but the difference has diminished. On the return of fibrinogen to preinjection level the usual relationship is restored.

Summary. The fibrinogen level of plasma and blood of large veins and arteries declines sharply and then gradually returns toward the preinjection level immediately after intravascular injection of histamine. The quantitative changes in fibrinogen are independent of those in other plasma proteins. They parallel the acute drop and gradual return in blood pressure referable to peripheral capillary alterations. This movement of fibrinogen is independent of anesthesia, splenectomy and cord transsection. It is not specific for histamine but occurs with other vasodepressors as well. With vasopressor agents a reversed movement of fibrinogen follows. The sudden changes in size of the capillary bed seem to be coordinated with the fibrinogen content of the circulating blood.

The two methods used for the estimation of fibrinogen yielded qualitatively similar but quantitatively differential values.

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Prophylactic and Curative Effects of Certain Sulfonamide Compounds on Exoerythrocytic Stages in *Plasmodium Gallinaceum* Malaria.

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The discovery by Raffaele[1] of a hitherto unrecognized form in *Plasmodium relictum* malarial infections has aroused considerable interest. They are non-pigmented and develop outside the red cell. They have been seen in *Plasmodium gallinaceum* infections by James and Tate[2] who called them exoerythrocytic schizonts. More recently these forms have been recognized in several other avian malarial infections, but with doubtful exceptions they have not been encountered in monkey or human malaria. A complete summary of recent literature has been compiled by Porter and Huff.[3]

The chief interest in these forms from the standpoint of chemo-therapy has been their refractoriness to quinine or atebrin. It is possible to obtain a marked therapeutic effect on the circulating *Plasmodium gallinaceum* parasites in young chicks by administering quinine or atebrin, yet death usually ensues, and at autopsy overwhelming numbers of exoerythrocytic forms can be found. For this reason it has been postulated by some that they are responsible for initiating relapses since they are not destroyed by the accepted antimalarial drugs. According to Kikuth and Mudrow[4] plasmochin possesses slight activity on the exoerythrocytic stages but no other drug has been shown to affect them.

Since these stages are found rarely in the circulating blood it has been difficult to study their development and to determine their response to chemo-therapy. This handicap has been overcome by perfecting a simple biopsy technic which permits multiple examination of brain tissue in individual animals and thus enables one to follow the course of development in treated and control chicks. The purpose of this preliminary report is to describe the methods used and to show that

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1 Raffaele, G., *Riv. di Malariol.*, 1936, **15**, 318.

2 James, S. P., and Tate, P., *Nature*, 1937, **139**, 545.

3 Porter, R. J., and Huff, C. G., *Am. J. Trop. Med.*, 1940, **20**, 869.

4 Kikuth, W., and Mudrow, L., *Z. f. Immun.*, 1939, **95**, 285.

TABLE I.
Development of Exoerythrocytic Stages in Quinine Treated and Untreated Chicks with *Plasmodium gallinaceum* Infections.

Age of chicks	No. of chicks	Dosage trophozoites	Route	Day of appearance exostages	No. deaths	No. recovered	Day of disappearance exostages
30 days	4	10,000	I.C.	20-25	2	2	35, 39
15	9 Q	100,000	I.V.	19-24	7	2	
13	8 Q	10,000	I.V.	17-20	6	2	26
20	6 Q	10,000	I.V.	17-21	6	0	
15	3 Q	1,000	I.V.	18-22	3	0	
7	2 Q	1,000	I.V.	20-22	2	0	
3	9 Q	200	I.V.	18-21	8	1	
7	7	20,000*	I.V.	8-11	6	1	18
7	12	10,000*	I.V.	8-11	12	0	

Q = Received quinine therapy during acute peripheral blood infection.
I.C. = Intracerebral.
I.V. = Intravenous.
* = Received sporozoites.

certain sulfonamide drugs prevent the appearance of exoerythrocytic forms. Likewise these drugs have a demonstrable curative effect on the forms when given after they have made their appearance in the brain capillaries. Neither quinine nor atebrin retards the appearance or alters the development of the forms. Plasmochin possesses slight activity.

