Purine-6-alkylsulfonamides Listed in Table II.—The appropriate amine (0.0747 mole) (see Table II) in 50 ml. of water was cooled to 15° and stirred as 5 g. (0.0247 mole) of 6-sulfonylfluoropurine[6] was added in small portions so that the temperature of the mixture did not exceed 20°. This mixture was stirred for 15 min. and then acidified with glacial acetic acid. The precipitate that formed was filtered, washed with water, dried at 60°, and then recrystallized from the indicated solvent (Table II).

Potential Hypoglycemic Agents: 1,3,4-Oxadiazoles and Related Compounds

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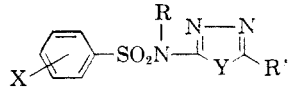
A number of thiadiazole and oxadiazole derivatives were synthesized and evaluated for oral hypoglycemic activity. All of the compounds were tested by single dose administration in rats and/or dogs by the oral route. Most of the derivatives exhibited some degree of activity. 5-Cyclohexyl-2-(*p*-toluenesulfonamido)-1,3,4-oxadiazole was fairly potent in both rats and dogs, and on daily administration in dogs produced and maintained a hypoglycemia. Some toxicity data are presented.

Over the years many substances have been examined for hypoglycemic properties. The observation by Watanabe[1] that guanidine caused a drop in the sugar levels in animals stimulated a search for orally active antidiabetic agents. Janbon[2] in 1942 found that the antibacterial sulfonamide 2-(*p*-aminobenzenesulfonamido)-5-isopropyl-1,3,4-thiadiazole lowered blood sugar. Loubatieres[3] and others studied this phenomenon, and in 1955 this compound was applied in the treatment of diabetes.[4] At the same time a series of N-arylsulfonyl-N′-alkylureas was found to have utility in the treatment of diabetes and at present, three compounds are in extensive use.[5] These are N-*p*-aminobenzenesulfonyl-N′-*n*-butylurea (carbu-

(1) C. K. Watanabe, *J. Biol. Chem.*, **33**, 65, 253 (1918).
(2) M. Janbon, P. Chaptal, A. Vedel and J. Schaap, *Montpellier Méd.*, **21–22**, 441 (1942).
(3) A. Loubatieres, *Comp. Rend. Soc. Biol.*, Paris, **138**, 766 (1944).
(4) A. Loubatieres, *Therapie*, **10**, 907 (1955).
(5) H. Ruschig, G. Korger, W. Aumüller, H. Wagner, R. Weyer, A. Bänder and J. Scholz, *Medizin und Chemie*, **6**, 61 (1959).

tamide), N-*p*-toluenesulfonyl-N′-*n*-butylurea (tolbutamide) and N-*p*-chlorobenzenesulfonyl-N′-propylurea (chloropropamide). Recently, further work on arylsulfonyl thiadiazoles by Matti[6] and also by Chubb and Nissenbaum[7] has yielded another clinically useful drug, namely, 2-(*p*-methoxybenzenesulfonamido)-5-isobutyl-1,3,4-thiadiazole.

The present work describes the synthesis and testing of a series of potential hypoglycemic agents of the general structure:



where X = hydrogen, halogen, alkyl, alkoxy, amino, acetamido or nitro; R = hydrogen or methyl, Y = S, O or NH; and R′ = alkyl, aryl, heterocyclo or cycloalkyl.

In all, 58 new compounds are described,[8] including 2-sulfonamido-5-substituted-1,3,4-thiadiazole, 2-sulfonamido-5-substituted-1,3,4-oxadiazoles and 3-sulfonamido-5-substituted-1,2,4-triazoles. Results of pharmacological evaluation of these compounds are presented, including a more detailed account and toxicity data of 5-cyclohexyl-2-(*p*-toluenesulfonamido)-1,3,4-oxadiazole, one of the more active compounds prepared.[9]

Experimental

General Preparative Directions. I. 2-Arylsulfonamido-5-substituted 1,3,4-Thiadiazoles.—Thiosemicarbazide reacted with the required carboxylic acid in the presence of a dehydrating agent to form the desired 2-amino-5-substituted-1,3,4-thiadiazole; this in turn was treated with an arylsulfonyl chloride in the presence of an organic acid acceptor.

II. 2-Arylsulfonamido-5-substituted 1,3,4-Oxadiazoles.—A carboxylic acid hydrazide was prepared by the usual procedure and then heated with a methanolic cyanogen bromide solution giving the desired 2-amino-1,3,4-oxadiazole derivative.[10] The final sulfonamide was obtained by treatment with the appropriate aryl sulfonyl chloride.

