CHEMOTHERAPY OF LEPROSY

II — Compounds structurally related to 4,4'-diaminodiphenylsulfone [2]

CHEMICAL STUDIES(*)

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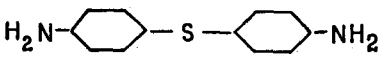
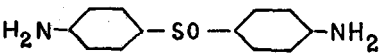
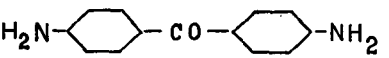
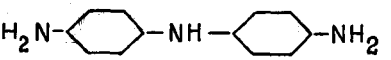
On developing our working program[1], several compounds were synthetized, trying to establish some relationship between chemical structure and the already recognized therapeutic activity of 4,4'-diaminodiphenylsulfone on human[3] and murine leprosy [2]. According to Mauri, Hadler and Carvalho[2], 4,4'-diaminodiphenylsulfone presents a comparable behaviour on rat and human leprosy, showing a very pronounced activity. Our substances were tested by the method of these Authors and the activities compared to that of 4,4'-diaminophenylsulfone [21].

The first part of our work covers some compounds that differ from 4,4'-diaminodiphenylsulfone only on the sulfonic group —SO_2—, as follows:

	Symbol	Formula	Name
I	AS		4,4'-diaminodiphenylsulfide
II	AX		4,4'-diaminodiphenylsulfoxide
III	AC		4,4'-diaminodiphenylcetone or 4,4'-diaminobenzophenone
IV	AA		4,4'-diaminodiphenylamine

On table 2 of the previous comunication[1] we reason why these substances were studied in first place.

(*) Realized in the Chemotherapic Section of Instituto Butantan, Caixa Postal 65, São Paulo — Brasil.

METHODS OF SYNTHESIS

The first two compounds (AS and AX), were synthetized by the process indicated on Table 3 of previous communication [1] and also by amination, under pressure, of the corresponding chloro-derivates, according to the following table:

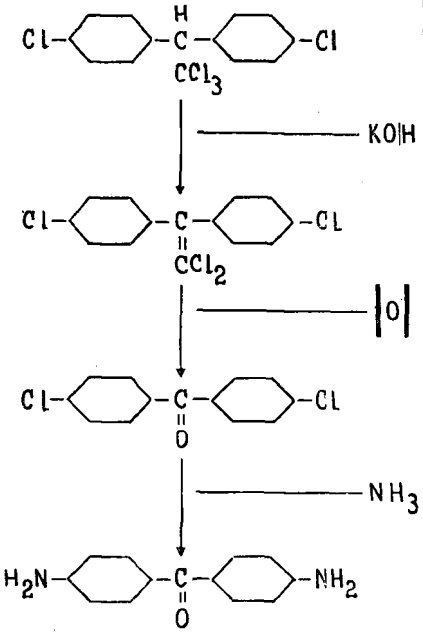
TABLE Nº 1

			Compounds obtained	Reaction	Average Yield	Reference	M.P. Found	M.P. Described	M.P. Reference
AX	Method described in literature	1st Phase	4-nitro-4'-amino-diphenylsulfide	Condensation of 4-nitrochloro-benzene with sodium 4-amino tiophenolate.	42% (2 preparations)	(4)	145/146º	145º	(4)
		2nd Phase	4,4'-diamino-diphenylsulfide	Reduction of 4-nitro-4'-amino-diphenylsulfide with metallic tin and HCl.	37% (7 preparations)	(4)	110/111º	108º	(4)
	Our modified method	1st Phase	4,4'-dichloro-diphenylsulfide	Condensation of chlorobenzene with sulfur monochloride by aluminium chloride (*).	50.3% (3 preparations)	(6)	93º	93º	(5)
		2nd Phase	4,4'-diamino-diphenylsulfide	Amination of 4,4'-dichlorodiphe-nylsulfide with ammonia under pressure.	64.8% (1 preparation)		110/111º	108º	(4)
AS	Method described in literature		4,4'-diamino-diphenylsulfoxide	Oxidation of 4,4'-diaminodiphe-nylsulfide with "Perhydrol" in acetone.	49% (2 preparations)	(7)	174º	175º	(7)
	Our modified method	1st Phase	4,4'-dichloro-diphenylsulfoxide	Condensation of chlorobenzene with thionyl chloride by alu-minium chloride.	93% (2 preparations)		143/147º	143º	(8)
		2nd Phase	4,4'-diamino-diphenylsulfoxide	Amination of 4,4'-dichlorodiphe-nylsulfoxide with aqueous am-monia under pressure.	56.6% (6 preparations)		177º	175º	(7)

(*) We adapted the method of preparation of diphenylsulfide, described in Org. Synth. 11, pg. 242 (1943).