Materials and Methods. White Rock chicks from a single hatchery were routinely used. The parasite was *Plasmodium gallinaceum.*

Infections were induced by injecting viable sporozoites from infected *Aedes aegypti* mosquitoes. Mosquitoes from 3 to 10 days old are allowed to feed upon infected chicks which show approximately 0.4% of the red cells containing gametocytes. It has not been found necessary to demonstrate exflagellation as practically all of the biting females become infected. The infected lots of mosquitoes are stored at 28°C for approximately 15 days, when they are lightly etherized, removed from the cages and rapidly dissected. An operator can remove the salivary glands at an average rate of 90 per hour. The number of infected mosquitoes usually employed to infect a group of chicks may be roughly approximated in the ratio of one mosquito for each chick. The glands are placed in a small vial containing 0.5 cc heparinized normal chick blood. The vial is approximately 2 inches long and contains 3 sections of glass stirring rod each 1½ inches long. The vial is then rolled slowly for 15 minutes and sporozoite counts are made from stained smears of a sample of the mixture. The sporozoites are counted and expressed as the number per 10,000 red cells. An effort is made to get 20,000 sporozoites in each 0.2 cc of the inoculum. If the lots are heavily infected, the number of sporozoites per unit volume can be reduced by dilution or, if scanty, additional infected glands added. The mixture is injected intravenously in 0.2 cc amounts. Parasites usually appear on the sixth or seventh day with death occurring on approximately the fourth day thereafter.

Exoerythrocytic Biopsies. The exoerythrocytic forms are most readily discernible in the brain capillaries. A 0.25 cc syringe containing 0.02 cc of saline fitted with a twenty-guage needle is inserted into the cortex of the cerebrum, and rotated 360°. At the same time slight suction is applied, and upon removal a satisfactory sample of brain tissue can be obtained. This biopsied material is emptied on a glass slide and smeared. Dried and stained with Giemsa stain, it is available for immediate study. Repeated biopsies on the same chick can be made with only occasional damage. Secondary infections are rarely encountered. This simple technic affords an unusual opportunity to observe the development of the exoerythrocytic forms in the living animal.

Development of Exoerythrocytic Forms. In the routine sporozoite-induced infections the exoerythrocytic forms first appear in the

TABLE II.

Effectiveness of Sulfonamides and Quinine on Exoerythrocytic Schizonts in Trophozoite-induced *Plasmodium gallinaceum* Infections. Drugs started on initial appearance of exoerythrocytic forms, one dose per day.

Chick No.		Dosage, mg/os	Days following inoculation										
			16	20	23	24	25	26	27	29	32	35	39
162	Sulfaguanidine	250*	0	+	+			0		0	0	0	0
170	”	250*	0	+	++			+		0	0	0	0
165	Sulfapyridine	500	0	0	+			+		0			0
182	”	500		0	+			c		0	0	0	0
173	Sulfathiazole	500	0	0	+			+d		0	0	0	0
191	”	500		0	+			++		0	0	0	0
169	Sulfadiazine	500			0	0	+	0		0	0	0	0
172	”	500		0	0	+		+		0	0	0	0
179	”	250*	0	++	+++	D							
180	”	250*	0	+	++		+++D						
156	Sulfanilamide	250	0	0	0	0	0	+		0	0	0	0
176	”	500		0	+				+++D				
181	”	500†		0	++					0	0	0	0
153	Quinine	40		0	0	+		++		+	+++	+++	+++
157	”	40	0	+	++	++++D							
161	”	40		++	++++D								
	Controls			0	++			++++D					
				+	+			+		++	+	0	0
				0	0	0	++			++	++	+	0
				+	+++			+++D					

*Dose increased to 500 mg on 23rd day.
†Dose reduced to 250 mg on 29th day.
+ - ++++ = Intensity of exoerythrocytic stages.
d = Damaged.
D = Died.

TABLE III.

Prophylactic Effect of Sulfonamides, Sulfones, Quinine, Atebrin and Plasmochin on Exoerythrocytic Stages following Sporozoite Inoculations. Drug fed in mash from fifth to fifteenth day.

Chick No.	Drug	% of drug in mash	Biopsies on days following inoculations								
			9	10	11	12	13	14	15	16	17
3418	Sulfadiazine	0.5			0			0			0
3419					0			0			0
3420					0			0			0
3421					0			0			0
3423	4,4′-Diamino diphenyl	0.12			++			+++D			
3424	sulfone				++		++D				
3427					++D						
3431	Sulfadiazine	0.5			0			0			0
3432	and Quinine	0.25			0			0			0
3433					0			0			0
3434					0			0			0
3435					0			0			0
3436	Quinine	0.25			+++		++D				
3437					+++	D					
3438					+++			++++			+++
3439					+++		++++D				
3440					++			++++			+++
3441	Atebrin	0.12			+++	D					
3442					+++		+++D				
3443					+++			D			
3444					++++D						
3446	Plasmochin	0.12	+D								
3447					+D d						
3449			0D								
3452	Diamino diphenyl	0.12			++	D					
3453	sulfone derivative				++	D					
3454					++			++			+++
3455					++			++		D	
3456	*Sulfadiazine	0.5			0						0
3458					+d			0			0

* Drug given seventh day to sixteenth day.
d = damaged exoerythrocytic forms.

