would be freely available to aid in the absorption of fat-soluble vitamins. This conclusion is supported by the data obtained in the fourth experiment of this study. These are added reasons for concluding that it is highly unlikely that cholestyramine will induce a Vit. K deficiency in man. From the experimental point of view, however, it is evident that large doses of cholestyramine may serve as a useful tool in the study of bile acid function and their effect on the absorption of fat-soluble vitamins.

Summary. Studies were undertaken to determine whether or not cholestyramine, a bile acid binding resin, would interfere with the intestinal absorption of fat-soluble vitamins. The studies were designed to give the resin an optimal opportunity to interfere with Vit. K_1 absorption. Administration of cholestyramine in a dose range recommended for human use had no effect on Vit. K_1 absorption. Large doses of the resin, administered before and shortly after feeding the vitamin, did delay and decrease the absorption of this vitamin. However, very large doses of cholestyramine given 17 hours before feeding Vit. K_1 had no affect on the availability of the vitamin. These studies suggest that cholestyramine would not be expected to interfere with the absorption of Vit. K when the resin is used under practical clinical conditions in man. However, the resin may be a useful experimental tool to investigate the role of bile acids in fat absorption.

1. Tennent, D. M., Siegel, H., Zanetti, M. E., Kuron, G. W., Ott, W. H., Wolf, F. J., *J. Lipid Research,* 1959-1960, v1, 469.

2. ———, *Circulation,* 1959, v20, 969.

3. Tennent, D. M., Kuron, G. W., Zannetti, M. E., Ott, W. H., PROC. SOC. EXP. BIOL. AND MED., 1961, v108, 214.

4. Bergen, S. S., Jr., Van Itallie, T. B., Tennent, D. M., Sebrell, W. H., PROC. SOC. EXP. BIOL. AND MED., 1959, v102, 676.

5. Van Itallie, T. B., Hashim, S. A., Crampton, R. S., Tennent, D. M., *New England J. Med.,* 1961, v265, 469.

6. Tennent, D. M., Hashim, S. A., Van Itallie, T. B., *Fed. Proc.,* 1962, v21, suppl. 11, 77.

7. Mushett, C. W., Kelley, K. L., Hirschmann, R. F., *Blood,* 1959, v14, 37.

8. Quick, A. J., *J. Am. Med. Assn.,* 1938, v110, 1658.

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Absence of Cross Reactivity Between Heterophile and Dye Test Antibodies.* (28846)

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One of the recognized clinical syndromes resulting from acute infection with *Toxoplasma gondii* closely resembles infectious mononucleosis(1,2,3). Although the laboratory findings in patients with these infectious diseases are similar, the presence of a positive heterophile antibody test titer, which is considered diagnostic of infectious mononucleosis, is seldom found in cases of acute toxoplasmosis(1). Of interest in this regard is a report of a case in which Toxoplasma organisms were isolated at a time when the serum was positive in both the dye and heterophile tests(4).

During recent studies performed in an attempt to determine the incidence of acute Toxoplasma infection among students with symptoms and signs suggestive of infectious mononucleosis, a number of sera were noted to be positive both in the dye test and het-

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erophile test(3). In some of these sera the dye test titer was significantly higher than is usually found in the normal student population. Because of these findings, it was considered important to determine if the antibodies measured by the dye and heterophile tests are cross-reacting.

Materials and methods. Samples of blood were obtained from students who were seen at the Stanford University Health Service with what was thought to be infectious mononucleosis. The samples were allowed to clot at room temperature and the serum separated by centrifugation at 2000 rpm for 20 minutes. The sera were stored at −20°C, and prior to serological testing, were inactivated at 56°C for 30 minutes.

The Toxoplasma dye test was performed as described by Frenkel and Jacobs(5). The lowest dilution of each serum tested in the dye test was 1:16 and a serum with a titer below this level was considered a negative.

Exclusion chromatography was performed on a column of Sephadex G 200 (150 × 2.5 cm) equilibrated with 0.15 M NaCl. Sera which were positive in both the dye and heterophile tests were dialyzed against 0.15 M NaCl and run either separately or as a pool. Columns were connected to a continuous flow ultraviolet absorption meter and the optical density of the eluate at 254 mμ recorded directly. Elution with 0.15 M NaCl was continued until there was no further ultraviolet absorption. Contents of tubes comprising portions of the elution patterns within each peak were combined, dialyzed against distilled water and lyophilized.