III. N-Methylation of 2-Arylsulfonamido-5-substituted 1,3,4-Oxadiazoles or 1,3,4-Thiadiazoles.—Two thiadiazoles and one oxadiazole were alkylated with dimethyl sulfate. A 50% molar excess of the alkylating agent was treated with

(6) J. Matti, C. Ledoux and E. Kesler, *Bull. soc. chim. France*, 477 (1959).

(7) F. L. Chubb and J. Nissenbaum, *Can. J. Chem.*, **37**, 1121 (1959).

(8) Chemistry reported in part by J. B. O'Neal, P. B. Russell, A. C. Adams and E. L. Buhle, Meeting, Division of Medicinal Chemistry, American Chemical Society, New York, N. Y., September 11–16, 1960.

(9) Pharmacology presented in part by H. Rosen, A. Blumenthal, J. A. Singley and C. S. Davies, Meeting, American Society for Pharmacology and Experimental Therapeutics, Seattle, Washington, August 21–25, 1960.

(10) A. P. Swain, U. S. Patent 2,883,391 (1959).

the sodium salt of the sulfonamide and the resulting oil was purified and recrystallized from ethanol and water.

IV. **Preparation of 3-Arylsulfonamido-5-substituted-1,2,4-triazoles.**—The 3-amino-5-substituted-1,2,4-triazoles were prepared by the method of Allen and Bell.[11] The resulting 1,2,4-triazole was treated with the appropriate arylsulfonyl chloride in pyridine, and was allowed to stand for 16 hr. at room temperature before being precipitated with 6 normal hydrochloric acid. The sulfonamidotriazoles are insoluble in alkali.

The structures, melting points and analyses of these compounds are detailed in Tables I through V.

Pharmacology.—All compounds were first tested for their acute hypoglycemic effects by the oral route in fasting rats, and some of the more active compounds were also tested for their acute effects in fasting dogs. The following methods were used:

Rat.—Male Wistar rats weighing 170 to 200 g. were deprived of food for 18 hr. Control blood samples were taken from the tail, and the compound was administered immediately by stomach tube as a suspension in 2% Methocel. Further blood samples were taken for sugar determinations at hourly intervals for 5 hr. All compounds were tested at 3 or more dose levels with 3 or more rats, usually 4, per dose.

Dog.—Acute tests were made in dogs of both sexes following a like schedule of fasting and blood sampling. The drug was given to dogs by stomach tube as a slurry in water, and blood samples were taken from the saphenous or cephalic veins. Three or more dose levels with 3 or more dogs per dose were used in this test.

Practically all of the thiadiazole derivatives produced a lowering of blood sugar by one dose on oral administration in rats; the minimum effective doses[12] ranged from 15 to 120 mg./kg. Likewise, most of the oxadiazole derivatives produced a lowering of blood sugar in the rat; the minimum effective doses ranged from 30–240 mg./kg. The thiadiazoles as a group were more potent in rats, never requiring a dose greater than 120 mg./kg. to produce hypoglycemia, while the oxadiazoles frequently required doses of 240 mg./kg. to produce a similar effect. Several compounds were inactive at this dose. Table VI shows the most active thiadiazole and oxadiazole compounds, giving the minimal effective dose for each, in rats and dogs, respectively.

Three triazole compounds Wy (1535, 1536 and 1538) were active in rats at 120 mg./kg., two were inactive. One other compound, 2-(N-methyl-*p*-methoxybenzenesulfonamido)-5-isobutyl 1,3,4-thiadiazole Wy (2583), was active at 120 mg./kg., in contrast to the unmethylated compound Wy (2340), which, in rats, was active at half this dose.

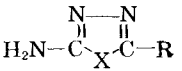
Two of the more potent oxadiazole derivatives Wy (1508 and 1509) were selected for further investigation. Table VII shows the effects of these compounds. Single oral doses in rats and dogs demonstrated that these compounds were approximately equiactive.

To compare these further, three groups of 3 dogs each were fasted for 18 hr. and control venous blood samples taken at zero time. One group received 25 mg./kg. of compound 1508 in capsules at zero time, the second group was given

(11) C. F. Allen and A. Bell, *Org. Syntheses*, **26**, 11 (1946).

