 blank urine samples would have been classified as positive.

In this study it was decided to collect urine samples 2 days after the day on which a dose of 300 mg DDS should have been taken. Thus positive results would also have been anticipated from patients who had taken only 100 or 200 mg DDS instead of the full 300 mg dose, or who had taken the correct dose a few days too early or one day late. However, it can be reasonably argued that adequate DDS blood levels were probably being achieved in most of those patients from whom urine samples were classified as being positive. Thus Ellard *et al.* (1971) have shown that daily dosage with 1 mg DDS results in the maintenance of DDS blood levels continously in excess of about 0.01 μg/ml and prevents the multiplication of drug sensitive leprosy bacilli in the body. The level of 0.01 μg/ml is considered to be the minimal inhibitory concentration. A dose of 300 mg DDS gives a mean blood level of more than 3 μg/ml (Powell *et al.*, 1967; Glazko *et al.*, 1968). In the present study the mean half-life of DDS determined among the 65 supervised patients was 38 ± 1.2 h for both men and women. This value is significantly higher than those of 21 h (Glazko *et al.*, 1968), 28 h (Peters *et al.*, 1974, 1975), 29 h (Peters *et al.*, 1972) and 31 h (Ellard *et al.*, 1974*b*) reported from determinations of the rates of fall of DDS plasma concentrations. Although the higher DDS half-life determined in the present study was based on renal excretion, DDS blood levels probably fall with the same half-life (Glazko *et al.*, 1968).

It is therefore probable that in the average patient in the Mwanza region of Tanzania DDS blood levels would take about 13 days to fall below the minimal inhibitory concentration against *M. leprae* after giving a 300 mg dose of the drug. Since the DDS/creatinine end-points chosen to discriminate between positive and negative urines were equivalent to the mean ratios in urines from supervised patients 7 days after the ingestion of 300 mg DDS (Table 1, Fig. 1), a positive result indicated that inhibitory DDS blood levels would probably be maintained for at least another 6 days, that is until the next scheduled intake of DDS. Although these calculations are based on mean values, they provide a context from which the possible therapeutic relevance of these results may be discussed.

Similar considerations raise the possibility that a significant proportion of patients providing urine samples that were classified as negative might still have been taking therapeutically significant doses of DDS. However, the similarity between the mean DDS/creatinine ratios of the field urine samples classified as negative and the blank urine samples from the controls (Table 2) indicates that the majority of the negative urine samples were correctly graded. Although no attempt was made in the present study to determine the consistency with which positive or negative urine test results would be given by individual patients, it is probable that the results obtained truly represent the overall picture of DDS self-administration among regularly attending out-patients in the Mwanza region of Tanzania.

If then one keeps the necessary restrictions in mind, it may be said that also *weekly* self-medication of leprosy patients can be monitored by DDS/creatinine ratios in urines. Thus it may be concluded from Table 2 that at any one time about 30% of the out-patients prescribed 300 mg DDS once-weekly are not taking their prescribed weekly doses of 300 mg DDS. The difference between men and women in this regard is not significant. It was also apparent that the mean values of the positive samples were significantly lower than the mean values of the ratios

for control urines taken 2 days after a 300 mg DDS dose. These data may be compared with the results of the study of Ellard *et al.* (1974*b*) in Malawi among patients on a self-medication schedule of 25 mg DDS daily. In the Malawi study 30% of the patients were found to take DDS grossly irregularly. The definitions given in the text are such that this group may be compared with the "negative" groups (30%) in the present study. If one compares our "positive" groups with the rest of the Malawian results, again the results of the 2 studies agree remarkably. The evaluation of the Malawian results in terms of the estimated percentage of doses taken by all out-patients (52-53%) may also be compared with a same evaluation of the present results in which about 50% of the doses were taken. This last figure was confirmed by determining the DDS/creatinine of pools of aliquots prepared from all the urine samples. An exact comparison of our results with those found in Ethiopia (Low and Pearson, 1974) is somewhat more difficult, due to the different way in which the Ethiopian results were presented. The trend of irregularity however is evidently the same.

