3. Rector, F. C., Jr., Van Giesen, G., Kiil, F., Seldin, D. W., ibid., 1964, v43, 341.
4. Earley, L. E., Friedler, R. M., ibid., 1964, v43, 1928.
5. ———, ibid., 1965, v44, 929.
6. Earley, L. E., Kahn, M., Orloff, J., ibid., 1961, v40, 857.
7. Goldsmith, C., Beasley, H. K., Whalley, P. J., Rector, F. C., Seldin, D. W., ibid., 1961, v40, 2043.
8. Goodman, B., Cohen, J. A., Levitt, M. F., Kahn, M., Am. J. Physiol., 1964, v206, 1123.
9. Stein, R. M., Levitt, H. B., Goldstein, M. H., Porush, J. J., Eisner, G. M., Levitt, M. F., J. Clin. Invest., 1962, v41, 2101.
10. Goldberg, M., McCurdy, D. K., Ramirez, M. A., ibid., 1965, v44, 182.
11. Hargitay, B., Kuhn, W., Z. Electrochem., 1951, v55, 539.

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19S and 7S Anti-Toxoplasma Antibodies in Diagnosis of Acute Congenital and Acquired Toxoplasmosis.* (30778)

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Because newborn infants with congenital toxoplasmosis may exhibit clinical signs and symptoms often found in other disease states, serological methods and/or isolation procedures have usually been employed in an attempt to establish the diagnosis. If specific therapy is to be used in such cases, in hope that it will prevent further tissue destruction by the parasite, early diagnosis (usually during the first days of life) is imperative. Unfortunately, early serologic diagnosis of toxoplasmosis in the newborn is complicated by the fact that maternal antibody is passively transferred to the fetus *via* the placenta. Because none of the serologic methods presently available differentiate between maternal and fetal antibody, a high anti-*Toxoplasma* antibody titer in the serum of an infant may merely reflect infection in the mother and does not therefore establish the diagnosis of congenital toxoplasmosis. This problem of interpretation of serologic tests is magnified in infants who do not exhibit the classical clinical picture of hydrocephalus, jaundice, hepatosplenomegaly and chorioretinitis often associated with fulminant congenital toxoplasmosis. In such infants, in whom irreparable damage may not yet have occurred, therapy may prevent crippling sequelae such as mental retardation and blindness.

An additional method used in diagnosis is the demonstration of *Toxoplasma* by isolation techniques. However, if the infecting strain is relatively avirulent for laboratory animals, the isolation procedure may not reveal the diagnosis in time to allow early initiation of specific therapy.

A no less complicated situation confronts physicians attempting to establish a diagnosis of acute acquired toxoplasmosis in individuals with signs and symptoms of this disease who, on initial examination, exhibit high serologic test titers to *Toxoplasma*. Since clinical manifestations in cases of acquired toxoplasmosis may mimic those of a variety of disease states and since dye test, hemagglutinating and complement fixing anti-*Toxoplasma* antibody titers may remain elevated for years following the acute infection(1,2), a high serologic test titer in such individuals does not necessarily indicate that the antibodies are related to the present illness. In addition, because the cyst form of *Toxoplasma* persists in extraneural tissues of chronically infected humans for years and perhaps for life following acute infection(3,4), successful isolation of the parasite from a patient with a high serologic test titer is difficult to evaluate and

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does not establish the diagnosis of acute acquired toxoplasmosis.

Because of these problems in interpretation of serologic techniques now in use, it was considered necessary to develop additional methods which might prove helpful in cases such as those referred to above. Since 19S antibodies are formed early in a variety of acute infectious diseases in infants(5,6) as well as in adults(7-9), the present study was performed to determine if 19S antibodies are produced during the acute stage of toxoplasmosis and, if so, whether demonstration of such macromolecular antibodies can be used in diagnosis of acute congenital and acquired toxoplasmosis.