(12) Minimum effective dose was taken as the lowest dose which produced a drop of between 15 and 20% in blood sugar, this drop being maintained for 1 hr. or longer.

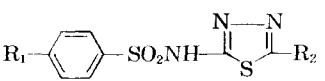
TABLE I



R	X	M.p., °C.	Formula	Calcd., % C	Calcd., % H	Calcd., % N	Found, % C	Found, % H	Found, % N
3-Cyclohexylpropyl	O	165–166	$C_{11}H_{19}N_3O$	63.1	9.1	20.1	63.3	9.1	20.2
Cyclopentyl	O	196–197	$C_7H_{11}N_3O$	54.9	7.2	27.4	54.7	7.2	27.4
Cyclobutyl	O	189–190	$C_6H_9N_3O$	51.8	6.5	30.2	51.6	6.6	29.9
Cyclopropyl	O	170–172	$C_5H_7N_3O$	48.0	5.6	33.6	48.4	5.8	33.7
p-Tolyl	O	265–267	$C_9H_9N_3O$	61.7	5.2	24.0	61.5	5.3	24.2
Isobutyl	NH	130–133	$C_6H_{12}N_4$[a]	51.4	8.6	40.0	51.1	8.2	39.5
Cyclohexyl	NH	193–195	$C_8H_{14}N_4$	57.8	8.5	33.7	57.7	8.4	33.8

[a] J. Rilley and D. Madden, *J. Chem. Soc.*, 819 (1929), prepared the nitrate, m.p. 171°.

TABLE II

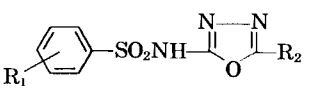


Wy number	R_1	R_2	M.p., °C.	Formulas	Calcd., % C	Calcd., % H	Calcd., % N	Calcd., % S	Found, % C	Found, % H	Found, % N	Found, % S
2340	CH_3O	$(CH_3)_2CHCH_2$	144–146	$C_{13}H_{17}N_3O_3S_2$[a]	47.7	5.2	..	..	47.5	4.9	..	..
2341	CH_3	$(CH_3)_2CHCH_2$	165–168	$C_{13}H_{17}N_3O_2S_2$[b]	50.2	5.5	..	..	50.0	5.6	..	.
2561	CH_3	2-(cyclo-C_6H_{11})C_2H_4	154–155	$C_{17}H_{23}N_3O_2S_2$	55.9	6.3	11.5	17.5	56.0	6.3	..	17.4
2562	CH_3O	2-(cyclo-C_6H_{11})C_2H_4	160–161	$C_{17}H_{23}N_3O_3S_2$	53.5	6.0	11.0	16.8	53.0	5.9	10.4	16.7
2592	CH_3	3-(cyclo-C_6H_{11})C_3H_6	134–135	$C_{18}H_{25}N_3O_2S_2$	..	..	..	16.9	..	..	..	16.8
2593	CH_3O	3-(cyclo-C_6H_{11})C_3H_6	170–171	$C_{18}H_{25}N_3O_3S_2$	..	..	..	16.2	..	..	..	16.4
2594	CH_3	cyclo-C_6H_{11}	203–204	$C_{15}H_{19}N_3O_2S_2$[c]	..	..	..	19.0	..	..	..	19.5