Immunoelectrophoresis was performed according to the micro-method described by Scheidegger(6). The antisera employed included the following:† Horse anti-human serum #G76138-53062; horse anti-human β_2-macroglobulin #H137-2-63; goat anti-human β_2-macroglobulin #G37-98/3-3163; horse anti-human Fraction II #H205PABS. The anti-β_2-macroglobulin sera were adsorbed with human fetal cord serum.

Adsorption studies were performed using washed sheep red blood cells. One-half ml of serum was incubated with 0.1 ml of the packed cells at 37°C and shaken gently for 4 hours. Cells were separated by centrifugation and the sera tested for the presence of antibody in the dye and heterophile tests. Additional adsorption procedures were performed with viable Toxoplasma organisms obtained from the peritoneal fluid of mice which had been inoculated 3 days previously with the RH strain of the parasite. Toxoplasma organisms were separated from the formed elements of the peritoneal fluid by use of glass filters and subsequently washed with 0.15 M NaCl to remove soluble antigen. Approximately 1×10^6 organisms were added to 0.5 ml of the serum samples and the mixture allowed to incubate at 37°C for 4 hours. The organisms were removed by centrifugation and the supernatant serum was examined for antibody titers.

Analytical ultracentrifugal analyses were made in a Spinco model E ultracentrifuge.

Results. A Toxoplasma dye test was performed on 197 sera which were positive in the heterophile test and on 128 sera which were negative in that test. The results of these tests are shown in Table I. The levels of dye test antibody titers were comparable in both the heterophile positive and heterophile negative sera and there was no significant difference in prevalence of dye test antibodies in the two groups. Dye test antibodies were present in 12% of the heterophile positive sera and in 14% of the heterophile negative sera.

Adsorption of 5 sera positive in both the dye and heterophile tests with washed sheep red blood cells resulted in complete loss of the heterophile antibodies but did not affect the dye test titers; adsorption of aliquots of the same sera with Toxoplasma organisms resulted in removal of dye test antibodies without a change in heterophile titers.

A representative elution pattern obtained by separation of a pool of 7 sera on a column of Sephadex G 200 resin is shown in Fig. 1. The dye test titer in this pool was 1:512 and the heterophile titer 1:224. Six ml of the serum pool were placed on the column and the elution pattern revealed 3 sepa-

† Hyland Laboratories, Los Angeles, Calif.

TABLE I. Toxoplasma Antibodies in Heterophile Positive and Heterophile Negative Sera.

Heterophile test titer*	Dye test titer*										
	<16	16	32	64	128	256	512	1024	2048	4096	8192
<112	110		2	8	5		2				1
112	45		1	2			4				
224	27				1		1				
448	24				1	1	1		1		
896	23		1	1		1			1		1
1792	18										
3584	23	1			1						
>3584	14		1		1		1				

* Reciprocal.

rate peaks. Three similar peaks were noted in all of the experiments. Dye test and heterophile antibodies were found to be completely separable by this technique; heterophile antibodies appeared in the material obtained from the first peak (Fraction I) and dye test antibodies from the material obtained from the second peak (Fraction II).

Immunoelectrophoretic patterns of the fractions obtained from these two peaks are shown in Fig. 2. When reacted with an anti-human serum, the fractions from the first peak were shown to contain both α_2 and β_2-macroglobulins and α and β-lipoproteins (Fig. 2B). The material from the first peak reacted with an antiserum prepared against β_2-macroglobulins (Fig. 2E) but no precipitin lines were observed when the fractions were reacted against an antiserum to 7S γ-globulins. The fractions from the second peak contained mainly γ-globulins and albumin, although precipitin bands were noted also in the alpha and beta areas which were not identified (Fig. 2C). The material from the second peak reacted with an anti-7S γ-globulin serum but no precipitin lines were evident when the same fractions were reacted with the anti-β_2-macroglobulin serum. The material from the third peak did not have antibody activity and was found to contain only α-globulins, β-globulins and albumin (Fig. 2D).

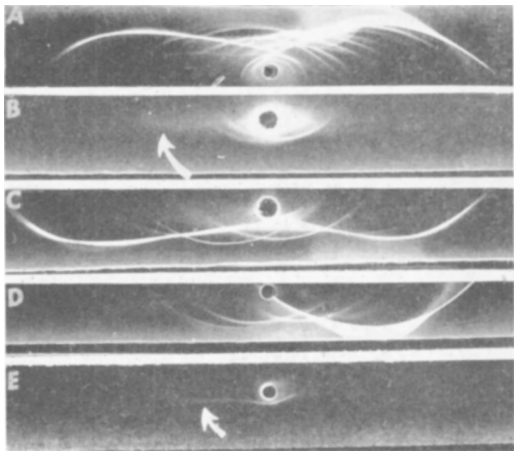

FIG. 2. Immunoelectrophoretic patterns of fractions obtained from Sephadex G 200 columns: A, Normal serum; B, E, fraction I; C, fraction II; D, Fraction III. Antisera: ABCD = horse anti-human serum; E = goat anti-human β_2-macroglobulin.

