barium acetate in the presence of 3 volumes of alcohol. The barium alcohol insoluble precipitate was collected, freed of barium and assayed for adenylic acid, adenosine diphosphate and adenosine triphosphate according to the method described by Albaum and Lipshitz(4). The method briefly consists in enzymatically converting adenosine diphosphate and adenosine triphosphate into adenylic acid and determining the latter as inosinic acid in the spectrophotometer.

Results. Data on the muscle phosphocreatine content of starved animals under various treatments are given in Table I. It is evident that adrenalectomy or cortisone treatment did not significantly alter the phosphocreatine content of the muscle; nor was there any effect of starvation. Well fed animals had the following phosphocreatine content in the muscle: Normal, 258 mg %, adrenalectomized, 281 mg %. It seems apparent that the adrenalectomized animal had as much phosphocreatine content in the muscle as the normal or cortisone treated animal and that, therefore, lack of phosphocreatine was not the cause of muscle fatigue or of increased sensitivity to stress. While the amounts of phosphocreatine found are very low (approximately 20 mg %) we were also able to find no change in the phosphocreatine content of kidney or liver upon adrenalectomy, with or without starvation.

TABLE II. Adenosine Triphosphate Content of Rat Muscle.

Treatment	ATP, mg %	Avg
Normal, no treatment	262 224 242	242 ± 15
Adrenalectomized	265 235 263	254 ± 14
Normal, treated with cortisone, 10 days	200 223 215	213 ± 10
Adrenalectomized, treated with cortisone, 10 days	262 236 202	233 ± 24

Since phosphocreatine is not altered one would expect no change in the ATP and since phosphocreatine is relatively high, one would further expect to find little adenosine-diphosphate or adenylic acid in the muscle. None of the muscle samples contained adenosine-diphosphate or adenylic acid. Their ATP content is given in Table II from which it is evident that there is no significant change in ATP content.

Conclusion. There was no significant effect of adrenalectomy or cortisone treatment upon the phosphocreatine or ATP content of rat muscle.

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Diasone and Promin as Therapeutic Agents in Experimental Toxoplasmosis.* (18552)

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Since this paper is concerned with the problem of therapy in toxoplasmosis, other pertinent information with respect to this protozoan disease should be sought elsewhere (1-4). 2(para amino benzene sulfonamide) pyridine (Sulfapyridine) and 2(p-acetyl-

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aminobenzene sulfonamide) thiazole (Sulfathiazole), reported by Warren and Sabin(5), still seem to be the accepted remedies in man(6), although in mice the carrier state and fatal relapse are usual(7). Both of these defects appear to have been avoided in an encouraging but limited number of mice treated with either p, p′ diamino diphenyl sulfone N, N′ (dextrose sodium sulfonate) (Promin, Parke Davis & Co.) or Disodium formaldehyde sulfoxylate diamino diphenyl sulfone (Diasone, courtesy of Abbott Laboratories). To the various antibiotics, arsenicals, sulfas and essential nutrients previously listed as ineffective for therapy(3,8-10), Pentaquine, Chaulphosphate, Desoxyribonucleic acid, Milibis, Aureomycin, Spleen Marrow and Sulfadiazine may be added. Biocca & Nobrega's favorable report(11) of the last named drug could not be confirmed by our trials. Sabin's strain of *Toxoplasma* has been used because no spontaneous recoveries have occurred during our 5 years of experimentation and because the survival period rarely varies (7 to 8 days following subcutaneous inoculation). Our technic for inoculations and for the administration of drugs has been reported earlier(10). No serial dilutions of the infective emulsion are made when inoculating because the nature of the parasite negates this customary estimation of virulence. An homogenous distribution of reagents particles, or parasites in the diluent is a pre-requisite for such determinations and the units of distribution in the emulsified tissue of a *Toxoplasma* infected animal consist of free parasites and the host's cells within which there may be anywhere from 0 to agglomerations of 90 parasites. That our inoculum is adequately uniform in virulence, however, is evidenced by the uniform lethality of a given inoculum. This investigation has extended over a year because long and repeated post therapeutic observations are necessary to avoid assuming asymptomatic carriers are cured animals. In the earlier experiments, only 3-6 mice were used to reduce the hazard from handling, which seems minimal since repeated trials of artificial "biting" failed to produce infections in mice (12).

Our hypothesis that toxoplasmosis is essentially (if not exclusively) a disease of the RES broadly defined(13) prompted the investigation of Promin and Diasone because these sulfones have been beneficial in leprosy, a disease with a similar predilection.

In accordance with the clinical usage of Promin(14), subcutaneous inoculations (40 mg in 0.4 ml) were made 6x a week with the interpolation of a resting week after each 2 weeks. Curative results were obtained if a "booster" injection was given midway in the resting week. One mouse from the first trial was living after 14 months (6 weeks of injections and 5 boosters). Delayed therapy in a second trial was unsuccessful but 2 of 3 mice treated within 24 hours (4 weeks of injections and 4 "boosters") were in good health after 13 months. The 3rd mouse was killed at 8 months as a donor for 5 recipients which also remained symptomless for 6 weeks and produced no symptoms when used as donors for additional recipients (Table II). Promin failed to protect in reduced dosage levels or in delayed therapy but surpassed Sulfapyridine in comparative trials. All but 1 mouse treated with 1% Sulfapyridine in the diet died after treatment stopped. The simultaneous use of Promin and Sulfapyridine

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TABLE I. Comparison of Diasone and Promin.

