TABLE I. Effect of Hemorrhage on Inhibition of ACTH Secretion Produced by α-Ethyltryptamine. Values are means ± standard errors.

		17-hydroxycorticoid output (μg/min) in response to:		
	BP rise* (mm Hg)	Stress	Restress	100 mU ACTH
6 dogs subjected to hemorrhage	0 ± 3.7	8.4 ± .5	7.4 ± 1.0	9.1 ± 1.3
6 control dogs	22 ± 3.8	9.0 ± 2.3	2.7 ± 1.5	9.5 ± 2.0

* Systolic blood pressure at time of restress specimen minus systolic blood pressure at time of stress specimen.

is the rise in blood pressure produced by α-ethyltryptamine that causes the inhibition of ACTH secretion.

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Complement-Fixing and Hemagglutinating Antibodies to *Treponema microdentium*.* (31790)

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Socransky, Loesche, Hubersak and MacDonald(1) reported that the oral spirochete *Treponema microdentium* could be grown in a considerably less complex medium than was hitherto possible. In this study we have utilized their medium and methods to grow these organisms. We then determined the antibody responses in actively immunized rabbits as well as the antibody levels found in a group of human sera using *T. microdentium* as the antigen.

Methods and materials. The *T. microdentium* used in the study was kindly supplied by Dr. S. S. Socransky, Harvard School of Dental Medicine.

Preparation of antigens. The spirochetes were carried in low concentrations of agar (0.1 to 0.7%) and transferred to broth for growth in quantity using the technics of Socransky *et al*(1). After anaerobic incubation for 6-7 days at 35°C, the broth cultures were checked for contamination by the use of dark-field microscopy and gram-stain. The spirochetes in broth culture were centrifuged at 3000 rpm for 1 hour at 4°, washed 3 times in cold 0.9% NaCl and resuspended in saline to 1/10 or 1/20 of the original volume (10× and 20× concentrates respectively). Thimerosal was added as a preservative in a final concentration of 1:10,000 and the vaccines were stored at 4°. The optical density of the 10× vaccine was >2.0.

Immunization of rabbits. Four New Zealand rabbits weighing 3-4 kg were used, 2 receiving vaccine in aqueous medium and 2 receiving vaccine in Freund's complete adjuvant. Rabbits 30 and 31 received twice weekly intravenous injections of the 10× vaccine (aqueous) over a 3-week period for a total dose of 9 ml. Their secondary booster series consisted of 3 injections of 1 ml of the 20× vaccine starting one month after the last injection of the primary series. The

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tertiary booster consisted of a single injection of 2 ml of the 20× vaccine given 3 months after the last injection of the secondary series. Rabbits 34 and 35 received primary immunization with equal volumes of 20× vaccine emulsified in complete adjuvant (Difco). These rabbits received 1.0 ml subcutaneously and 0.1 ml intradermally in 2 sites; the injections were repeated one week later. Both rabbits received a booster injection of 2 ml of 20× aqueous vaccine intravenously three and one-half months after the primary series.

Bleedings were done by cardiac puncture one week after the last injection in a given series. In the rabbits receiving adjuvant, blood was obtained 2 weeks and 5 weeks after primary immunization.

Complement-fixation (CF) test. Anticomplementary activity and antigenic titrations followed the methods of Kolmer, Spaulding and Robinson(2), using one-fifth volume of the reagents, and hemolytic activity of the antigen was determined in a similar way. Hemolysin titration, complement titration and Kolmer one-fifth volume quantitative tests with serum were carried out using standard methods(3). The diluent was veronal buffer(4). All CF tests were done at least twice.

Hemagglutination (HA) test. Formalinized human type O red cells were prepared and coupled by the method of Ingraham(5). A 10× suspension of *T. microdentium* without thimerosal was subjected to ultrasonic treatment for twenty minutes at 1.4 amperes.† The resulting lysate was added to the formalinized cells at the rate of 5 ml of lysate per 100 ml of 10% washed cells. The red cell-antigen mixture was placed in a polyethylene bottle and rotated for 4 days at room temperature. The rotator held the bottle at a 45° angle and revolved 11 times per minute. The red cell-antigen mixture was then stored at 4°.

