TABLE VI. Effect of Chlortetracycline on Weight and Number of Young (Second Litter of Dams in Table V).

Level of chlortetracycline in dams diet.

Litter No.	Control No./litter	Control Wt/litter, g	0.5 g/kg No./litter	0.5 g/kg Wt/litter, g	2.0 g/kg No./litter	2.0 g/kg Wt/litter, g
1	8	52.0	11	65.0	10	62.0
2	9	60.0	11	62.0	10	59.0
3	9	60.0	8	56.0	2	19.0
4	10	63.0	12	67.0	5	37.0
5	7	36.0	10	68.0	13	82.0
6	5	32.0	3	23.0	8	44.0
7	9	63.0	14	76.0	12	77.0
8	5	33.0	4	32.0	7	50.0
9			13	84.0	13	80.0
10					3	20.0
Avg	7.7	49.9	9.5	59.2	8.3	53.0

than for the young rats that were sacrificed at 24 hours after birth.

The high level of chlortetracycline supplement appeared to aid in conception on second breeding. In the second experiment (Table V), 4 of the 10 females received did not bear young. When these same females were continued on the same supplement while being rebred, all conceived within a 3-week period and went full term (Table VI). In the unsupplemented group conception was slower and only 8 of the 10 females conceived over a period of 3 months.

Summary. Pregnant female rats were fed diets containing different amounts of chlortetracycline. When the prenatal diet contained 2 g/kg the antibiotic was present in the fetal tissues. At levels of 0.5 and 2.0 g/kg of diet, detectable amounts of the antibiotic were present in the mammary glands of the females and in tissues of the suckled young. The higher level of antibiotic (2 g/kg) improved the conception rate but did not affect the size of the fetuses or the number of young per litter.

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Radioactive Tracer Studies on Uptake of Diamino-diphenyl-sulphone by Leprosy Patients. (22875)

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Diamino-diphenyl-sulphone and a number of its derivatives are now well-established in the treatment of leprosy. But there is much confusion as regards the mode of action of sulphone on leprous lesions(1-5). The present radioactive tracer study on uptake of sulphone by leprosy patients was undertaken with the hope that it might throw some new light and may be helpful in removing the confusion. The investigation has been concerned with (a) concentration of the drug in blood, bone-marrow and skin tissues, the rate of excretion through kidneys and (b) the localization, if any, of the drug in affected tissues and invading micro-organisms. This paper deals with the first part of the investigation.

Material and methods. We studied 22 cases of leprosy—8 lepromatous and 14 non-lepromatous. They were adult males, weighing 40-60 kg. Blood volume of each patient was determined by Evans blue and thiocyanate technics. *Diamino-diphenyl-sulphone (DDS)* tagged with S-35 was used. This was procured from Amersham, England and had an initial specific activity of 1 μc/mg. For *oral administration,* a single dose of 4 μc/mg was given as a powder. For parenteral use, 2-7 μc were injected subcutaneously as a 5% suspension in 0.1% agar solution. *Blood* was

taken at 1, 2, 4, 6, 10, 24 hours after administration and then every 24 hours for several days until the concentration of the drug could not be recorded. *Urine* of each patient during 24 hours was pooled to study the cumulative renal excretion for 10 consecutive days. Urine passed at different times within the first 24 hours was also collected for study of rate of excretion. *Bone marrow* was obtained by usual sternum puncture technic. *Skin tissues* (both healthy and affected) were obtained by biopsy under local anesthesia employing nerve blocking technic. The tissues were finely minced and homogenized with distilled water in a specially devised homogenizer. Blood, urine, bone-marrow or suspensions of skin tissues were then uniformly spread over previously-weighed copper planchets of 5 cm^2 area and dried under an infra-red lamp. The planchets were then reweighed. Drying and weighing was repeated till final weight became constant. The difference of final and initial weights divided by area of planchets gave the thickness of dried samples in mg/cm^2. Thicknesses varied from 1-5 mg/cm^2. Radioactive assay: Each planchet was placed in a fixed position under an end-window G-M counter (window thickness 2.5 mg/cm^2) surrounded by 1″ of lead. The number of counts/min. n, due to beta particles of S-35 of radioactive DDS in the sample was then determined. The corresponding activity, a, in μc, could be deduced from the following formula:

$$a = [(1.44t)/(1 - e^{-0.225t})] \times n \times 10^{-6} \quad (1)$$

Where t is thickness of sample in mg/cm^2. This relation had been derived from previous calibration experiments.