Materials and methods. Sera of suspect cases of congenital toxoplasmosis were obtained from newborns on the pediatric service of the Palo Alto-Stanford Hospital and from Dr. Georges Desmonts, Hospital Saint Vincent de Paul, Paris. Sera of suspect or established cases of acute acquired toxoplasmosis were obtained from patients seen by physicians in the Palo Alto area, from Mr. Milford Lunde, Laboratory of Parasitic Diseases, Nat. Inst. of Health, Bethesda, Md., from Dr. Ben Kean, Tropical Disease Unit, Cornell University School of Medicine, New York City, and from a bank of specimens collected in previous studies(13,14).

The Sabin-Feldman dye test was performed as described by Frenkel and Jacobs(10). The *Toxoplasma* hemagglutination test was performed according to the method of Jacobs and Lunde(11).

Exclusion chromatography was performed on columns of Sephadex G-200, as previously described(12).

Preparative ultracentrifugation was performed using a Spinco Model L ultracentrifuge with a swinging bucket rotor (SW-39). 0.25 ml of serum was mixed with 0.25 ml of 13% sucrose and the mixture layered on linear gradients of sucrose, 13% to 37%. The samples were centrifuged at 35,000 rpm for 18 hours. Following centrifugation, 5 drop fractions were collected from the bottom of the tubes with a #25 needle. The fractions were dialysed against distilled water, lyophylized, and the resulting powders resuspended in 0.25 ml of 0.15 M NaCl. The linearity of the gradients was checked by refractometry using an Atago protometer (National Instrument Co., Baltimore, Md.).

Results. When 1 ml to 5 ml of serum was placed on columns of Sephadex G-200, elution patterns revealed 3 separate peaks(12). Immunoelectrophoretic analysis of the materials obtained from the first peak revealed α_2- and β_2-macroglobulins and α- and β-lipoproteins, and from the second peak γ_2-globulins, albumin and nonidentified α- and β-globulins. Analytical ultracentrifugal analysis of the material obtained from the first peak revealed 2 components with sedimentation coefficients of approximately 9S and 19S, and from the second peak 2 components of about 4S and 7S. When only 7S antibodies were demonstrable in fractions obtained by gel filtration in the present study, the antibodies in these same sera were not demonstrable below fraction 6 of the sucrose gradient preparations. Therefore, sucrose gradient fractions 1 through and including fraction 6 were considered to contain 19S antibodies. This was supported by comparative studies using gel filtration experiments and by results noted in cases of acquired toxoplasmosis in whom there was a transition from 19S to 7S antibodies. Analysis of sucrose gradient fractions by double diffusion in agar with an antiserum prepared against IgG and IgM revealed IgG in fractions 8 through 17, 18, or 19, and IgM in fractions 4 through 6.

Individuals from whom samples of serum were obtained were placed into 1 of 3 categories according to their suspected or established diagnosis: (1) acute congenital toxoplasmosis, (2) acute acquired toxoplasmosis, and (3) chronic toxoplasmosis (long-standing positive *Toxoplasma* serologic test titers). In Table I are depicted the data obtained in 9 representative cases in these 3 categories. The first 3 cases were members of a single family. G.D. and D.D., a girl and boy, were products of a twin pregnancy. A single specimen of serum obtained from G.D. 36 hours after birth had a dye test titer of 1:4096. Separation of this serum by sucrose gradient ultracentrifugation revealed dye test antibodies in both 7S and 19S fractions of the

TABLE I. Data in 9 Cases of Toxoplasmosis.