Before use the sensitized cells were washed 5 times in 40 volumes of cold phosphate saline(5) and to the final packed cells was added 4 volumes of phosphate saline. HA tests were done in acid-cleaned 12 × 75 mm tubes by serially diluting 0.5 ml of the test serum in 0.5 ml of a 1:200 dilution of normal rabbit serum‡ in phosphate-saline. To the 2-fold serial dilutions and appropriate cell controls was added 0.1 ml of the washed sensitized cells. After shaking, the tubes were left at room temperature and the patterns read at three hours and again at 24 hours. High titered sera gave sharp end-points at three hours while low titered sera gave better end-points after 24 hours. All HA tests were also done at least twice.

In our experience serum HA titers of less than 10 are often non-specific(6-8); hence the lowest dilution of serum used was 1:10.

Hemagglutination inhibition tests (HAI). The specificity of hemagglutination for *T. microdentium* was determined by adding 0.1 ml of 1:10 dilution of the 10× *T. microdentium* lysate to each dilution of serum 30 minutes prior to addition of sensitized cells. As a control system, a culture of *Staphylococcus epidermidis* was washed and lyzed following the same procedures used with *T. microdentium* and 0.1 ml of this antigen added to duplicate tubes. The lysates of neither *T. microdentium* nor *S. epidermidis* produced agglutination of sensitized cells by themselves.

Human sera. The human sera studied were samples submitted to the serology laboratory for standard syphilitic tests. Only sera negative to such tests were included in the current study; assays for antibody to *T. microdentium* in sera containing syphilitic reagin will be reported separately.

Results. Treponema microdentium produced high antibody titers in rabbits when measured by either the CF or HA test (Table I) irrespective of the use of adjuvant. In the rabbits immunized with the aqueous vaccine the titers reached a peak following the first booster series and were lower after the second booster injection. The HA/CF ratios vary from less than 0.06 to 32 or over 500-fold. Rabbits 30 and 31 were identically immunized and the CF titers were quite similar. In striking contrast the HA titer in R30 never reached 100 while a titer of over 25,000 was attained in R31.

† Raytheon sonic oscillator, model DF 101.

‡ Cappel Laboratories.

TABLE I. CF and HA Titers of Antisera Against *T. microdentium.**

	Primary immunization						Secondary immunization			Tertiary immunization		
	1st bleeding			2nd bleeding								
Rabbit #	CF	HA	HA/CF	CF	HA	HA/CF	CF	HA	HA/CF	CF	HA	HA/CF
30	100	<100	<1	—	—	—	1,600	<100	<0.06	400	<100	<0.25
31	400	1,600	4	—	—	—	2,400	25,600	10.7	800	12,800	16
34†	320	800	2.5	640	3,200	5	1,600	25,600	16	—	—	—
35†	160	800	5	1,280	6,400	5	1,600	51,200	32	—	—	—

* Titers are reciprocals of the serum dilutions. † With Freund's complete adjuvant.

The specificity of the HA test is shown in Table II. The antisera to *T. microdentium* were titrated in triplicate. The first column presents the results of a standard titration, the second the results found when the homologous antigen is first added and the third presents the results found when a completely unrelated antigen is added. The homologous antigen reduced the original titer by over 99% while the heterologous antigen produced a 50% fall. Almost identical figures have been obtained with other antigen-antibody systems using an HA assay. For instance, an antiserum to bovine-γ-globulin (BGG) had an HA titer of 12,600. Prior addition of BGG caused a fall to 400 while addition of heterologous antigen (casein) produced a titer of 6400(6).

An attempt was made to purify the antigen(s) by centrifugation of the ultrasonic lysate for one hour at 40,000 rpm at 4°. The supernatant so prepared had slightly less antigenic activity in both the CF and HA tests than did the original material. Hence the whole lysate was used in all reported studies.