\+ - ++++ = Intensity of exoerythrocytic forms. D = Died.
Controls shown in Table IV.

TABLE IV.

Effect of Sulfonamides, Diamino diphenyl sulfone, and Plasmochin after the Appearance of Exoerythrocytic Forms. Drug fed in mash from ninth day to fifteenth day.

Chick No.		% of drug in mash	Biopsies on days following inoculation									
			8	9	10	11	12	13	14	15	16	17
3460	Sulfaguanidine	0.5	++			++	+++D					
3461			+			++	D					
3463			++			+++	D					
3469			+			++	D					
3465	Sulfathiazole	0.5	++		+++D							
3466			++			+++	D					
3472			+				+++D					
3480			+			++	D					
3467	Sulfadiazine	0.5	++			++d			+d			0
3468			++			++d			+d			0
3481			+			+++d			++d	D		
3485			+			++d	D					
3470	Sulfapyrazine	0.5	++			++			+d			+d
3473			++			++++D						
3486			+			++d			0			0
3487			+			++d			++d			0
3462	Plasmochin	0.25	0	+		+		+D				
3477			0	+		++		++D				
3493			0	++		++		++++D				
3475	4,4′-Diamino-	0.25	0	++		+++D						
3482	diphenyl		0	+		++		+	D			
3495	sulfone		0	+		++	D					
3459	Controls		0	+		0			+++D			
3476			++			++	D					
3478			++			+++		++++D				
3479			++			++++	D					
3484			0	++		+++	D					
3488			0	+		++		+++D				
3489			+			+++			++++	D		
3490			0	0		++			++D			
3491			0	0		0			+	+D		
3492			++			+++	D					
3494			++			+	D					
3496			+				+++D					

d = Damaged.
+ - ++++ = Intensity of exoerythrocytic forms.
D = Died.

brain capillaries from the seventh to ninth day after inoculation. There is a direct relationship between the number of sporozoites injected and the appearance of these forms as a greater number of sporozoites decreases the time of appearance of exoerythrocytic parasites. Two chicks inoculated with 6,500,000 sporozoites each died on the fifth day with minimal blood infections but with an advanced degree of exoerythrocytic involvement.

If chicks are inoculated with infected red cells, exoerythrocytic forms do not appear until much later, approximately the eighteenth day. Deaths before this interval are usually negative for exoerythrocytic forms. These findings confirm earlier reports based on autopsy examinations. However, by the above described technic it was discovered that an occasional animal inoculated either with sporozoites or trophozoites, will show some exoerythrocytic stages and spontaneously recover. (See Table I.)

Effect of Various Drugs on Exoerythrocytic Forms on Induced Infections. Sulfaguanidine, sulfapyridine, sulfathiazole, sulfadiazine, sulfanilamide and quinine were first tried for their effect on trophozoite-induced infections. The inoculating dose contained 10,000 parasites. Treatment was withheld until exoerythrocytic stages appeared in biopsy specimens, then was given *per os*. Details of the experiment are summarized in Table II. It is shown that of 13 chicks receiving sulfonamide drugs 10 recovered and 3 died. All 3 quinine-treated chicks died. Two of 4 controls recovered although exoerythrocytic stages were demonstrable in all. It was of interest to note that effective drugs could be detected before there was any diminution in the numbers of exoerythrocytic stages. Visible damage recognized by vacuolation, loss of definition, and poor staining was very evident in the stained smears. In the normals which recovered there was a gradual disappearance of the exoerythrocytic stages.

The above experiment was repeated several times, with differences in dosages and variety of compounds. Instead of giving drugs in interrupted doses in some instances they were incorporated in mash. It was found that quinine and atebrin and a wide variety of other compounds were totally without effect. A positive effect with plasmochin was barely demonstrable. The above-mentioned sulfonamides were regularly effective and 4,4′-diamino diphenyl sulfone was also partially effective in destroying the exoerythrocytic stages after they had made their appearance in the brain capillaries.

Effectiveness of Sulfonamides, Sulfones, Quinine, Atebrin, and Plasmochin on Exoerythrocytic Stages in Sporozoite-Induced Infections. Because of the differences in appearance of the exostages depending upon whether sporozoites or trophozoites were used to induce an infection, the experiments were repeated using sporozoites as the infecting agent. As mentioned above, in infections following sporozoite inoculations in routine dosages, exoerythrocytic stages appeared on the seventh to ninth day. In Table III details of drug dosages and results of biopsy examinations are given.