We found that our modified synthesis of compounds AS and AX give: 1) better yields; 2) reactions of more simple execution; 3) products that are easily purified.

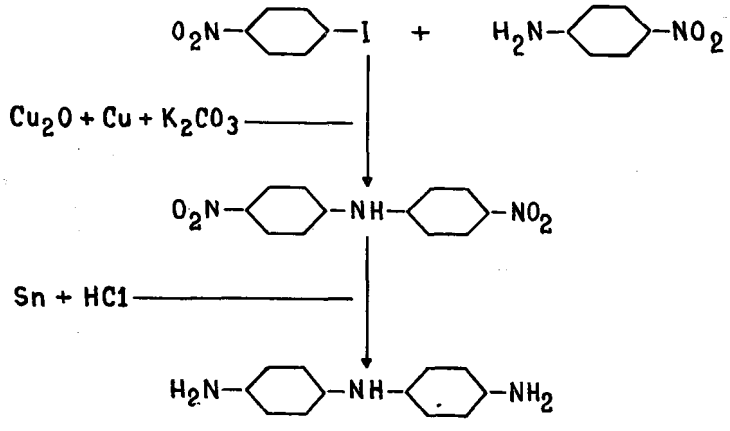
Third compound (AC) was obtained as shown below:



Described			Our observations		
Yield	M. P.	Ref.	Yield	M. P.	
97%	83-86°	(9)	a) 87% crude average of 3 preparations	80-88° crude	Using ethylene glycol as solvent.
			b) 99.3% recristall. from acetone or ethanol	82,1-85,1° recristall.	Using hexylene glycol as solvent (*).
	143-144°	(10)	a) 96% average of 3 preparations	146° crude	Oxydation with chromic anhydride in glacial acetic acid.
			b) 94.8%	141,6-146° crude	Oxydation with sodium bichromate and sulfuric acid.
	246,5-247,5° (12)	(11)	78.5% average of 2 preparations	243-245° recristall.	Amination with aqueous ammonia under pressure.

(*) We noted that using hexilen-glycol [2-metil-2,4-pentandiol (Shell)] instead of diethylene-glycol, a smoother reaction and better yelds are obtained.

AA was prepared through the dinitroderivate as shown below:

		Yield %	M. P.	
	(*)	216,5-218°	62-67.5%	(16)
	(15)	152-154°	65.3%	(17)

(*) Goldberg [13] using 4-chloronitrobenzene and Smiles and Hilditch [14] using 4-bromonitrobenzene, and heating for 20 hours, describe a similar process.

EXPERIMENTAL STUDIES (*)

With 4,4'-diaminodiphenylcetone (AC) the following tests were made:

T O X I C I T Y

In 24 hours, AC did not kill any of twenty rats when administered orally up to 8g/kg. However with the dosages from 4 to 8 g/kg nervous symptoms of intoxication were noted as paralysis of posterior feet, in practically all animals treated (**). In 48 hours one rat died with the dosage of 8g/kg. Macroscopic autopsia of some animals which had received 4 and 8g/kg revealed a mechanical intestinal occlusion caused by the drug on the last portion of the small intestine and the beginning of the large intestine. In the 4g/kg group one animal was observed with strong yellow pigmentation of the fatty tissue (jaundice?).

	HYSTOPATHOLOGIC FINDINGS		
Single dosis administered	Liver	Kidney	Spleen
4g/kg.	Chronic passive congestion (first stage). Infiltrative microglobular fatty degeneration in moderate degree.	Strong hyperemia. Cloudy swelling of the cells of the tubules [Albuminous nephrosis (?)].	Hyperplasia of the white pulp. Strong hyperemia.
8g/kg	Hyperemia. Marked degenerative, microglobular steatosys.	Marked hyperemia. Intensive cloudy swelling of the tubular cells [marked albuminous nephrosis (?)].	Marked hyperemia of the Billroth cords. Moderate hyperplasia of the white pulp. Small hemosiderosis.