Seventy-seven human sera were studied for the presence of antibodies to *T. microdentium* by both CF and HA tests. Five percent were positive by CF test and 12% were positive by HA test. All titers were low: the highest CF titer was 1:4 and the highest HA titer was 1:40. Prior addition of *T. microdentium* lysate decreased these HA titers to <10 while *S. epidermidis* antigen had no significant effect. A few sera showed positive HA titers which were unaffected by *T. microdentium* antigen, one having a titer of 1:320. These sera were classified as negative since there was no evidence that the observed hemagglutination was due to *T. microdentium* or a serologically related microbe.

TABLE II. Specificity of the Hemagglutination Inhibition Test.*

	Hemagglutination titers		
Antisera to *T. microdentium*	HA	HAI with *T. microdentium*	HAI with *Staph. epidermidis*
#31	25,600	200	12,800
#34	12,800	100	6,400
#35	25,600	200	12,800

* Titers are reciprocals of the serum dilutions.

There was no correlation between the CF and HA tests in these human sera; in fact only one serum was positive for both tests and this serum contained cold agglutinins.

Discussion. The wide range in the HA/CF ratio shown in Table I (over 500-fold) coupled with the dissociation between HA and CF antibodies in human sera strongly indicate that the CF test and HA test measure different antigens of *T. microdentium.*

Our study of a group of human sera indicated that 5% had low CF antibody and 12% had low HA antibody titers to *T. microdentium.* In 1941, Kolmer, Kast and Lynch (9) reported that 80% of human sera had CF antibody to *Spirochaeta microdentium;* no titers were reported. Our results with only 5% of the sera having CF antibodies to *T. microdentium* are entirely different but our results are internally consistent with our HA assay results which also showed a low incidence of antibody. Two factors may bear on this discrepancy between our results and those of Kolmer *et al.* The oral spirochetes have been very difficult to classify and it is possible that the organism designated *S. microdentium* by Kolmer *et al* may be different from the one designated *T. microdentium* which we have used. In addition, oral treponemes are most abundant in people with periodontal disease and the oral hygiene of

their 1940 population may have been poorer than that of our 1966 population.

The mere presence of bacteria within the oral cavity does not necessarily indicate that detectable humoral antibodies will be present. Even in cases of clinical cystitis due to *Escherichia coli,* only 6% of the patients showed appreciable antibody responses(10). But if pyelonephritis developed, then 82% of the patients showed humoral antibody responses(10). Hence we may anticipate that the humoral antibody responses to *T. microdentium* in man will be correlated with active infection. There is support for this view in the work of Steinberg, Socransky, Gerskoff and Weinstock(11). Using a different HA technic for detecting antibodies to *T. microdentium,* they found that none of five patients with normal gingivae had HA titers >10 while 8 of 9 with periodontal disease had titers of 10 to 40. Although no inhibition studies were reported, their results are consistent with the concept that antibody formation requires infection.

Summary. 1. Rabbits heavily immunized with *Treponema microdentium* develop high titers of both complement-fixing (CF) and hemagglutinating (HA) antibody. 2. Of 77 human sera, 5% had low titers of CF antibody and 12% had low titers of HA antibody to *T. microdentium.* 3. The patterns of antibody response in both the rabbits and patients indicate that the CF and HA antibodies are produced in response to different antigens of this spirochete.

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Quantification of Cellular Influx into the Croton Oil-Induced Pouch. Effects of Prednisolone.* (31791)

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A number of models have been developed to study inflammatory processes in both man and experimental animals. The modified Selye "granuloma" pouch of Roberts and Nezamis has been extensively used to assay for anti-inflammatory agents(1,2,3). Glenn and his associates analyzed the chemical composition of exudates obtained from pouches induced by the local administration of croton oil and d-α-tocopherol(3). The leukocyte content of these fluids has not, however, been examined. Moreover, the influence of anti-inflammatory agents on the cellular composition of the exudates remains unexplored. It was the purpose of these investigations to examine leukocyte influx into croton oil-induced pouches and to study the effect of locally administered prednisolone on the numbers and types of cells mobilized into this inflammatory site.

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Materials and methods. Male rats of the Long-Evans strain weighing approximately