2595	CH_3O	cyclo-C_6H_{11}	145–146	$C_{15}H_{15}N_3O_3S_2$	..	..	..	18.1	..	..	..	18.3
2607	CH_3	2-(cyclo-C_5H_9)C_2H_4	153–154	$C_{16}H_{21}N_3O_3S_2$	..	..	12.0	18.2	..	..	12.0	18.3
2608	CH_3O	2-(cyclo-C_5H_9)C_2H_4	168–170	$C_{16}H_{21}N_3O_3S_2$	..	..	11.4	17.4	..	..	11.7	17.4
2628	Cl	cyclo-C_6H_{11}	182–184	$C_{14}H_{16}ClN_3O_2S_2$[d]	..	..	..	..	..	..	..	..
2643	CH_3O	(cyclo-C_6H_{11})CH_2	177–179	$C_{16}H_{21}N_3O_3S_2$	..	..	..	17.4	..	..	..	17.2
2644	CH_3	(cyclo-C_6H_{11})CH_2	180–182	$C_{16}H_{21}N_3O_2S_2$	..	..	..	18.2	..	..	..	18.2
2645	H	3-(cyclo-C_6H_{11})C_3H_6	132–133	$C_{17}H_{23}N_3O_2S_2$	..	..	..	17.5	..	..	..	17.7
2646	Cl	2-(cyclo-C_5H_9)C_2H_4	162–163	$C_{15}H_{18}ClN_3O_2S_2$	..	..	..	17.2	..	..	..	17.4
2647	H	cyclo-C_6H_{11}	203–205	$C_{14}H_{17}N_3O_2S_2$	..	..	..	19.8	..	..	..	20.3
2660	CH_3	$(C_2H_5)_2CH$	150–151	$C_{14}H_{19}N_3O_2S_2$	..	..	12.9	19.7	..	..	13.1	19.8
2661	CH_3O	$(C_2H_5)_2CH$	109–111	$C_{14}H_{19}N_3O_3S_2$	..	..	12.3	18.8	..	..	12.8	18.6
2673	Cl	$(C_2H_5)_2CH$	109–110	$C_{13}H_{16}ClN_3O_2S_2$	..	..	..	18.5	..	..	..	18.7
1702	Br	$(CH_3)_2CHCH_2$	155–157	$C_{12}H_{14}BrN_3O_2S_2$	..	..	..	17.1	..	..	..	17.1
1703	F	$(CH_3)_2CHCH_2$	149–150	$C_{12}H_{14}FN_3O_2S_2$	..	..	..	20.3	..	..	..	20.6

[a] R. Jasmin and W. Johnson, *J. Am. Pharm. Assoc.*, **48**, 113 (1959); equiv. wt.: calcd., 311; found, 313. [b] F. J. Macrae and D. J. Drain, British Patent 811,522 (4/8/59) give m.p. 173–174°; equiv. wt.: calcd., 327; found, 331. [c] Ref. *b*, m.p. 201.5–202°. [d] H. Ruschig, G. Kerger, W. Aumüller, H. Wagner, R. Weyer, A. Bänder and J. Scholz, *Arzneimittel-Forsch.*, **8**, 448 (1958), give m.p. 183–185°.

TABLE III

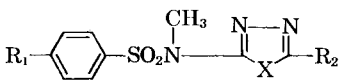


Wy number	R_1	R_2	M.p., °C.	Formula	Calcd., % C	Calcd., % H	Calcd., % N	Found, % C	Found, % H	Found, % N
1508	*p*-CH_3	cyclo-C_6H_{11}	163–164	$C_{15}H_{19}N_3O_3S$	56.1	6.0	13.1	56.1	6.1	12.9
1509	*p*-Cl	cyclo-C_6H_{11}	168–169	$C_{14}H_{16}ClN_3O_3S$	49.2	4.7	12.3	49.2	4.8	12.1
1530	*p*-Cl	$(CH_3)_2CHCH_2$	105–106.5	$C_{12}H_{14}N_3ClO_3S$	45.6	4.5	13.3	45.7	4.4	13.7
1531	*p*-CH_3	$(CH_3)_2CHCH_2$	110–111	$C_{13}H_{17}N_3O_3S$[a]	52.9	5.8	14.2	52.7	5.6	14.6