Carrying out analyses on representative pools of urine samples can reduce the cost of this type of study. On the other hand it also reduces the amount of information gathered. Another way of reducing expenditure is to use the services of local medical helpers to collect the urine samples. This was tried in two other districts of the Mwanza region where 154 urine samples were collected by Health Home Visitors. However, many difficulties were encountered, especially as regards communication and although the overall results did not differ significantly from those presented in Table 2, the authors fear that too much uncertainty was associated with these collections. Nevertheless in some situations local helpers may be able to undertake a great part of the work.

Finally it should be emphasized that the actual situation regarding the regularity of DDS self-administration in the Mwanza region is probably more unfavourable than is suggested by our results. Of the estimated 13,700 leprosy patients in the region only 4312 are registered and of the latter only about 50% attend regularly for treatment. The sample in the present study was taken from this minority who attend regularly for treatment. Those patients who either do not attend at all or who do not attend regularly are obviously not receiving effective treatment.

On the other hand the proportions of these groups of patients still requiring treatment is not known. It is evident however that irregularity is one of the main factors that promote the emergence of DDS-resistant strains of *M. leprae* (Rees, 1967; Jacobson, 1973). Although there are great differences between control schemes, the similarity between the results obtained in the present study and those of studies conducted in Malawi (Ellard *et al.*, 1974*b*) and Ethiopia (Low and Pearson, 1974) suggests that these findings are probably applicable to many other similar schemes. This should be a cause for concern and reflection. Methods of improving the regularity of both clinic attendance and self-medication are clearly required. If such methods cannot be found, the objectives of many current leprosy control schemes may be incapable of realisation.

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References

Documenta Geigy, Scientific Tables, 7th Ed. (1970), p. 665.

Ellard, G. A., Gammon, P. T. and Harris, J. M. (1974*b*). The application of urine tests to monitor the regularity of dapsone self-administration. *Lepr. Rev.* **45**, 224.

Ellard, G. A., Gammon, P. T., Helmy, H. S. and Rees, R. J. W. (1974*a*). Urine tests to monitor the self-administration of dapsone by leprosy patients. *Am. J. trop. Med. Hyg.* **23**, 464.

Ellard, G. A., Gammon, P. T., Rees, R. J. W. and Waters, M. F. R. (1971). Studies on the determination of the minimal inhibitory concentration of 4,4′-diamino-diphenyl-sulphone (Dapsone, DDS) against Mycobacterium leprae. *Lepr. Rev.* **42**, 101.

Jacobson, R. R. (1973). Sulphone-resistant leprosy: Etiology, incidence and treatment in the United States. *Int. J. Lepr.* **41**, 684.

Low, S. J. M. and Pearson, J. M. H. (1974). Do leprosy patients take dapsone regularly? *Lepr. Rev.* **45**, 218.

Peters, J. H., Gordon, G. R., Ghoul, D. C., Tolentino, J. G., Walsh, G. P. and Levy, L. (1972). The disposition of the antileprotic drug dapsone (DDS) in Philippine subjects. *Am. J. trop. Med. Hyg.* **21**, 450.

Peters, J. H., Gordon, G. R., Levy, L., Storkan, M. A., Jacobson, R. R., Enna, C. D. and Kirchheimer, W. F. (1974). Metabolic disposition of dapsone in patients with dapsone resistant leprosy. *Am. J. trop. Med. Hyg.* **23**, 222.

Peters, J. H., Gordon, G. R. and Karat, A. B. A. (1975). Polymorphic acetylation of the antibacterials, sulfamethazine and dapsone, in south indian subjects. *Am. J. trop. Med. Hyg.* **24**, 641.

Powell, R. D., De Gowin, R. L., Bennet Eppes, R., McNamara, J. V. and Carson, P. E. (1967). The antimalarial and hemolytic properties of 4,4′-diamino-diphenyl sulfone (DDS). *Int. J. Lepr.* **35**, 590.

Rees, R. J. W. (1967). Drug resistance of *Mycobacterium leprae*, particularly to DDS. *Int. J. Lepr.* **35**, 625.

World Health Organisation Expert Committee on leprosy, Fourth Report. Technical Report Series No. 459, Geneva 1970 p. 9.