(*) We are indebted to Dr. F. Saliba for the histopathologic findings and to Dr. Baeta Henriques for valuable help and advice for the toxicity tests and the colorimetric dosages.

(**) Similar effects were noted by Kuhn et al. (loc. cit.[12], pg. 716) who also found that the toxicity of AC injected subcutaneously in the mice is 7 mg/20g.

COLORIMETRIC DOSAGE: COMPARED ABSORPTION OF 4,4-DIAMINODIPHENYLCETONE AND 4,4-DIAMINODIPHENYLSULFONE

According to Marshall's method [18], the following determinations were made :

1. Standard curve for 4,4'-diaminodiphenylsulfone

2. Standard curve for 4,4'-diaminodiphenylcetone

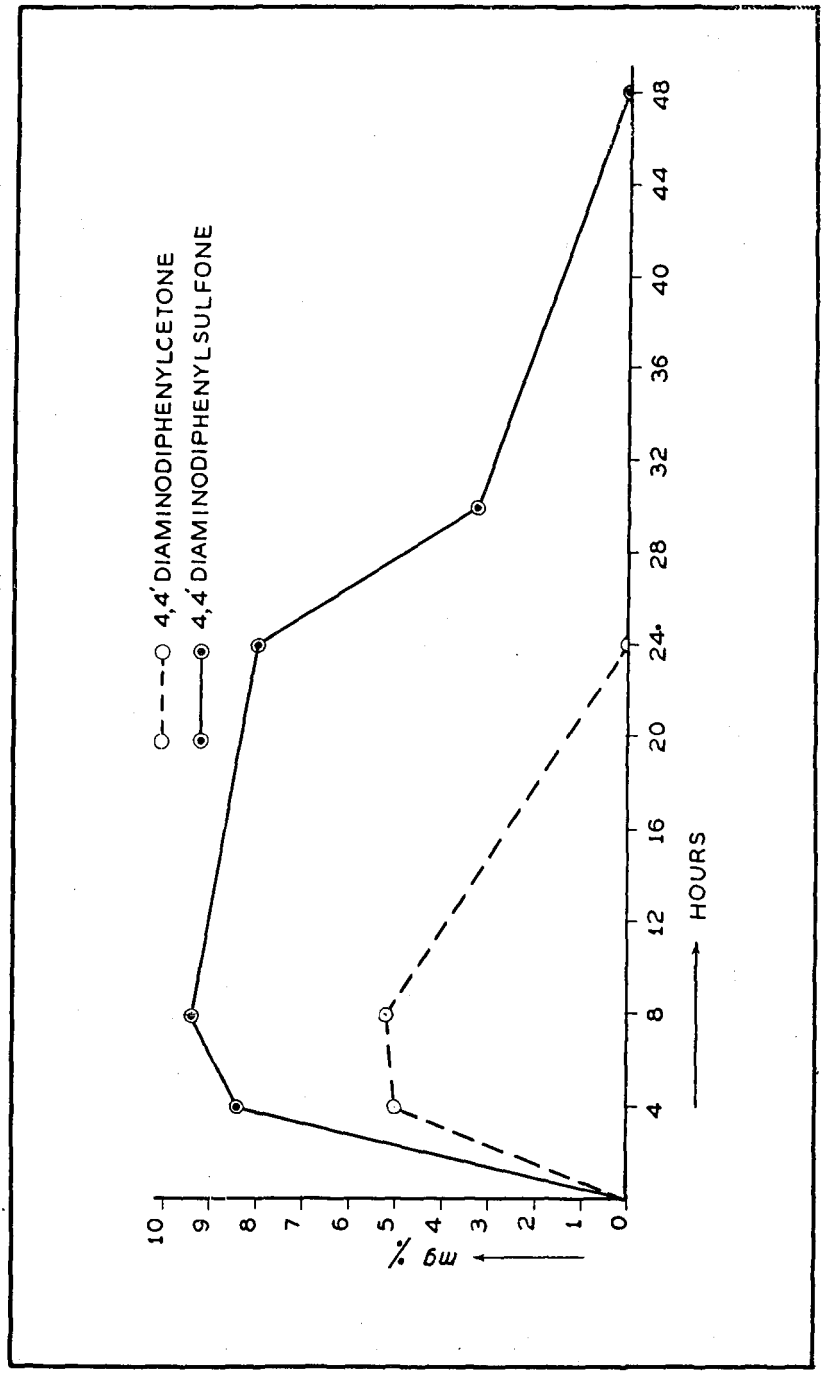
3. Blood concentrations in rabbits, on basis of established standard curves.