No. mice	No. infected	Promin, 40 mg in .4 ml	.5% diasone mg/day/mouse	Days survived
5	5	—	—	7
3	—	6x wk 6x '' Booster	—	28 Killed
5	5	6x wk 6x '' Booster	—	1 for 16, 2 for 18 1 for 19, 1 for 25
3	—	—	23.7 mg 1st 6 wk 20.3 mg 8th wk	All thrived* 1 destroyed in 32
15	15	—	17.3 mg 1st 6 wk 17.5 mg 8th wk 500 mg/kg	1 for 22 1 for 55, fighting? 1 destroyed, 32 2 donors, 44 3 donors, 60 2 challenged, 62 1 for 85 1 for 88 1 for 101 2 thriving at 130†

Therapy delayed 2½ days after inoculation.
* 1 littered at 4 wk, 6 young thrived, killed at 4 wk.
† 1 littered at 7 wk, 6 young thrived at 110 days.

gave erratic results which were surpassed by those of Diasone.

After preliminary trials demonstrated that Diasone (0.5% in the diet)(15) protected mice even when therapy was delayed, a comparison of the effectiveness of Diasone and Promin was made. As Table I shows, the 5 untreated mice died 7 days after inoculation. Survival following delayed treatment with Promin was 16 to 25 days. One escaped, Diasone-treated mouse was killed because of its questionable identity, but 13 mice were thriving and one female littered when the Diasone therapy was interrupted at 6 weeks. Two of the symptom-free, treated mice served as donors for 10 recipients which were also used as donors after 28 symptom-free days. By error, only 3 final recipients were used, but these were symptomless at 33 days (Table II). Even the nurslings thrived when Diasone was again administered during the 8th week. Interrupted therapy was used to counteract the possibility of the drug's discouraging the emergence of parasites from the supposedly resistant intracellular position. Following the 8th week, 3 symptom-free mice were used as donors for 10 recipients which all developed symptoms and died (Table II). Two symptom-free, Diasone treated mice were challenged at this time and died at 7 days, which was in agreement with results with animals treated with Sulfapyridine and Promin (Table III) and with Weinman's report that recovery from toxoplasmosis did not guarantee immunity(16).

Diasone was much more effective than Promin in protecting mice from toxoplasmosis when therapy was delayed a third of the survival time of untreated mice, but the results with Promin are of value because they surpassed those of the presently favored Sulfapyridine and are based on over a year's observation of symptom-freed mice. Diasone has not been investigated as long but appears equally capable of suppressing symptoms and eliminating the carrier condition in a rapidly fatal infection with *Toxoplasma*. Mice treated with Diasone thrived and produced and suckled healthy litters. Leprosy in man has been treated successfully with Diasone for a considerable time and the trial of this drug in therapeutically resistant cases of human toxoplasmosis in as high a dosage as is compatible with the known standards of toxicity would seem to be indicated.

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TABLE II. Investigation of Symptom-free, Treated Mice. 1. For carrier state.

	Donors		Recipients #1		Recipients #2	
Therapy	No. of mice	Description	No. of mice	Results	No. of mice	Results
Diasone	1	150 days, PI	10	NS	8	NS
	2	105 days, PI		6 donors at 28 days		Killed 47th day
	1	35 days, litter				
	2	44 days, PI	10	NS	3	NS
				6 donors at 28 days		Killed 33rd day
	3	60 days, PI	10	All dead at 12 days		
Promin	1	242 days, PI	5	NS	5	NS
				donors at 61 days		Killed 28th day
Promin and sulfapyridine	3	Newborn from mother, 91 days, PI	4	NS Killed at 28 days		—
	5	Newborn from mother, 86 days, PI	4	NS Donors at 2 mo.	4	NS Killed 28th day

PI = Post inoculation. NS = No symptoms.

TABLE III. Investigation of Symptom-Free, Treated Mice. 2. For immunity.

Therapy	No. of mice	*Toxoplasma* challenge interval	Results
Diasone	2	62 days	Dead* in 7 days (Controls in 4 days)
Promin and sulfapyridine	1	113 days and her	1 of young died in 8
	3	26 day old young	days, others in 4
	1	84 days and her	All died in 5 days
	5	20 day old young	(Controls in 4 days)
	1	247 days	Dead in 6 days
	3	214 "	Dead in 51, 67, 70 days (symptoms atypical)
	1	203 "	Dead in 6 days (Controls in 4 days)

* Preparations from ascitic fluid contained *Toxoplasma.*

Summary. In an encouraging but limited number of white mice infected with a rapidly fatal toxoplasmosis, Diasone and Promin have not only suppressed the symptoms but have repeatedly eliminated the carrier state. Symptom-freed, Promin-treated mice have survived over a year but Diasone appears to be less toxic and more potent since it protected mice when therapy was delayed for ⅓ of the survival time of simultaneously infected, untreated mice.

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