Patient(*)	Age	Sex	Onset of illness	Date of serum	Dye test titer	Dye test antibodies† 19S	Dye test antibodies† 7S	Isolation results	
G.D.	36 hr	♀	Congenital	10/22/64	1:4096	+	+	Cysts seen in many organs at autopsy	
D.D.	2 wk	♂		11/ 4/64	1:4096	—	+		
	6 "			11/20/64	1:1024	—	+		
	5 mo			3/ 3/65	1:16384	—	+		
M.D.	26 yr	♀		11/20/64	1:4096	—	+		
				3/ 3/65	1:16384	—	+		
M.L.(17)	31 "	♂	10/ 9/55	10/25/55	1:1024	+	+	+ (lymph node)	10/19/55
				12/ 1/55	1:1024	—	+	+ (blood)	10/15/55
				1/16/56	1:16384	+	+	— (lymph node)	1/16/56
				10/30/56	1:1024	—	+		
L.B.(17)	48 "	♀	2/10/56	3/26/56	1:262,000	+	+	+ (lymph node)	2/25/56
								+ (muscle)	3/12/56
				5/22/56	1:64000	+	+		
				7/ 3/56	1:16384	+	+		
				7/27/56	1:16384	+	+		
S.C.(18)	23 "	♀	1/15/58	2/22/58	1:1024	+	+	+ (blood)	1/24/58
									1/25/58
				4/25/58	1:16384	—	+		
				2/10/59	1:4096	—	+		
S.S.	23 "	♀	1/—/61‡	5/11/61	1:16384	+	+	+ (lymph node)	4/20/61
				5/18/61	1:64000	+	+		
				9/ 7/61	1:2048	—	+		
E.C.	29 "	♀		10/ 9/62	1:1024	—	+		
				2/26/65	1:4096	—	+		
W.H.(15)	19 "	♂		9/—/61	1:32000	+	+		
				3/—/62	1:2048	—	+		
				3/ 5/63	1:2048	—	+		

* Reference No.
† Sera separated by gel filtration and sucrose density gradient ultracentrifugation.
‡ Actual date of onset not known. May have been as late as March, 1961.

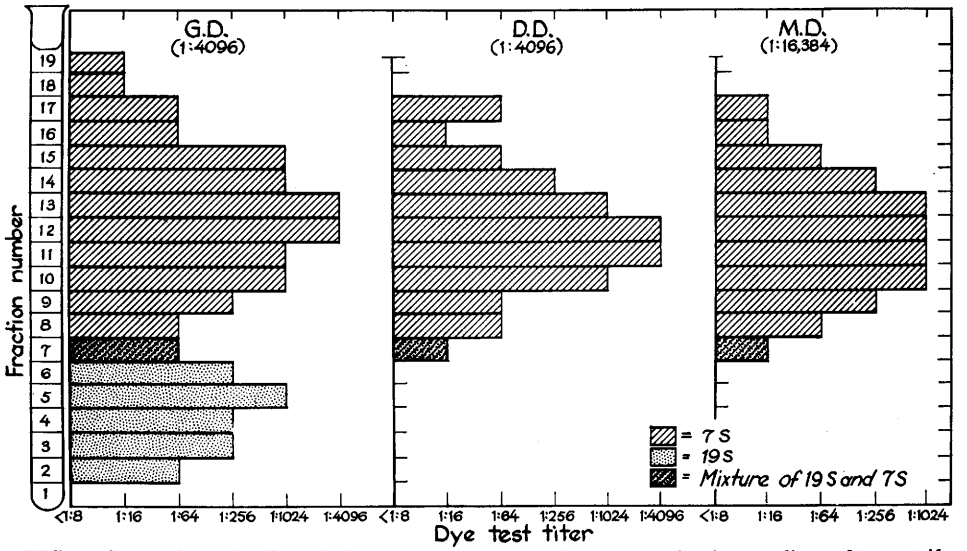

FIG. 1. Separation of 19S and 7S dye test antibodies by sucrose density gradient ultracentrifugation in a case of acute congenital toxoplasmosis (G.D.) and in the patient's twin brother (D.D.) and mother (M.D.) (See text and Table I.)

immunoglobulins (Table I and Fig. 1). Antibodies, first demonstrable in fraction 2 of the gradient, were present in 2 peaks, the highest titers appearing in fractions 5 and in 12 and 13. The antibodies in the serum of the brother (D.D.) and mother (M.D.) of this patient first appeared in fraction 7, and the peak titers were in approximately the 11th and 12th fractions (Table I and Fig. 1). Infant G.D. died 46 hours after birth with a clinical diagnosis of erythroblastosis. At autopsy, typical cysts of *Toxoplasma* were noted in her brain, eyes, suprarenals, kidneys and heart. The patient's brother (D.D.) had received an exchange transfusion for hyperbilirubinemia on the 4th day of life and was clinically well with a dye test titer of 1:16384 at 5 months of age.