The following table and respective graph 1, made on basis of mean values found, show that, on rabbits, the blood concentration of AC administered by gastric tubage (1g; kg in aqueous suspension) reaches its maximum in the first 8 hours and then drops to zero soon after, while the concentration of 4,4'-diaminodiphenylsulfone with the same dosage reaches its maximum 8 hours after administration and maintains detectable values during 30 hours. Therefore 4,4'-diaminodiphenylcetone is more rapidly eliminated than 4,4'-diaminodiphenylsulfone. It was also observed that the sulfone maintains, with the above dosage, a level of 8 mg% during 24 hours following administration, and it may be inferred that a single daily does by mouth is also sufficient to maintain a constant level on man. This observation is of value for the oral therapy of leprosy and suggests further studies about the best blood level of sulfone in relation to therapeutic activity.

Hours after administration	4,4'-diaminodiphenyl-sulfone	4,4'-diaminodiphenyl-cetone
	mg.%	mg.%
4	8.26	5.05
8	9.33	5.20
24	8.07	0
30	3.20	0
48	0	0

EXPERI MENTAL

1) — *Preparation of 4,4'-diaminodiphenylsulfone:* 72 g of recrystallized dichlorodiphenylsulfide were placed in a high pressure autoclave with 1,680 ml of 25% aqueous ammonia and 30.8 g of commercial



Graph 1: — Compared blood level in mg.% of 4,4'-diaminodiphenylcetone and 4,4'-diaminodiphenylsulfone, during several hours after oral administration to rabbits (1 g/kg).

copper sulfate. The mixture was heated to 200-210° and then rocked. Heating was continued during 4 hours. The obtained product was filtered and thoroughly washed. Purification was made by dissolving in a mixture of 500 ml of concentrated hydrochloric acid and 500 ml of water. The heated solution was filtered through glass wool and neutralized with ammonia. Yield: 40 g crude (64.8% of theory). M.P. — 110-111°.

2) — *Preparation of 4,4'-dichlorodiphenylsulfoxide:* This substance was prepared similarly to the corresponding dibromo derivate [19]: In a three necked flask of 3 Its., fitted with a reflux condenser, mechanical stirrer and thermometer, 187 g (115 ml) of recently distilled thionyl chloride were dissolved in 896 g (820 ml) of chlorobenzene. To this solution, cooled to 10-15° by means of an ice bath, 600 g of commercial anhydrous aluminium chloride were added under constant stirring during a period of 2 hours. The temperature during the reaction was maintained between 10 and 15°. After addition, the ice bath was withdrawn and stirring of the mass continued for 1 hour and a half, at room temperature. Finally the mass was heated on the water bath to 40-50°, under stirring for one hour. The product was poured in 1 kg of ice and water containing 50 ml of concentrated hydrochloric acid, filtered and washed. A small quantity of unreacted chlorobenzene was separated by filtration. Crude yield: 397 g (92.7% of theory). M.P. — 174°.

3) — *Preparation of 4,4'-diaminodiphenylsulfoxide:* 140 g of recrystallized dichlorodiphenylsulfoxide were placed in a high pressure rocking autoclave with 1,680 ml of 25% aqueous ammonia and 60 g of commercial copper sulfate. The mixture was heated to 210° and then agitated at that temperature during 5 hours. The product was filtered and washed. Purified through the chlorhydrate with 400 ml of hydrochloric acid and 400 ml of water in the same way as the 4,4'-diaminodiphenylsulfide, the product weighed 93 g (77.7 of theory). Treated with decolorizing carbon in aqueous ethanol, the recrystallized product melted at 177°.

4) — *Preparation of 4,4'-dichlorodiphenylcetone:* Oxydation with sodium bichromate and sulfuric acid: In a glass lined reactor, fitted with stirrer and reflux condenser 2,100 g of 1,1-di-p-chlorophenyl-2,2-dichloroethylene and 6,300 g of sodium bichromate were mixed with 22.3 Its. of conc. acetic acid. To that mixture, 3.2 Its. of concentrated sulfuric acid (d. 1.8) were added through a dropping funnel, in 30 minutes. As soon as the vigorous reaction ceased, the mixture was boiled during 4 hours. The reaction product

was poured in 200 Its of water. Yield: 1,575 g (crude) = 94.8% of the theory.

