of any particular case, nor to the type of therapy, whether with serum or drug. Its titer did not parallel that of either the type-specific or species-specific agglutinins. Incidentally, we found, as have others,[5] no indication that the heterophile titer was directly related to or responsible for serum-sickness. There was no correlation between the heterophile titer and the positivity or negativity of the cutaneous reaction to polysaccharide. There was some increase in its titer following the administration of species-specific serum high in heterophile-antibody content.

Summary. 1. The immune responses that occur naturally in the course of an untreated pneumonia, studied by determining type-specific and species-specific agglutinins and by the dermal reactivity to type-specific polysaccharides, are apparently unaltered by treatment with sulfapyridine. 2. There seems to be no relation of the heterophile-antibody titer to the clinical course of pneumococcal pneumonia or its immune responses following sulfapyridine-therapy.

10967

Toxicity of the Ortho, Meta and Para Isomers of Sulfanilamide.

EDWIN P. LAUG AND HERMAN J. MORRIS (Introduced by Erwin E. Nelson.)

From the Division of Pharmacology, Food and Drug Administration, U. S. Department of Agriculture, Washington, D. C.

While the therapeutic potency of p-sulfanilamide has been amply demonstrated, reports by a group of French investigators[1] indicate that the protective action of the ortho and meta isomers against experimental infection in mice is practically nil. The present investigation was undertaken to determine whether there is any corresponding difference in the toxicity of the 3 isomers.

The acute toxic effects for mice were determined by oral administration of the isomers in 33% suspension in 5% gum acacia to fasted animals. The manifestations of toxic action were quite different among the 3 isomers. When the para-isomer was given there usually resulted, in from 30 minutes to an hour, a preliminary stage of stimulation, with muscular incoordination and spastic paralysis of

[5] Powell, H. M., Jamieson, W. A., and Kempf, J. F., *J. Immunol.*, 1935, **29**, 267.

[1] Trefouel, J., Trefouel, J., Niti, F., and Bovet, D., *Ann. Inst. Pasteur*, 1937, **58**, 30.

the hind limbs. This was followed by coma, sometimes lasting 18 to 24 hours, and death. After the ortho isomer, however, there was no evidence of either stimulation or spastic paralysis. Instead the animals became comatose within 30 minutes, and died after 18 to 24 hours, with no additional symptoms other than slow and labored respiration. The above effects were induced by a dosage level of from 4 to 6 g per kilo for both ortho and para isomers. The meta isomer was without effect at this dosage, but at 10 g per kilo there were symptoms suggestive of the action of para isomer, but with prompt recovery.

The results of the toxicity studies have been tabulated in Table I. Calculation of the LD50's and the regression coefficients from the 3 respective dosage-mortality curves were obtained by treating the data according to the method outlined by Bliss[2] and described by us[3] in detail elsewhere. The ascending order of toxicity is meta, para and ortho, and it should be noted that the differences between the respective LD50's are significant. The toxicity of the meta isomer proved to be so low that sufficient quantities could not be administered to effect even 50% mortality without danger of mechanical injury to the animals. Consequently the LD50 value had to be ob-

TABLE I.
Acute Toxicity of Ortho, Meta, and Para Isomers of Sulfanilamide for Mice.

	Dose, g/kg	Mortality Ratio No. mice died / No. mice used	LD50 g/kg	Fiducial* limits	Regression Coefficient	Fiducial* limits
	2.5	1/10				
	3.0	1/10		4.15		11.5
Ortho	3.5	2/10	3.84		8.58	
	4.0	16/25		3.55		5.65
	5.0	22/25				
	6.0	23/25				
	4.0	1/25				
	5.0	3/25		19.3		6.06
Meta	6.0	3/25	12.5		3.36	
	7.5	2/10		8.04		0.65
	10.0	4/10				
	3.5	0/10				
	4.0	8/25		4.86		15.4
Para	4.5	5/10	4.61		11.5	
	5.0	24/40		4.39		7.54
	5.5	7/10				
	6.0	24/25				

* For odds of 19 out of 20 trials.

[2] Bliss, C. I., *Quart. J. Pharm. and Pharmacol.*, 1938, **11**, 192.

[3] Laug, E. P., Calvery, H. O., Morris, H. J., and Woodard, G., *J. Ind. Hyg. and Toxicol.*, 1939, **21**, 173.

TABLE II.
Solubility of Ortho, Meta and Para Isomers of Sulfanilamide at 28°C and 38°C.

	Grams dissolved per 100 g H_2O	
	28°C	38°C
Ortho	0.808	1.317
Meta	1.508	2.796
Para	0.940	1.731

tained by interpolation of the rectified mortality curve. This accounts in part for the wide limits of error for the value given. In the case of the regression coefficients, there is a significant difference between meta and para only. Thus, it would appear that the observed toxicities show no correlation with the reported therapeutic potencies.

In Table II are given the results of solubility measurements at 28° and 38°. These represent averages of closely agreeing triplicate determinations. While it could be expected that solubility might modify the toxicity of a compound, no correlation was noted in the case of the isomers. M-sulfanilamide, roughly twice as soluble as either o- or p-, is the least toxic, while o-sulfanilamide, the least soluble, is the most toxic. Thus it would appear that solubility, in so far as it may influence absorption from the gut, is not a factor to be considered in the toxicity of these three isomers.

Dyke[4] has reported on the acute toxicity of the 3 isomers for mice with LD50's ranging from 3 to 5 g per kilo. However, examination of the data reveals considerable scattering of the results, as well as the use of restricted numbers of animals. No statistical analyses of the results, nor exact calculation of LD50's were attempted. For p-sulfanilamide alone, Marshall and coworkers[5] give 3.8 g per kilo, Wien,[6] 4.2 g per kilo. Our value, 4.61 g per kilo is somewhat higher.

Conclusions. (1) The LD50's for oral administration to mice of the isomers of sulfanilamide are: ortho, 3.84 g; meta, 12.45 g; para, 4.61 g per kilo. (2) There is no correlation between solubility and toxicity of the 3 isomers. (3) There is no relation between toxicity as here found and therapeutic efficiency as reported by others.

[4] Dyke, W. J. C., *Chemist and Druggist*, 1937, **127**, 126; *Quart. J. Pharm. and Pharmacol.*, 1937, **10**, 319.

[5] Marshall, E. K., Cutting, W. C., and Emerson, K., *J. A. M. A.*, 1938, **110**, 252.

[6] Wien, R., *Quart. J. Pharm. and Pharmacol.*, 1938, **11**, 217.