composed of yolk-sac, outgrowths of allantoic origin, and much embryonic material. Medullary tubes, occasionally with chorda, somites, dense mesenchyme and differentiating muscle, lateral plate material split to form coelomic cavities, and possibly nephric tubules, are found in such masses.

Evidently at this stage, the levels of the axis are all equally capable of differentiation *in vitro* to form primitive axial structures of characteristic level. Histogenesis of both medullary and muscular elements may proceed quite normally.

A few cases of explants of pieces in the primitive streak stage (stage 13) are available. The anteriormost third of the anterior half may form pulsating heart tissue and yolk-sac epithelium. The entire anterior half may form well-differentiated lobed brain tube, foregut, heart and mesenchyme. Only one explant of the posterior half at this stage was successful; it contained yolk-sac and a necrotic outgrowth probably allantoic in nature. The posterior half of the primitive streak stage appears deficient in potency when compared with the anterior half of the embryo which may develop quite as well as the same material in later stages.

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Toxicity of Sulfanilamide. A Study of the Pathological Lesions in White Mice.*

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Since the introduction of sulfanilamide and related substances as chemotherapeutic agents, there have appeared in the literature several reports on their toxicity for experimental animals. Domagk[1] observed that 0.5 gm. per kilo of 'Prontosil Insoluble,'† given orally was well tolerated by mice and dogs, whereas the cat tolerated only 0.2 gm. per kilo. The urine of dogs which were exposed to varying amounts of 'Prontosil Insoluble' over a period of 14 days showed no red or white blood cells or casts. Similar studies were carried on

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1 Domagk, G., *Deutsche. Med. Wchnschr.*, 1935, **61**, 250.

† Hydrochloride of 4-sulfamido-2,4-diamino azo benzene.

by Buttle, Gray and Stephenson,[2] who found oral dosages of 0.6 gm. per kilo of 'Prontosil Insoluble' innocuous for mice. Larger doses, 1.25 and 5 gm. per kilo proved fatal in the majority of instances. These investigators also studied the toxicity for mice of sulfanilamide given orally and found 2.5 gm. per kilo well tolerated, whereas 5 gm. per kilo killed 2 of 6 animals and 10 gm. per kilo killed 6 out of 6. Rosenthal,[3] studying the toxicity of sulfanilamide for mice, reported 6 gm. per kilo as the fatal dose when given subcutaneously in olive oil. Two gm. per kilo produced spastic extremities, flexion of the spine, excitability and incoördination which disappeared after 12 hours. In a similar manner, Raiziss and his coworkers[4] found 1-2 gm. of sulfanilamide given subcutaneously to be well tolerated by mice, whereas 2.5 and 3 gm. proved to be a fatal dose. Maximum tolerated oral dosage for rabbits was found to be 1.5 gm. per kilo.

Up to the present time all published data relevant to toxicity of sulfanilamide for animals has presented maximum tolerated and lethal dosages. No studies of gross or microscopic pathology produced by sulfanilamide in animals have appeared in the literature.

The present report deals with the histology of various organs of mice exposed to varying doses of sulfanilamide.

Normal Swiss mice of approximately 20 gm. each in weight in groups of 5, were given large parenteral doses of Prontosil or sulfanilamide over a period of 14 days (Table I). At the end of this time, one of the surviving animals of each group was sacrificed and the remaining animals at weekly intervals thereafter. Five normal mice were studied as controls. Microscopic sections‡ of liver, spleen, kidney and vertebral and femoral bone marrow were made at the time each animal expired or was sacrificed. Mice to be sacrificed were given a lethal dose of ether in each instance. Tissues were fixed in Zenker-Formol and stained with hematoxylin and eosin unless otherwise indicated.

Results. 'Prontosil' was well tolerated in the dose given. The rapid absorption of the dye was evidenced by the fact that the ears became pink 5-10 minutes after the injection. Stools were dark red in color for the first 6-8 hours after injection, whereas the urine remained faintly pink as long as 18 hours. No reactions to the drug were noted.

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2 Buttle, G., Gray, W., and Stephenson, D., *Lancet*, 1936, **1**, 1286.

3 Rosenthal, S., *Pub. Health Rep.*, 1937, **52**, 48.

4 Raiziss, G. W., Severac, M., and Moetsch, J., *J. Chemotherapy*, 1937, **14**, 1.

‡ The author is indebted to H. A. Weiner, M.D., for assistance in interpretation of the pathological material.