1532	H	$(CH_3)_2CHCH_2$	107–108	$C_{12}H_{15}N_3O_3S$[b]	51.2	5.4	14.9	51.1	5.3	15.2
1534	*p*-CH_3O	$(CH_3)_2CHCH_2$	97–99	$C_{13}H_{17}N_3O_4S$[c]	50.2	5.5	13.5	50.5	5.7	13.1
1555	H	3-(cyclo-C_6H_{11}) C_3H_6	119–120	$C_{17}H_{23}N_3O_3S$	58.4	6.6	12.0	58.7	6.5	12.0
1557	H	cyclo-C_6H_{11}	163–165	$C_{14}H_{17}N_3O_3S$	54.7	5.6	13.7	54.6	5.6	13.6
1559	*p*-CH_3O	cyclo-C_6H_{11}	139–140	$C_{15}H_{19}N_3O_4S$	53.4	5.7	12.5	53.7	5.6	12.4
1627	H	cyclo-C_4H_7	143–144	$C_{12}H_{13}N_3O_3S$	51.6	4.7	15.1	51.6	4.7	15.2
1628	H	cyclo-C_5H_9	164–165	$C_{13}H_{15}N_3O_3S$	53.2	5.2	14.3	53.2	5.2	14.4
1629	*p*-Cl	cyclo-C_5H_7	151–153	$C_{12}H_{12}ClN_3O_3S$	45.9	3.9	13.4	46.1	3.7	13.6
1630	*p*-Cl	cyclo-C_5H_9	177–178	$C_{13}H_{14}ClN_3O_3S$	47.6	4.3	12.8	47.7	4.0	12.9
1631	*p*-CH_3	cyclo-C_5H_9	167–169	$C_{14}H_{17}N_3O_3S$	54.7	5.6	13.7	54.5	5.8	13.3
1632	*p*-CH_3	cyclo-C_4H_7	146–148	$C_{13}H_{15}N_3O_3S$	53.2	5.2	14.3	53.0	4.9	14.0
1648	*p*-$(CH_3)_2CH$	cyclo-C_6H_{11}	152–154	$C_{17}H_{23}N_3O_3S$	58.4	6.6	12.0	58.2	6.6	12.3
1649	*p*-CH_3	cyclo-C_5H_7	144–145	$C_{12}H_{13}N_3O_3S$	51.6	4.7	15.1	51.1	4.7	15.0
1650	*p*-C_2H_5	cyclo-C_6H_{11}	129–131	$C_{16}H_{21}N_3O_3S$	57.3	6.3	12.5	57.2	6.1	12.9
1658	*p*-CH_3CONH	cyclo-C_6H_{11}	193–195	$C_{16}H_{20}N_4O_4S$	52.7	5.5	15.4	52.8	5.7	15.3
1659	3,4-benzo	cyclo-C_6H_{11}	149–151	$C_{18}H_{19}N_3O_3S$	60.5	5.4	11.8	60.4	5.3	12.3
1661	*p*-NH_2	cyclo-C_6H_{11}	199–200	$C_{14}H_{18}N_4O_3S$	52.2	5.6	17.4	52.2	5.5	17.6
1662	*o*-CH_3	cyclo-C_6H_{11}	122–124	$C_{15}H_{19}N_3O_3S$	56.1	6.0	13.1	55.9	5.8	13.0
1665	2,5-Cl_2	cyclo-C_6H_{11}	159–160	$C_{14}H_{15}Cl_2N_3O_3S$	44.7	4.0	11.2	44.6	3.9	11.4
1666	*p*-NO_2	cyclo-C_6H_{11}	228–229	$C_{14}H_{16}N_4O_5S$	47.7	4.6	15.9	47.6	4.5	15.9
1667	*p*-Br	cyclo-C_6H_{11}	180–181	$C_{14}H_{16}BrN_3O_3S$	43.5	4.2	10.9	43.7	4.1	10.8
1668	*m*-NH_2	cyclo-C_6H_{11}	196–198	$C_{14}H_{18}N_4O_3S$	52.2	5.6	17.4	51.9	5.2	16.9
1669	*m*-NO_2	cyclo-C_6H_{11}	165–167	$C_{14}H_{16}N_4O_5S$	47.7	4.6	15.9	47.7	4.6	16.1
1671	*p*-CH_3	C_6H_5	233–235	$C_{15}H_{18}N_3O_3S$	57.1	4.2	13.3	57.2	4.4	13.1
1672	*p*-CH_3	*p*-$CH_3C_6H_4$	221–223	$C_{16}H_{15}N_3O_3S$	58.4	4.6	12.8	58.2	4.7	12.8
1682	*p*-CH_3	2-Furyl	219–221	$C_{13}H_{11}N_3O_4S$	51.2	3.6	13.8	51.0	3.9	14.5

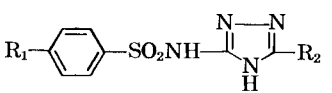
[a] Astra A. B., Kemiska Fabriker, British Patent 826,539 (1/13/60) gives m.p., 113.5–114.5°. [b] Reference *a*, m.p., 105.5–106.5°. [c] Reference *a*, m.p., 97–97.5°.

