between birth and their transfer to the Veterinary Science Department.

Summary. These experiments indicate that infection with the hookworm parasitic in calves takes place by way of the skin as well as orally and that the larval stages are capable of producing severe symptoms of parasitosis. The prepatent period may be as short as the 57th day and may extend to at least 79 days.

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Derivatives of Diphenylsulfone, Related Sulfoxides and Sulfides in Experimental Tuberculosis.*

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Many investigators have reported favorable results in experimental tuberculosis with derivatives of 4,4'diaminodiphenylsulfone, including promin and diasone, and the related sulfone, promizole. In the following experiments several new derivatives of diphenylsulfone, a number of related sulfoxides and other allied compounds were evaluated for their effect in experimental tuberculosis, both *in vitro* and *in vivo*. An effort was made to determine the relationship between chemical structure and chemotherapeutic activity.

Methods. The bacteriostatic studies were initially performed on the avirulent strain of tubercle bacilli, No. 607, using Proskauer and Beck synthetic media, at a pH of 7.2. The chemical compounds were dissolved in propylene glycol to make 0.5% solutions. Subsequent dilutions were made directly into the media. Each tube was inoculated with one loopful of fresh culture of tubercle bacilli. The appearance of a pellicle was taken as the criterion of growth; the cultures were read at the end of 2 and of 4 days. Compounds showing a favorable bacteriostatic action were repeated, using the virulent culture H37Rv. The cultures were read at the end of 3 weeks. As the differences between the bacteriostatic effect on the avirulent and the virulent cultures were insignificant, only the results of the experiments performed with the avirulent culture are reported.

Animal Experiments. Approximately 9 guinea pigs, averaging 350 g weight, were used to evaluate each compound. The animals were inoculated in the left groin with 0.01 mg of H37Rv tubercle bacilli. The drugs were administered either orally or hypodermically. For oral administration, in most cases, the finely ground drug was mixed with ground rabbit pellets; in the other cases the drugs were suspended in tragacanth solution and given with a pipette. The food was supplemented by fresh vegetables. For hypodermic administration the drugs were ground with either cotton seed oil or a 1% beeswax-sesame oil mixture. All experiments were terminated at the end of 6 weeks. Pathology was evaluated numerically by the method of Sweany, Sher, and Kloeck,[1] in which the maximum gross involvements of the organs were given the following numerical ratings: lymph nodes 8, spleen 24, liver 28 and lungs 40. Hemoglobin determinations were made at the end of the experiments.

Results. The *in vitro* bacteriostatic results are recorded in Table I. The com-

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[1] Sweany, H. C., Sher, B. C., and Kloeck, J. M., *Am. Rev. Tuberculosis*, 1946, **53**, 250.

TABLE I.
Tuberculostatic Action.

Compound	Chemical formula	Conc. 1-part-in:
4,4'Diaminodiphenylsulfone	$H_2NC_6H_4SO_2C_6H_4NH_2$	60,000
2,4' "	"	40,000
2,2' "	"	10,000
3,3' "	"	10,000
4-Nitro,4'acetylaminodiphenylsulfone	$O_2NC_6H_4SO_2C_6H_4NH \cdot COCH_3$	10,000
4-Benzylideneamino,4'aminodiphenylsulfone	$C_6H_5 \cdot CH{=}N{-}C_6H_4SO_2C_6H_4NH_2$	10,000
4,4'Diaminodiphenylsulfoxide	$H_2NC_6H_4SOC_6H_4NH_2$	40,000
4,4'Diacetylaminodiphenylsulfoxide	$H_3COCHNC_6H_4SOC_6H_4NHCOCH_3$	10,000
4-Aminodiphenylsulfoxide	$C_6H_5SOC_6H_4NH_2$	100,000
4-Nitro,4'aminodiphenylsulfoxide	$O_2NC_6H_4SOC_6H_4NH_2$	60,000
4-Nitro,4'acetylaminodiphenylsulfoxide	$O_2NC_6H_4SOC_6H_4NHCOCH_3$	10,000
4-Chloro,4'aminodiphenylsulfide	$ClC_6H_4SC_6H_4NH_2$	800,000
4-Iodo,4'aminodiphenylsulfide	$IC_6H_4SC_6H_4NH_2$	800,000
4-Chloro,4'acetylaminodiphenylsulfide	$ClC_6H_4SC_6H_4NHCOCH_3$	400,000
4-Chloro,4'aminodiphenylsulfone	$ClC_6H_4SO_2C_6H_4NH_2$	10,000
4-Iodo,4'aminodiphenylsulfone	$IC_6H_4SO_2C_6H_4NH_2$	10,000
4-Iodo,4'Nitrodiphenylsulfone	$IC_6H_4SO_2C_6H_4NO_2$	10,000