5) — *Preparation of 4,4'-diaminodiphenylcetone:* 2,700 g of 4,4'-dichlorodiphenylcetone, 28 liters of 25% aqueous ammonia and 700 g of cristallized commercial copper sulfate were placed in a 40 Its. high pressure rocking autoclave and heated at 230° for 2 hours [pressure ± 60 atm. (1,000 lbs)]. The obtained product was filtered, washed and dried. Crude yield: 1,800 g = 73.1% of theory.

6) — *Preparation of 4-iodonitrobenzene [20]:* To a mixture of 24 g of 4nitroaniline (0.17 moles), 12.1 g sodium nitrite (0.17 moles) and 35 ml of water, cooled in an ice bath, was added in small portions a mixture of 160 [89 ml (1.83 moles)] of concentrated sulfuric acid and 80 g of cracked ice. During the addition the temperature did not rise higher than 10°. The solution was filtered through glass wool, and added to a cooled solution of 48 g of iodine and 48 g of potassium iodide in 50 ml of water. The mixture was heated on the water bath, for one hour and after having been cooled in the refrigerator the excess iodine was destroyed by adding small portions of solid sodium bisulfite. The product was filtered by suction, washed and dried at 100°. Yield: 28 to 32.5 g (65 to 75% of theory). M.P. 170 to 172°.

7) — *Preparation of 4,4'-dinitrodiphenylamine:* 3.8 g (0.1 mot) of 4-nitroaniline, 25 g (0.1 mot) of 4-iodonitrobenzene, 7 g (0.05 mot) of cuprous oxide, 6 g (0.1 mot) of powdered copper metal, 2] g (0.15 moles) of potassium carbonate (anhydrous) and 100 g (83 cc) of recently distilled nitrobenzene were heated under reflux in a metal bath to 190-200° for five hours. 75 ml of toluene were added and heating continued on the water bath. The inorganic residue was separated by filtration and extracted three times with 75 ml portions of hot toluene and finally with 50 ml of hot nitrobenzene. The combined extracts were cooled in the refrigerator over night. The crystals were separated by suction and washed with 150 ml of 60% ethanol in water. Yield 16-17.5 g (61.8 - 67.8% of the theory). M.P. 212-217°.

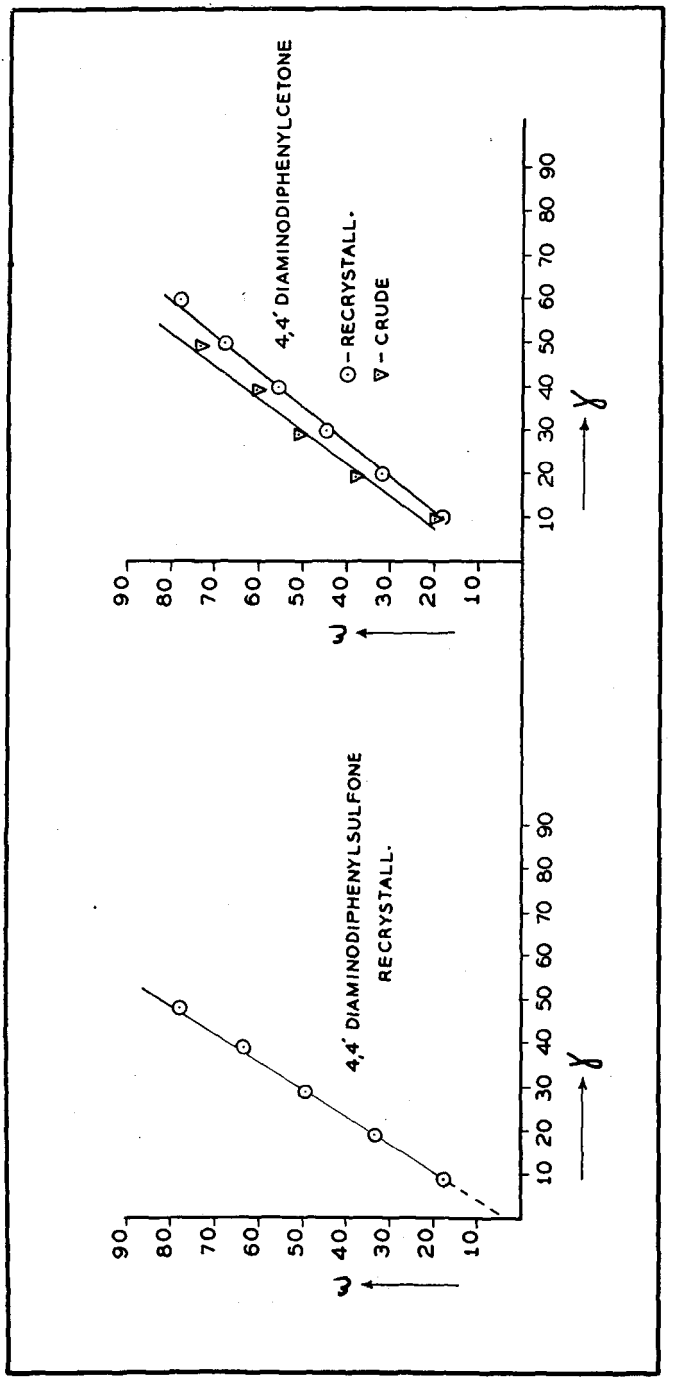
8) — *Preparation of 4,4'diaminodiphenylamine:* 25.9 g (0.1 mol) of 4,4'-dinitrodiphenylamine, 71 g of tin (0.6 mol), 100 ml of concentrated hydrochloric acid and 500 ml of ethanol were heated under reflux on the water bath for 6 hours. The reaction product was filtered through glass wool and the filtrate was evaporated in a