Observations. Concentration of DDS in blood. From the G-M counter data the concentration of DDS/ml of blood could be obtained from the equation (1). Total concentration, *i.e.*, amount of DDS in total blood was determined by multiplying this figure by blood volume of patient. The latter varied from 3,250 to 5,400 ml, average 4,333 ml. Variation of average total concentration of patients having a single oral dose is shown in Fig. 1. The vertical bars indicate standard

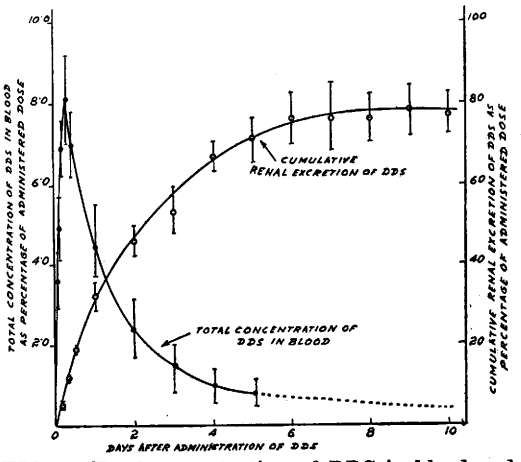

FIG. 1. Total concentration of DDS in blood and its cumulative renal excretion expressed as % of administered dose.

deviations of average values. The concentration increased very rapidly during initial hours and reached maximum level of 8.1 ± 1.1% of administered dose at about 6 hours after administration. Then it began to decrease somewhat slowly to 4.6 ± 0.9% at the end of 24 hours. After 4 days, it reached a negligibly low level of 0.96 ± 0.4%. At the end of 5 days the amount of tagged DDS in blood was too low to be detected by our method.

Renal excretion. Since it is known from the chemical estimation(6) that most of the sulphone is excreted through the kidneys, only renal excretion was studied in this experiment. The variation of average cumulative excretion after a single oral administration is presented in Fig. 1. As before, the bars indicate standard deviation of the average. As shown in this Figure most of the sulphone (about 76%) had been excreted within 6 days, and a slight increment, ~ 2% in the next 4 days.

Concentration in bone marrow. Bone marrow samples were collected from 6 patients, at 24, 72, 120 hours respectively. The level of activity was roughly that of blood at corresponding periods.

Concentration in skin. Uptake of the drug by skin tissues was investigated to elicit whether there was preferential fixation of DDS and if there was any significant difference in concentration of the drug in healthy

TABLE I. Concentration of Radioactive Diamino-Diphenyl-Sulphone in Blood and Skin Tissues.

Dose	Hr after admin.	Conc. (in μc/g) Blood	Healthy skin	Affected skin	Nature of lesion
Oral admin.:					
4 μc/kg body wt	6	.035	.013	.143	Single
	6	.036	.016	.067	Multiple
	18	.019	.014	.140	Single
	18	.008	.005	.064	Multiple
	24	.008	.009	.084	Single
	24	.010	.005	.083	Multiple
	24	.019	×	.050	Generalized
	72	.001	0	.004	Multiple
	144	0	0	.010	Single
	144	0	0	.005	Multiple
Subcut. inj.:					
2 μc	3	.001	.001	.012	Single
5 "	6	.002	.003	.016	Multiple
7 "	48	.001	.002	.050	Single

and affected tissues. Patients suffering from single and multiple lesions were studied. The findings in Table I show that the concentration in healthy skin during the first 6 hours was about 2 to 3 times less than that of blood. The difference was gradually made up and at the end of 18 hours the concentration of DDS in blood and healthy skin tissue was about the same. However, uptake of the drug by the affected tissues was always about 10 times greater than that of healthy tissues. No significant difference in concentration was observed in single versus multiple lesions.