pounds, 4,4'diaminodiphenylsulfone and diasone, were included for purposes of comparison and internal standardization. 2,4'-Diaminodiphenylsulfone was slightly less bacteriostatic than the 4,4' derivative; 2,2'diaminodiphenylsulfone and the 3,3' derivative were the least tuberculostatic of the 4 isomers. 4-Nitro,4'acetylaminodiphenylsulfone and 4-benzylideneamino,4'aminodiphenylsulfone showed only minimal bacteriostatic action *in vitro*. 4,4'Diaminodiphenylsulfoxide was slightly less inhibitory than the corresponding sulfone. 4-Nitro,4'aminodiphenylsulfoxide was slightly superior in tuberculostatic action to the 4,4'diaminodiphenylsulfoxide. Acetylation of the above compounds, in general, prevented the inhibitory effect. 4-Aminodiphenylsulfoxide showed a higher bacteriostatic effect than did the corresponding diamino derivative.

The halogenated derivatives of the sulfides inhibited growth in very high dilutions; 4-chloro,4'aminodiphenylsulfide, as well as the corresponding iodo derivative was bacteriostatic in dilutions of 1:800,000. Acetyl derivatives of these sulfides showed high bacteriostatic action. The halogenated sulfones, on the other hand, showed no tuberculostatic action. *p*-Aminobenzoic acid antagonized the bacteriostatic action of the sulfones and sulfoxides, but not that of the halogenated sulfides. All compounds lost most of their inhibitory action in the presence of 20% horse serum.

The *in vivo* results are summarized in Table II. The isomers of diaminodiphenylsulfone showed the following order of decreasing therapeutic effect: 4,4'; 2,4'; 2,2'. This order is the same as the order of the *in vitro* tuberculostatic effects. 4-Nitro,4'acetylaminodiphenylsulfone and 4-benzylideneamino,4'aminodiphenylsulfone were approximately as effective as 4,4'diaminodiphenylsulfone and diasone. 4,4'Diaminodiphenylsulfoxide, given hypodermically, was as effective as the corresponding sulfone. Given orally, however, its therapeutic effect was comparatively slight. 4,4'Diacetylaminodiphenylsulfoxide showed a considerable effect orally but it was much less effective when given subcutaneously. While the spleens of the animals treated with 4,4'diaminodiphenylsulfone or its benzylidene derivative were large and cyanotic, the spleens of the animals treated with the sulfoxides did not show this effect. None of the halogenated compounds were effective *in vivo*. 4-Iodo,4'-aminodiphenylsulfone was considerably less toxic than the corresponding sulfide. Guinea pigs tolerated a daily dose of 75 mg of the sulfone and only 8 mg of the sulfide.

Discussion. It is interesting to note that

TABLE II.
Summary of *in Vivo* Experimental Data.

Group	Compound	Dosage, mg	Avg path. rating	Path. ratio controls / treated	Avg wt gain	Hglb.
A	4,4′Diaminodiphenylsulfone	50 daily, oral	33.7	1.77	240	11
	4-Nitro,4′acetylaminodiphenylsulfone	100 " "	25.8	2.33	213	10.5
	Controls		60		167	11.4
B	2,4′Diaminodiphenylsulfone	50 " "	43	1.09	179	12
	2,2′Diaminodiphenylsulfone	50 " "	55.2	0.85	141	11
	Controls		47		118	11.4
C	Diasone*	180 " "	19.9	2.26	194	9.4
	Controls		44		175	10.1
D	4-Benzylideneamino,4′aminodiphenylsulfone	100 " "	18.9	2.53	213	10.4
	Controls		48		223	10.9
E	4,4′Diaminodiphenylsulfoxide	100 " "	47	1.28	113	10
	4,4′Diacetylaminodiphenylsulfoxide	100 " "	21	2.86	54	9
	4-Aminodiphenylsulfoxide	50 " "	53	1.13	74	10.6
	4-Nitro,4′aminodiphenylsulfoxide	100 " "	44	1.36	103	11
	4-Nitro,4′acetylaminodiphenylsulfoxide	80 " "	53	1.13	96	11
	Controls		60		167	10.9
F	4,4′Diaminodiphenylsulfoxide	130 hypo 3× wk	18	2.17	172	11.5
	Controls		39		259	10.6
G	4,4′Diaminodiphenylsulfoxide	90 " q. 4 days	22	2.60	191	11.1
	4,4′Diacetylaminodiphenylsulfoxide	90 " " " "	34	1.70	201	9.7
	Controls		57.3		160	11.1
H	4-Iodo,4′aminodiphenylsulfone	75 daily oral	44	1.09	191	11
	" " "	50 hypo, q. 2 days	42.1	1.14	142	11.1
	Controls		48		223	11
	4-Chloro,4′aminodiphenylsulfide	8 daily, oral	36.7	1.13	116	11.2
	" " "	8 " hypo	41.4	1.06	60	11
	4-Chloro,4′acetylaminodiphenylsulfide	20 " oral	41.2	1.06	101	11.6
	" " " "	8 " hypo	37	1.18	87	11.8
	4-Iodo,4′aminodiphenylsulfide	10 " oral	41.6	1.05	115	13
	Controls		43.7		169	12.8