Only 7S anti-*Toxoplasma* antibodies were demonstrable in samples of cord blood or blood obtained by venipuncture shortly after birth from 15 newborns whose mothers had chronic toxoplasmosis. Similar sera obtained from babies born of mothers whose sera were negative in the dye test did not show anti-*Toxoplasma* antibody activity before or after they were separated by gradient ultracentrifugation or gel filtration.

Of special interest is a mother whose infant died at birth with a clinical diagnosis of *hydrops foetalis*. *Toxoplasma* infection in the infant was proven by isolation of the parasite and by recognition of the cyst form in microscopic sections of tissues obtained at autopsy. The mother's titer at the time of parturition was 1:16384, and rose to a titer of 1:256,000 4 months postpartum. Both 19S and 7S anti-*Toxoplasma* antibodies were present in all samples of blood obtained during this period. Despite the presence of both classes of antibody and of the remarkable high titer, *Toxoplasma* was isolated from her blood at 1, 3 and 4 months postpartum and from lochia, endometrium and menstrual blood at 1 month postpartum.

Sera were obtained from 11 cases of suspect acute acquired toxoplasmosis and from 10 cases in whom that diagnosis had been established by serologic methods, isolation of the parasite, or history of inoculation with the parasite in a laboratory accident. 19S anti-*Toxoplasma* antibodies were demonstrable in all cases in which a previous diagnosis of acute acquired toxoplasmosis had been established. In Table I are shown data obtained in 4 representative cases of proven acute acquired toxoplasmosis [M.L.(15), L.B.(15), S.C.(16) and S.S.] and one [W.H.(14)] who was in perfect health at the time his first blood specimen was obtained during a study of the prevalence of antibodies to *Toxoplasma* among college students. Patient M.L. accidentally inoculated himself with a virulent strain of *Toxoplasma* on October 9, 1955. 19S and 7S anti-*Toxoplasma* dye test and hemagglutinating antibodies were present in his serum obtained 13 days later, and, as shown in Fig. 2, by December 1, 1955, only 7S antibodies were demonstrable. Of interest is the fact that on January 16, 1956, a serum obtained because of an acute exacerbation of the patient's symptoms revealed a rise in titer from 1:1024 to 1:16384, and a reappearance of 19S antibodies in high titer (Fig. 2). *Toxoplasma* was isolated from his blood on October 13, 1955, and from a lymph node on October 19, 1955.

In 2 instances 19S anti-*Toxoplasma* antibodies persisted as long as 5 months following the onset of illness (*e.g.*, Case L.B., Table I). Although additional sera were not available from L.B., they were from 7 other patients in whom 19S antibody persisted for 2-4 months. 19S dye test and hemagglutinating antibodies were not demonstrable in any of their sera obtained after the fifth month post inoculation or of illness (*e.g.*, Cases M.L. and S.C., Table I).

Sera obtained from 13 individuals with known chronic toxoplasmosis and persistent antibody titers over a period of years contained only 7S anti-*Toxoplasma* antibodies (*e.g.*, Case E.C., Table I). In a similar study of 4 cases of classical Waldenstrom's macroglobulinemia, whose sera were positive in the dye and hemagglutination tests, anti-*Toxoplasma* antibodies were found only in the 7S fraction of their immunoglobulins.