The much larger localization of DDS in affected skin tissues after oral administration suggested a study of the same localization after subcutaneous injection of the drug. The injection was given at a site adjacent to the affected zone but about 4" away from it. It was found that the drug was, as before, preferentially localized in the affected tissues.

Discussion. It has been noted earlier that diamino-diphenylsulphone when administered by mouth could be detected in blood within a short time after its ingestion. Concentration of the drug in blood reached its maximum in about 6 hours, but the total quantity of the drug in blood constituted only about 8% of the total dose. The corresponding concentration in blood was only about 0.6 mg%. As the solubility of the drug in water is 10 mg% and had the drug passed directly from stomach to blood stream, the maximum concentration in blood should have been much larger as well as quicker. It therefore seems probable that the drug is first localized in the cells of gastrointestinal tract from where it is slowly taken up by the blood.

When the daily rate of diminution in blood is compared with the rate of excretion through kidneys during this period, another interesting feature of sulphone uptake becomes apparent. These rates could be determined in the following way: When the average total concentrations in blood (Fig. 1) were plotted against time in a semilog graph, it was found that, barring initial interval of 10-12 hours, the points approximately fell on a line of slope 0.62 per day. This showed that the concentration in blood diminished more or less exponentially with time, the daily rate of diminution being 62%. To obtain renal excretion rate, a hypothetical kidney system had to be visualized, from which the drug was gradually excreted. The initial concentration in this hypothetical kidney system should be taken as equal to total amount of drug actually excreted through the kidneys *i.e.* about 78% (Fig. 1). By deducting the cumulative total excretion from this value (*i.e.* 78%), the percentages of the drug retained at any instant in this system could be obtained. On plotting these percentages on semilog paper against time, an approximate straight line curve of slope 0.42/day was obtained. This meant that the drug cleared out of the kidneys almost exponentially, the rate of clearance being about 42%/day.

Up to about 18 hours after administration, concentration in blood remains higher than that of skin, nerve, and bone marrow but after that period, concentration stabilizes and the drug was more or less in the same concentration in blood, bone marrow, healthy skin tissues. However, that the concentration in leprous tissues was about 10 times more than in healthy ones, explains, at least partially, the fate of the extra quantity of the drug which disappears from blood, but could not be detected in urine. As regards mode of localization of the drug in the affected tis-

sues it might be mentioned that Chatterjee *et al.*(7) had shown that the *M. leprae* and leprous tissue contained mucopolysaccharides. Leprous tissue and bacilli containing mucopolysaccharides might have some special affinity for DDS.

Of the total quantity of drug administered, about 78% could be accounted for in the urine. It may be argued that a certain percentage was excreted through other channels and a portion might have been lost in manipulation and the balance localized in sensitive sites. As recorded above, the concentration in blood and urine was too low to be detected by our method 10 days after administration. Is it possible that the drug was localized in a sensitive zone and was excreted at a very slow and reduced rate? Cornbleet(8) while studying the pharmacology of sulpha drugs found that after a sweat bath the drug could be detected in blood, skin, sweat and urine several days after its disappearance. In at least one of our cases a skin biopsy from the affected site, 14 days after administration of the drug showed presence of DDS, though concentration in blood and urine was not detectable several days before date of biopsy. It may, therefore, be concluded that the drug was localized in affected tissues from where it is slowly excreted through excretory organs.

Summary. 1. The uptake of sulphone by leprosy patients was investigated, employing S-35 tagged diamino - diphenyl - sulphone (DDS). After oral administration: (i) total amount of drug in blood increased rapidly during initial hours reaching a maximum of about 8% of dose at 6 hours and then gradually decreased at an average rate of 62% per day to a negligible level in about 5 days; (ii) on an average 78% of the drug was excreted through the kidneys within about a week; the average rate of excretion being 42% per day; (iii) level of drug in bone-marrow and healthy skin was almost parallel to that in blood; (iv) the drug was preferentially localized in diseased skin tissues, where concentration was about 10 times greater than that in healthy tissues. 2. Subcutaneous injection also revealed similar preferential localization in affected regions. The significance of these findings is discussed.

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