* Diasone obtained through the courtesy of Abbott Laboratories, Chicago, Ill.

in the sulfoxide group therapeutic effects have been found which are as significant as the therapeutic effects of diaminodiphenylsulfone and its derivative, diasone. Diaminodiphenylsulfoxide has been shown, previously, to exhibit a high therapeutic effect in streptococcus and gonococcus infections in mice.[2-4] This compound has also been found to inhibit tumor growth in mice.[5] Buttle *et al.*[2] found that acetylation did not reduce the therapeutic activity of 4,4′diaminodiphenylsulfoxide in animal streptococcus infections to the same degree as did acetylation of the corresponding sulfone. We have found 4,4′-diacetylaminodiphenylsulfoxide given orally to be as effective as the nonacetylated sulfoxide administered hypodermically in tuberculous guinea pigs.

The compound, 4-nitro,4′aminodiphenylsulfoxide, showed only a slight therapeutic effect on oral administration. The acidity of the gastric juice may have decomposed this derivative. In this connection we have observed that this compound is altered on contact with acids or on long standing. Buttle[2]

[2] Buttle, G. A., Dewig, T., Foster, G. E., Gray, W. H., Smith, S., and Stephenson, D., *Bioch. J.*, 1938, **32**, 1101.

[3] Rosenthal, S. M., Bauer, H., and Elvove, E., *U. S. Publ. Health Rep.*, 1939, **54**, 1317.

[4] Girard, A., Ray, A., and Richard, G., *Nature*, 1937, **140**, 283.

[5] Boyland, E., and Mauson, E. H., *Biochem. J.*, 1938, **32**, 1982.

found the benzylidene derivative of 4,4′-diaminodiphenylsulfone to be low in toxicity and to be more effective than the parent compound in pneumococcus infections in mice and monkeys. They reported that mice would tolerate daily repeated doses of approximately 500 mg/kg. In our experiments on tuberculous guinea pigs a favorable therapeutic effect was obtained with doses of 200 mg/kg per day.

Summary. A series of derivatives of diphenyl sulfone, sulfoxide and sulfide were tested for *in vitro* tuberculostatic activity and for antituberculous therapeutic activity in guinea pigs. The following compounds, in addition to the reference materials 4,4′-diaminodiphenylsulfone and its derivative diasone, showed high *in vivo* activity: 4-benzylideneamino,4′aminodiphenylsulfone; 4-nitro,4′acetylaminodiphenylsulfone; 4,4′diaminodiphenylsulfoxide (hypo); 4,4′diacetylaminodiphenylsulfoxide (oral). 4-Chloro, 4′aminodiphenylsulfide and the corresponding iodo derivative were highly tuberculostatic *in vitro* but were devoid of *in vivo* activity.

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Biological Factors Involved in Poisoning Rats with Alpha-Naphthyl Thiourea (ANTU).*

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From modern studies in the fields of nutrition and biochemistry much has been learned regarding the principles involved in the feeding of animals to keep them alive and in good health; but little has been learned regarding the principles involved in feeding animals substances to destroy them. When animals are kept in captivity this latter objective can be readily attained, often unwittingly; when, however, animals are not confined and have full access to all kinds of foods in their natural states it is less readily attained.

The use of poisons for killing animals by feeding involves at least the following considerations: (1) The poisonous compound must be highly toxic to the animals that are to be killed; (2) it must be readily acceptable in lethal amounts; (3) after surviving the ingestion of sublethal amounts of the compound animals must not be able to develop too high or too prolonged a tolerance; (4) the poison must be as free as possible from taste and odors, by means of which animals once having survived poisoning, are able to recognize it on a second exposure and this devolep a "refusal" response.

In the present paper an attempt has been made to place the study of these different qualifications of a poison—toxicity, acceptance, tolerance and refusal—on a controlled experimental basis.

The substance used for this purpose was alpha-naphthyl thiourea (ANTU), a substance which has been shown to be highly toxic for domestic and wild Norway rats, doses of 1-5 mg killing the average-sized rat. On the basis of the results of these and of other similar tests this substance was recommended as a rat poison and is now actually being used as such on a wide scale.[1]

Method. Sixty-four adult hooded and albino male domestic rats, ranging in age from 82 to 228 days and in weight from 227 to 313 g were used for these experi-

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† With the assistance of Mrs. Romaine Randall.

[1] Richter, C. P., *J. A. M. A.*, 1945, **129**, 927.