Discussion. The results described above establish the fact that 19S and 7S dye test and hemagglutinating anti-*Toxoplasma* antibodies are produced in the human in response

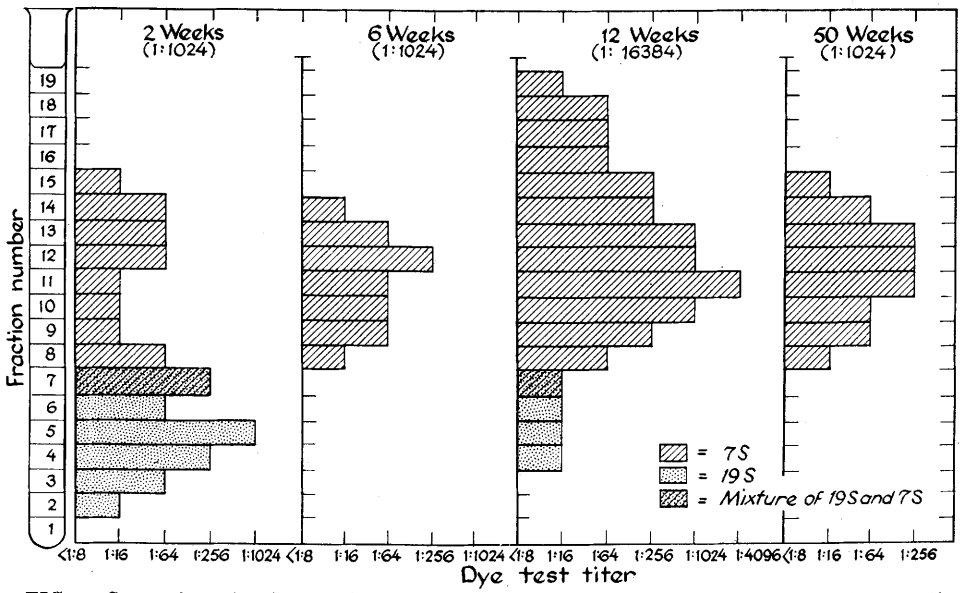

FIG. 2. Separation of 19S and 7S dye test antibodies by sucrose density gradient ultracentrifugation in a case of acute toxoplasmosis acquired in the laboratory (M.L.). Note reappearance of 19S antibodies and rise in titer at 12 weeks. (See text and Table I.)

to acute acquired toxoplasmosis and confirm the finding of Eichenwald and Shinefield that 19S anti-*Toxoplasma* antibodies may be produced by the fetus *in utero* in response to acute congenital toxoplasmosis(17). In the present studies only 7S anti-*Toxoplasma* antibodies were demonstrable in sera of newborn infants born of mothers with chronic toxoplasmosis whose antibodies were of the 7S class.

In the case of acute congenital toxoplasmosis in patient G.D. (Table I and Fig. 1) the diagnosis could not have been established in the early postnatal period without the demonstration of 19S anti-*Toxoplasma* antibodies. That the 19S antibodies were formed by the infant *in utero* is supported by the facts that these macromolecular antibodies were present in a sample of blood obtained 36 hours after birth and were lacking in the blood of the mother. The 7S antibodies in the serum of the infant can be accounted for by transplacental passage of maternal IgG. The finding of a titer of 1:16384 in the twin D.D. (Table I and Fig. 1) 5 months after birth cannot be accounted for by maternal transfer of antibody. As discussed by Sabin *et al*(1), such a titer this long after birth establishes the diagnosis of congenital toxoplasmosis. Unfortunately the child was lost to followup for several months so that serial samples of serum could not be obtained. The presence of only 7S anti-*Toxoplasma* antibodies in his serum obtained at 2 weeks of age might be attributable to the exchange transfusion. At 6 weeks of age his titer had dropped from the initial level of 1:4096 to 1:1024. Another serum was not available until age 5 months at which time the antibodies were all of the 7S class.