TABLE IV



Wy number	R_1	R_2	X	M.p., °C.	Formula	Calcd., % C	H	N	S	Found, % C	H	N	S
2582	CH_3	$(CH_3)_2CHCH_2$	S	110–112	$C_{14}H_{19}N_3O_2S_2$	51.7	5.9	..	..	51.6	5.7	..	..
2583	CH_3O	$(CH_3)_2CHCH_2$	S	109–111	$C_{14}H_{19}N_3O_3S_2$	49.3	5.6	..	..	49.3	5.7	..	..
1670	CH_3	cyclo-C_6H_{11}	O	154–156	$C_{16}H_{21}N_3O_3S$	57.3	6.3	12.5	9.6	57.4	6.1	12.5	9.6

TABLE V



Wy number	R_1	R_2	M.p., °C.	Formula	Calcd., % C	H	N	Found, % C	H	N
1535	CH_3	$(CH_3)_2CHCH_2$	165–166	$C_{13}H_{18}N_4O_2S$	53.1	6.2	19.0	52.8	6.0	19.5
1536	Cl	$(CH_3)_2CHCH_2$	170–172	$C_{12}H_{15}ClN_4O_2S$	45.8	4.8	17.8	45.7	4.7	17.7
1537	H	cyclo-C_6H_{11}	145–147	$C_{14}H_{18}N_4O_2S$	54.9	5.9	18.3	54.6	5.9	18.9
1538	Cl	cyclo-C_6H_{11}	198–203	$C_{14}H_{17}ClN_4O_2S$	49.3	5.0	16.4	49.1	4.9	16.5
1539	CH_3	cyclo-C_6H_{11}	167-169	$C_{15}H_{20}N_4O_2S$	56.2	6.3	17.5	56.1	4.8	17.6

TABLE VI

ACTIVE THIADIAZOLE AND OXADIAZOLE COMPOUNDS

Compound Wy no.	Minimum effective oral dose, mg./kg. Rat	Dog
	Thiadiazole derivatives	
2340	60	25
2341	—	25
2561	30	—
2562	60	—
2594	30	50
2595	60	25
2608	60	—
2628	30	12.5
2643	60	—
2644	15	—
2645	60	—
2646	60	—
2647	30	—
	Oxadiazole derivatives	
1508	30	12.5
1509	30	12.5
1559	60	—
1667	30	12.5

TABLE VII

ACUTE ORAL EFFECTS OF (1508), 2-(*p*-TOLUENESULFONAMIDO)-5-CYCLOHEXYL-1,3,4-OXADIAZOLE AND (1509), 2-(*p*-CHLOROBENZENESULFONAMIDO)-5-CYCLOHEXYL-1,3,4-OXADIAZOLE, IN RATS AND DOGS, PERCENTAGE BLOOD SUGAR CHANGE

Compound	No. rats used	No. dogs used	P.O. mg./kg.	Time in hours 1	2	3	4	5
	7	—	15	−9	−6	−6	0	+11
Wy 1508	8	—	30	−19	−15	−9	−6	−5
	7	—	60	−32	−25	−13	−2	+7
	5	—	120	−32	−38	−20	−1	+12
	4	—	15	−9	−4	−2	0	+4
Wy 1509	4	—	30	−18	−6	−3	+1	+2
	4	—	60	−25	−15	−11	−12	0
	4	—	120	−34	−39	−22	−19	−7
Control	5	—	—	−3	+1	+5	+7	+10
	—	4	12.5	−18	−21	−9	−7	−8
Wy 1508	—	4	25	−12	−21	−22	−25	−20
	—	3	50	−26	−37	−32	−31	−36
	—	4	12.5	−11	−18	−16	−13	−6
Wy 1509	—	4	25	−19	−23	−24	−20	−14
	—	3	50	−22	−29	−23	−28	−34
Control	—	8	—	+1	−2	−3	−4	−4

25 mg./kg. of Wy-1509 and the third group served as control. At three hr. and 12 hr. later, blood samples again were taken, and drug administration was repeated after the 12 hr. sampling. Medication was continued at 12-hr. intervals

for a total of nine doses, and blood samples for sugar determinations were taken just prior to drug for a total of 108 hr. Figure 1 shows the results obtained in this experiment. Wy-1508 has a longer duration of action than 1509 and with repeated doses 12 hr. apart, 1508 produces the greater effect.

An additional experiment was made in which compound Wy-1508 was administered to dogs over a longer period of time (104 days). Five groups of 3 dogs each were utilized. Preceding drug treatment, 2 complete blood counts were made with each dog at 2 week intervals as well as 3 control blood sugar determinations during the week preceding drug treatment. One group of 3 dogs served as control while the other 4 groups received 25, 50, 100 and 200 mg./kg. per respective groups. Blood sugar values were determined twice per week for 6 weeks and then once per week for the duration of the experiment. Figure 2 shows the results of this experiment.

Table VIII shows acute toxicity data in mice and rats.