TABLE I.
Exposure of Normal 20 Gram Swiss Mice to "Prontosil" and Sulfanilamide.

Mouse No.	Dosage—Sulfanilamide					Time Sections Were Made			
	mg. per day	Route	Days	gm./kg./day	Total mg.	Death— Day of Exposure	Sacrificed— Weeks after onset of Exposure	Hemosiderin in Spleen	Eosinophils in Bone Marrow
1	....	....	....	....	....	....	....	—	+
2	....	....	....	....	....	....	....	—	++
3	....	....	....	....	....	....	....	—	+++
4	....	....	....	....	....	....	....	—	+++
5	....	....	....	....	....	....	....	—	++
6	50*	i.p.†	14	2.5	700	....	2	—	+++
7	50*	"	14	2.5	700	....	3	....	++
8	50*	"	14	2.5	700	....	D-4‡	++	++
9	50*	"	14	2.5	700	....	5	±	+++
10	50*	"	14	2.5	700	....	6	+	++
11	20	"	14	1	280	....	2	±	+++
12	20	"	14	1	280	....	3	±	+++
13	20	"	14	1	280	....	4	+	+++
14	20	"	14	1	280	....	5	++	+++
15	20	"	14	1	280	....	6	++	+++
16	30	subq.§	3	1.5	90	4	....	++	+++
17	30	"	5	1.5	150	6	....	++	+++
18	30	"	14	1.5	420	....	3	++++	++++
19	30	"	14	1.5	420	....	4	+++	+++
20	30	"	14	1.5	420	....	5	++++	+++
21	50	"	1	2.5	50	1	....	....	....
22	50	"	4	2.5	200	5	....	±	+++
23	50	"	7	2.5	350	8	....	±	+++
24	50	"	7	2.5	350	8	....	±	+++
25	50	"	10	2.5	500	11	....	++++	++++

*"Prontosil"—Disodium salt of 4-sulfamido-phenyl-2-azo-7 acetylamino-1-hydroxy-naphthalene-3,6 disulfonic acid.
†i.p.—intraperitoneal.
‡Died of mouse typhoid infection.
§subq.—subcutaneous.

Sulfanilamide was not well tolerated in large doses and produced unsteadiness, incoördination, paralysis, acute anterior flexion of the spine, spastic extension of the legs, prostration, convulsions, and sometimes death. With the smaller dosages the above symptoms were transient, coming on 30-60 minutes after the injection and disappearing in 12-18 hours.

Mice receiving sulfanilamide intraperitoneally suspended in saline showed a fibroblastic foreign body reaction with crystals scattered through the area of reaction. These areas appear as milky white spots on the surfaces of the liver, spleen and peritoneum.

Liver: sections of this organ showed no definite abnormalities.

Kidneys: no pathological changes were found.

Spleen: golden-yellow pigment in varying amounts was noted in areas adjacent to the malpighian bodies. By the aid of Turnbull's stain, this pigment was proven to be hemosiderin. As will be seen in Table I, the amount of hemosiderin was roughly proportional to the size of the total dose and the duration of life after onset of exposure. This suggests that the reaction was progressive after the drug was discontinued.

Bone marrow: A considerably greater incidence of eosinophils was found in the marrows of the animals exposed to 'Prontosil' or sulfanilamide than in the controls. No other differences were noted. Femoral and vertebral marrows were essentially identical in every instance.

Under the above experimental conditions it appears that sulfanilamide and 'Prontosil' exerted no definite toxic action on the liver or kidney.

The occurrence of hemosiderin in the spleen of animals exposed to various amounts of sulfanilamide suggests that increased blood destruction had taken place. Blood counts were not done in this series. Such a mechanism may explain the anemia seen clinically during prolonged sulfanilamide administration.

The greater number of eosinophils noted in the bone marrows of mice exposed to sulfanilamide and 'Prontosil' may possibly indicate an allergic response. If this is true it might be interpreted as further evidence in favor of the antigenic nature of sulfanilamide as suggested in a report on drug fever[5] following sulfanilamide.

These observations are preliminary but should serve as a guide for more complete and thorough studies in the future.

[5] Hageman, P. O., and Blake, Francis G., *J. A. M. A.*, 1937, **109**, 642.