19S anti-*Toxoplasma* antibodies were demonstrable in the sera of all individuals in whom the diagnosis of acute acquired toxoplasmosis had previously been established. Whether 19S antibodies appeared before 7S antibodies in these individuals was not answered in the present study. Unfortunately sera were not available earlier than the second week of infection from the 2 cases who acquired their infection in the laboratory. However, at that time both had 19S and 7S anti-*Toxoplasma* antibodies. 7S antibodies were present in all samples of serum in which 19S antibodies were demonstrated. The 19S antibodies appeared to persist for at least 1 or 2 months following infection or onset of illness, and in 2 instances were demonstrated for as long as 5 months.

19S antibodies were demonstrated in a number of cases of suspect acquired toxoplasmosis in whom dye test and/or hemagglutination test titers were already at high levels when the patients were first seen by their physicians. The demonstration of such macromolecular anti-*Toxoplasma* antibodies in these individuals helped to establish the diagnosis and appear to be of diagnostic significance when they are demonstrated in the sera of individuals presenting clinical signs and symptoms compatible with the diagnosis of toxoplasmosis(2).

It is noteworthy that 19S antibodies were present in sera of individuals some months following their acute infection and/or illness and at a time when they appeared to be clinically well (*e.g.*, W.H., Table I). Thus the demonstration of macroglobulin anti-*Toxoplasma* antibodies indicates that the individual was infected probably within the past 1 or 2 months but may have been initially infected as long as 5 months before. Two cases have been observed in which 19S antibodies reappeared following a period of time when only 7S antibodies were demonstrable. One of these occurred 12 weeks following acute infection (M.L.) and the other in a patient during pregnancy almost 2 years following the delivery of a toxoplasmic infant. The latter patient had a stable dye test titer of 1:64 (7S) just prior to and in the first trimester. At a time when she was clinically well, at 9 months of gestation, her titer rose to 1:1024, and 19S as well as 7S antibodies were present. She gave birth to a full-term infant who appeared normal and whose cord blood showed only 7S anti-*Toxoplasma* antibodies. The cause of these reappearances of 19S antibodies is not clear. It may be that the individuals were reinfected shortly before the rise in dye test titer was noted or that they experienced a reactivation of parasites already within their tissues.

Treatment of fractions containing 19S anti-*Toxoplasma* antibodies (obtained by exclusion chromatography or gradient ultracentrifugation) with mercaptoethanol resulted in marked reduction in titer or complete destruction of activity of antibody in these fractions. However, since all sera tested in the present study contained 7S antibodies in high titer, the use of reductive cleavage to demonstrate 19S antibody activity did not prove useful. The laborious procedures necessary for the separation of the 19S and 7S antibodies do not allow for the ready availability of this test. However, studies are now in progress to develop such a technique. A fluorescent antibody method appears to be the most promising. If an easy approach to the recognition of 19S and 7S anti-*Toxoplasma* antibodies can be found, it is hoped that the observations reported here will be not only of academic interest but will offer a significant additional method for precise diagnosis of acute congenital and acquired toxoplasmosis.

Summary. An attempt was made to determine if macroglobulin (19S) anti-*Toxoplasma* antibodies are produced during the acute stage of infection and, if so, if their demonstration is of diagnostic significance. Sera were obtained from established cases of acute congenital and acquired toxoplasmosis and separated by gradient ultracentrifugation or gel filtration. Serologic examination of the fractions obtained by these methods revealed that 19S anti-*Toxoplasma* antibodies are formed in both acute congenital and acquired infection with *Toxoplasma*. Only 7S antibodies were demonstrated in infants born of mothers with chronic toxoplasmosis and in adults with longstanding infection with this organism. The demonstration of such macroglobulin antibodies in suspect cases of acute toxoplasmosis appears to be a useful adjunct to the presently available serologic tests.