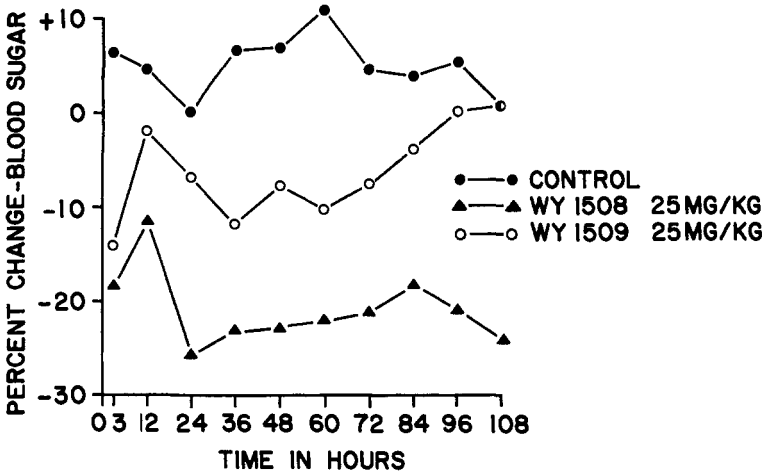


Fig. 1.—Five day-dog test.

In a chronic feeding study of compound Wy-1508, groups of rats, male and female, were fed the drug continuously at concentrations of 0.05, 0.1, 0.2 and 0.4% admixed to their diet, for a period of 52 weeks. Growth, food consumption and blood counts were satisfactory in the test groups as well as the control group. A few of the rats at the highest concentration were killed and autopsied at 6, 10 and 52 weeks and histopathological examination of their tissues revealed no abnormalities attributable to the drug treatment.

The 15 dogs used for daily drug administration and blood sugar determination, also served as a subacute toxicity test. All dogs at 25, 50, and 100 mg./kg. daily appeared to be in good health throughout the 15 weeks of drug administration. No emesis, depression or muscle weakness were observed. Blood counts were within normal range and food intake and weight were satisfactory in all except one dog at 100 mg./kg. This dog exhibited some signs of anorexia with a concomitant loss of 13% of her weight. Months after cessation of drug, these dogs are still in our colony and appear to be normal in all respects.

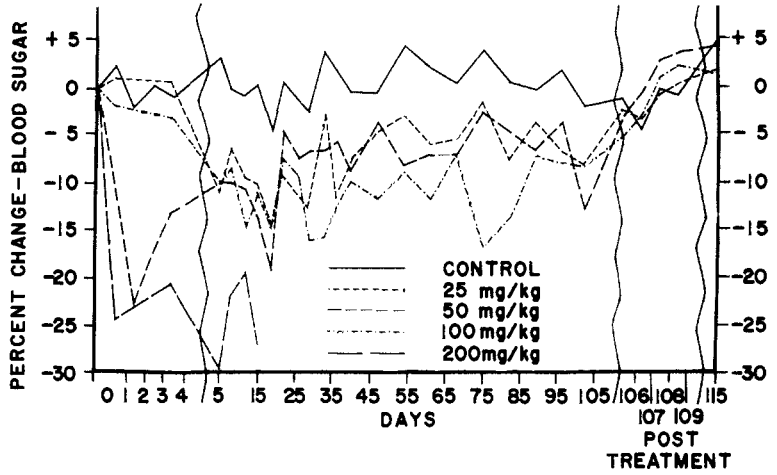


Fig. 2.—WY 1508 115 day dog study.

TABLE VIII

QUANTITATIVE ESTIMATION OF THE LETHAL DOSE-50 OF WY-1508 IN MICE AND RATS

Route	Species	No. used	Quant. est. LD_{50} mg./kg.	Range (for odds 19/20) limits —mg./kg.— Lower	Upper	Slope (dose-mort. curve)
Intraperitoneal	Mouse	60	398.7	365.5	434.9	19.9
Oral	Mouse	60	705.4	600.4	828.9	5.9
Intraperitoneal	Rat	40	620.8	589.2	654.1	24.9
Oral	Rat	50	1085.2	760.0	1549.4	8.7

The dogs treated with 200 mg./kg. daily all showed signs of toxicity which included vomiting for the first 10 days, anorexia, loss of weight, muscle weakness and depression. One dog died after 14 days of drug treatment, while the other 2 dogs were killed after 18 and 30 days of treatment respectively. At autopsy both showed low hemoglobin and red cell counts.

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