porcelain dish on the water bath. The residue was treated with 700 ml of 10% sulfuric acid on the water bath during a half hour. The obtained sulfate was left over night in the refrigerator and then centrifuged. The precipitate, after washed by centrifugation, was treated with a 10% sodium hydroxide solution until weakly alcaline. After being cooled on ice the product was filtered by suction and with two 50 ml portions of water. Yield 16-19 g = 80-95% of the theory. Purification by suspension in water (250 ml) and solution with hydrochloric acid added drop, by drop and re-precipitation with 40% sodium hydroxide, yielded 13 g (63% of theory) with M.P. = 152-154°.

9) — *Determination of standard curves:* The graph 2 shows the standard curves which were obtained as follows: 1) 4,4'-diaminodiphenylsulfone (recrystallized) with a melting point of 175.6-177° (Anschütz thermometer) ; 2) 4,4'-diaminodiphenylcetone crude; 3) 4,4'-diaminodiphenylcetone (recrystallized) with a melting point of 243.4-245.3° (Anschütz thermometer). The indicated values represent the average of two determinations. The estimation of 4,4'-diaminorliphenylsulfone and 4,4'-diaminodiphenylcetone in rabbit's blood was based on these curves.

10) — *Administration of drugs and collection of blood sample:* The drugs were given, by gastric tubage, lg/kg of animal weight suspended in 20 ml of water, the syringe and sound being washed with 5 ml of water. The animals were bled at the heart, and approximately 1.5 ml of blood was obtained per animal. To the sample 0.02 ml of 30% potassium oxalate was added.

11) — *Colorimetric determination:* Following technique was used: to 1.0 ml of the oxalated blood, diluted to 15.0 ml with distilled water, 4 ml of a 15% solution of trichloroacetic was added in order to deproteinize the blood. After filtering through a folded filter, 5.0 ml of 2N hydrochloric acid were added to 5 ml of the filtrate. The mixture was heated on the boiling water bath for one hour, and after cooling to room temperature the following solutions were added at intervals of 3 minutes each other: 1.0 ml of a 0.2% solution of sodium nitrite, 1.0 ml of a 2% ammonium sulfamate, 10.0 ml of a 0.02% solution of N-(1-naftyl)-ethylenediamine di-hydrochloride (20 mg in 10 ml of water and 90 ml of ethanol). Readings were made on an "Evans" colorimeter, green filter, compared with a blank prepared with the animals blood before the drug was given.



Graph 2: — Standard curves of 4,4'-diaminodiphenylsulphone and of 4,4'-diaminodiphenylcetone.

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