ADDENDUM. Since submitting this manuscript, sera have been obtained from 5 additional cases of acute congenital toxoplasmosis; 19S as well as 7S antibodies were demonstrated in each case. Two of the mothers had only 7S antibodies and in three, they were of both the 19S and 7S class. 19S antibodies were demonstrated in acute phase sera in each of ten additional cases of acquired infection.

1. Sabin, A. B., Eichenwald, H., Feldman, H. A., Jacobs, L., J. Am. Med. Assn., 1952, v150, 1063.

2. Remington, J. S., Jacobs, L., Kaufman, H. E., New Engl. J. Med., 1960, v262, 180.

3. Remington, J. S., Melton, M. L., Jacobs, L., J. Lab. Clin. Med., 1960, v56, 879.

4. Remington, J. S., Cavanaugh, E., New Engl. J. Med., 1965, v273, 1308.
5. Smith, R. T., Eitzman, D. V., Pediatrics, 1964, v33, 163.
6. Uhr, J. W., Dancis, J. W., Franklin, E. C., Finkelstein, M. S., Lewis, E. W., J. Clin. Invest., 1962, v41, 1509.
7. Schluederberg, A., Nature, 1965, v205, 1232.
8. Murray, E. S., O'Connor, J. M., Gaon, J. A., J. Immunol., 1965, v94, 734.
9. Reddin, J. L., Anderson, R. K., Jenness, R., Spink, W. W., New Engl. J. Med., 1965, v272, 1263.
10. Frenkel, J. K., Jacobs, L., Arch. Ophthal., 1958, v59, 260.
11. Jacobs, L., Lunde, M. N., J. Parasitol., 1957, v43, 308.
12. Remington, J. S., Merler, E., Proc. Soc. Exp. Biol. and Med., 1964, v115, 115.
13. Remington, J. S., Barnett, C., Meikel, M., Lunde, M., Arch. Int. Med., 1962, v110, 744.
14. Remington, J. S., Dalrymple, W., Jacobs, L., Finland, M., New Engl. J. Med., 1963, v269, 1394.
15. Kayhoe, D., Jacobs, L., Beye, H., McCullough, N. B., ibid., 1957, v257, 1247.
16. Frenkel, J. K., Weber, R. W., Lunde, M. N., J. Am. Med. Assn., 1960, v173, 1471.
17. Eichenwald, H. F., Shinefield, H. R., Pediatrics, 1963, v63, 870 (abst.).

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Sedimentation of *Eperythrozoon coccoides.** (30779)

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Eperythrozoon coccoides is a minute organism of somewhat uncertain taxonomy, presently classified among the Bartonellaceae(1). It is indigenous to mice, inducing in them a mild disease characterized by splenomegaly and general lymphadenopathy(2). The organism is frequently present in transplantable tumors(3) and may, in part, be responsible for the splenomegaly which follows their transplantation. In association with certain other organisms of normally low pathogenicity, disorders of high morbidity and mortality may result(4).

Knowledge of the biology of this ubiquitous but relatively little known organism has advanced slowly since its discovery in 1928(5). Recently, the splenomegaly induced by *E. coccoides* was quantitatively related to certain extracellular annular structures in the blood of infected mice, and it was inferred that of the various structures observed in blood, these particular ones were the infectious particles(6). As an approach to their concentration, purification and characterization, the behavior of *E. coccoides* in moderate and high gravitational fields was investigated, using biological activity to determine the results.

Materials and methods. The preparation of infectious material and the determination of biological activity have been described(6). The latter depends upon the increase in spleen weight of mice 7 days after intraperitoneal inoculation of 0.1 ml of material. The average response of 5 mice determines a single experimental point. Justification for using spleen weight as a measure of activity of *E. coccoides* has already been discussed(6).

Differential and density gradient centrifugation were performed in the Spinco Model L ultracentrifuge at about 4°C. Gradient columns were prepared by consecutively layering 0.6 ml of aqueous sucrose solutions of increasing concentration. The tubes were allowed to stand 18 hours at 4°C before use.

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