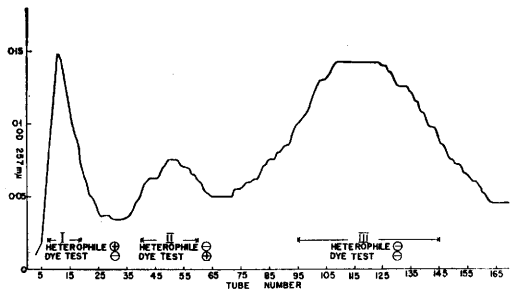

FIG. 1. Elution pattern from Sephadex G 200 of a pool of sera containing heterophile and dye test antibodies. Eluting solvent: 0.15 M NaCl.

Analytical ultracentrifugal analysis of the material obtained from the first peak revealed a component with a sedimentation coefficient of approximately 19S and one of about 9S. The fractions obtained from the second peak contained two components, a major one with a sedimentation coefficient of about 7S and a minor one with an S of about 4.

Discussion. The results of this study indicate that the antibodies measured in the Toxoplasma dye test and the heterophile agglutination test do not cross react and that

they are completely separable by specific adsorption techniques and by gel filtration. These findings should prove helpful when antibody titers to both of these serological tests are positive in patients with the clinical syndrome of infectious mononucleosis who are being studied for the presence of acute toxoplasmosis(3,4,8).

In the present study, the prevalence of dye test antibody titers did not vary significantly among students whose sera were positive in the heterophile determination when compared with students whose sera were negative in that test. In contrast, in a study at the University of California at Berkeley, there was a less frequent occurrence of positive Toxoplasma dye tests among patients in whom the diagnosis of infectious mononucleosis was confirmed by the findings of a positive heterophile antibody test (6%) as compared with those in whom the latter test was negative (20%)(3). The apparent discrepancy of results in these two studies may be accounted for by the smaller number of students in the California study.

The results obtained in the analytical ultracentrifuge with the fractionated serum proteins agree with those reported by Flodin and Killander(7), who found that "19S" antibodies were eluted in the first peak and the "7S" antibodies in the second one. The component from the first peak with a sedimentation coefficient of about 9S may be comparable to a similar peak described by these same authors who concluded that these materials are lipoproteins. Immunoelectrophoretic analysis of Fraction I did not reveal a precipitin line corresponding to that found with normal serum 7S γ-globulins.

Summary. The prevalence and level of dye test antibodies was found to be comparable in sera from students with and without a positive heterophile test. Dye test antibodies were completely separable from heterophile antibodies present in the same sera by means of specific adsorption techniques and by exclusion chromatography on Sephadex G 200.

1. Siim, J., *Ann. N. Y. Acad. Sci.,* 1956, v64, 185.
2. Beverley, J. K. A., Beattie, C. P., *Lancet,* 1958, v2, 379.
3. Remington, J. S., Barnett, C. G., Meikel, M., Lunde, M. N., *Arch. Int. Med.,* 1962, v110, 744.
4. Garin, J. P., in *Human Toxoplasmosis,* Proc. Conf. on Clinical Aspects and Diagnostic Problems of Toxoplasmosis in Pediatrics: VIII Internat. Cong. Pediatrics, Copenhagen, 1956. Munksgaard Co., Copenhagen, 1960, p86.
5. Frenkel, J. K., Jacobs, L., *Am. Med. Assn. Arch. Ophthal.,* 1958, v59, 260.
6. Scheidegger, J. J., *Internal Arch. Allergy,* 1955, v7, 103.
7. Flodin, P., Killander, J., *Biochim. Biophys. Acta,* 1962, v63, 403.
8. Lelong, M., Bernard, J., Desmonts, G., Couvreur, J., *Arch. Francaises de Pediat.,* 1960, v17, 1.

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Defective 5-Hydroxylation of Tryptophan in Phenylketonuria.* (28847)

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Knowledge of the exact mechanism by which mental defect is produced in phenylketonuria is not available. A detailed understanding might not only lead to improvements in treatment of this disease, and of other forms of mental deficiency involving disorders in amino acid metabolism, but might also shed light on some fundamental biochemical factors which play a role in mental function.

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