The results of this test showed that sulfadiazine alone or with quinine prevented the appearance of exoerythrocytic forms, although the drugs were withheld until the fifth day after inoculation. Quinine and atebrin were without effect. Plasmochin had a minimal retarding effect.

In order to subject the drugs to a more rigid test, a number of chicks from the same group were selected and therapy was withheld until the appearance of the exoerythrocytic stages. The details and results of this test are furnished in Table IV.

From this experiment it can be seen that sulfadiazine and sulfapyridine destroyed the exoerythrocytic forms even after they had commenced to occlude the brain capillaries. Quinine and atebrin were unable to influence their development, although they were effective on the asexual forms in the blood.

Summary and Conclusions. A method is described for obtaining biopsied brain tissue containing exoerythrocytic stages of *Plasmodium gallinaceum* throughout their course of development in individual chicks. With this method it is feasible to examine individual animals repeatedly without appreciable harm, thus making it a valuable technic for any study on exoerythrocytic schizogony. Earlier

studies made from autopsies have been confirmed. In addition it has been shown that in occasional animals exoerythrocytic stages were encountered which spontaneously disappeared, although this finding is infrequent. From the standpoint of chemotherapy, it has been found that sulfadiazine will prevent the appearance of these forms. Likewise this same drug or sulfapyrazine will effect a cure and cause a disappearance of exoerythrocytic schizonts even after they have made their appearance. Quinine and atebrin have no prophylactic or curative action. Plasmochin has a questionable therapeutic effect. The implications of this study are evident if it is eventually shown that exoerythrocytic schizogony is an integral part of human malarial infections. However, regardless of its place in the life cycle of plasmodial infections, the fact that these forms respond so readily to some of the sulfonamide compounds and yet are totally refractory to quinine or atebrin furnish another lead in the study of malarial chemotherapy.

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Some Chemotherapeutic Properties of Sulfaquinoxaline.

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Following the synthesis of sulfaquinoxaline by Weijlard, Erickson, and Tishler[1] and the report by Seeler *et al.*[2] on the pharmacological properties of this compound, it was thought of interest to determine the *in vitro* and *in vivo* efficacy of this drug against certain bacteria.

In Vitro. Using a modification of Kolmer's method[3] and a strain of *E. coli* grown in a synthetic medium,[4] sulfaquinoxaline was found to be 4 times as effective as sulfanilamide and 32 times less effective than sulfadiazine. As reported for other sulfonamides, sulfaquinoxaline was mainly bacteriostatic and not bactericidal. Thus over a 72-hour incubation period, sulfaquinoxaline, sulfanilamide, and sulfadiazine caused complete inhibition of the test organism in dilutions of 1:64,000, 1:16,000, and 1:2,000,000 respectively. As previously observed with the sulfonamides,[5] sulfaquinoxaline caused definite morphological changes in dilutions far greater than those required to inhibit the growth of the organism.

In Vivo. For the *in vivo* tests, mouse-virulent strains of *Diplococcus pneumoniæ* Type I, *Streptococcus hemolyticus* 1685, *Salmonella schottmülleri,* and *Salmonella aertrycke* were used. Details of maintaining the growth and virulence of these cultures have been described in a previous paper.[6]

The mice were infected by intraperitoneal injections of 0.5 cc of 10^{-4} to 10^{-6} dilutions of a six-hour culture of the above organisms. The amounts of culture were adjusted to equal approximately 10,000-100,000 lethal doses, as determined by titration in mice. All experiments were performed using suspensions of sulfaquinoxaline, sulfadiazine, and sulfathiazole prepared immediately before use by grinding the powder in 10% gum acacia. Treatment with the 3 drugs was given by oral administration immediately following the bacterial inoculation. Subsequent therapy with sulfaquinoxaline was given once every 24 hours, together with 0.5 mg/mouse of vitamin K_1. It has been shown by Seeler *et al.*[2] that sulfaquinoxaline produces a hypoprothrombinemia which can be prevented by feeding

[1] Weijlard, J., Erickson, A. E., and Tishler, M., in press.

[2] Seeler, A. O., *et al.*, in press.

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[5] Lockwood, J., *J. Immunol.,* 1938, **35**, 155.

[6] Robinson, H. J., *J. Pharm. and Exp. Ther.,* 1943, **17**, 70.