intracellular compartments of body(10).

Initiation of lactation in hypophysectomized rats was accomplished by Bintarningsih *et al.*(11) using combinations of lactogenic hormone and HCA or prednisolone acetate and growth hormone. Five mg lactogenic hormone plus 1 mg HCA or 0.4 mg prednisolone acetate maintained lactation at approximately 50% of normal. On basis of present work, levels of glucocorticoids used in above study were adequate for maintenance of normal lactation.

Significantly higher than normal milk production was found on day 14 of lactation in dams maintained on 1 mg HCA per day and 1% saline. Since the litters of these dams did not weigh any more than normal litters, question arises as to the explanation for this. Perhaps the composition of milk was changed; pups did not consume all milk that was available; or pups did not begin to eat solid food on day 16 of lactation because milk was more readily available.

Summary. Sprague-Dawley-Rolfsmeyer rats were adrenalectomized on day 4 of lactation and lactation was maintained by daily subcutaneous injections of corticosteroids from day 4 to 19. Data suggest that requirements for normal lactation in rats are 250 to 500 μg hydrocortisone acetate or equivalent in glucocorticoid activity and 250 μg desoxycorticosterone acetate or equivalent in mineralocorticoid activity. Either steroid alone at these levels was unable to support lactation but combination was effective.

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Received February 8, 1962. P.S.E.B.M., 1962, v110.

Treponemal Antigens as Related to Identification and Syphilis Serology. (27515)

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Since the introduction of the Treponema Pallidum Immobilization (TPI) test by Nelson and Mayer(1), a renewed interest in treponemal antibodies has been evidenced by the development of new test procedures. The objective has been to develop methods resulting in greater sensitivity and specificity of antibody detection in terms of syphilis. The Treponema Pallidum (T.p.) Agglutination (TPA) test(2), the T.p. Complement Fixation (TPCF) test, the Reiter Protein Complement Fixation (RPCF or KRP) test and the Fluorescent Treponemal Antibody (FTA) test are presently being used or evaluated(3). In spite of new developments and apparent improvements in the field of syphilis serology the need for more basic information in this area led to the present inquiry into the antigenic relationship of treponemes and their operation in current treponemal tests for syphilis. The following treponemes were selected for investigation: The "Reiter treponeme" (R.t.), a representative of several named spirochetes originally isolated from syphilis lesions and purported to be a cultivatable, non-pathogenic

variant of T.p.; *Treponema microdentium* (T.m.), a representative of the normal human flora that may be morphologically indistinguishable from T.p.; *Treponema zuelzerae* (T.z.), a free-living spirochete isolated from California mud, which is unrelated to human disease and has previously been used in antigen comparisons(4), and T.p., the etiologic agent of syphilis, was chosen as a standard for antigen comparisons.

Methods. T.p. (Nichol's Rabbit Strain) was grown in rabbit testicles and harvested according to the TPI test procedure(3). The R.t. and T.m.* were grown in Difco-NIH thioglycollate broth enriched with 10 to 12% rabbit serum according to the general methods for R.t. cultivation and harvest(3). The Veldkamp media, containing no animal serum, was used for T.z.* propagation(5). All cultivated for approximately 72 hours were frozen at –40°C and thawed at room temperature at the time of use. First injections were made subcutaneously with 5 ml of broth culture. Subsequent intravenous injections were started 4 days later with a beginning dose of 0.5 ml. Each additional dose was increased by 0.5 ml until a 2 ml dose was reached. A total of 6 injections was administered giving 2 injections per week, the last 2 injections being 2 ml each. After one week of rest, animals were bled by cardiac puncture. T.p. antiserums were obtained from rabbits which had been infected according to the TPI test procedure(3) and were bled 2 weeks to 2 months following evidence of orchitis. All antiserums, pre-inoculation and normal rabbit control serums were preserved at –40°C.

Serum globulins were prepared, labeled with fluorescein-isothiocyanate and absorbed with Difco beef bone marrow as previously described(6). Both fresh and preserved (frozen or lyophilized) treponemal suspensions were used for smears. These were made according to the FTA test procedure(7). Staining or reaction times were based on the use of one gram percent globulin conjugates and ranged from 30 minutes to one hour at room temperature. Direct staining was used in all cases. Conjugates were used either unabsorbed (except for bone marrow), treponeme absorbed or inhibited with unlabeled antibody. Inhibition or blocking tests were performed by the one-step procedure using equal portions of conjugate and unlabeled antiserum. A Leitz Ortholux microscope equipped with a high pressure mercury arc lamp (HB-200) and appropriate filters was used for determining fluorescent reactions.

The absorption technic consisted of using saline washed, packed treponemes equal to about 0.25% of the conjugate volume. Cells were in contact with the conjugate for 2 hours at 37°C. Absorption efficiency was determined by observation of cross-reactions. Extinction of fluorescence was used as the endpoint. In some cases, but not all, a second absorption was necessary. This was accomplished as described.

Where human serums were involved, FTA test quantitations were accomplished(8). Where absorptions of serums were indicated, these were performed in the same way as described for conjugates.

Results. Table I, Block A (Unabsorbed Serums), depicts cross-fluorescent reactions observed when treponemes were stained with homologous and heterologous labeled antibodies. It will be observed that T.m. reacts only with its own labeled antibody. On the other hand, antibodies derived from T.m. react with all of the treponemes tested. This may be interpreted as meaning that all of the treponemes possess a common antigen. The serologic behavior of T.m. indicates that the common cross-reacting antigen may be inadequate in amount or is so different in structure that too small amount of fluorescent antibody is bound to give visible localization.

The nature of the common or shared component is explained in Block B. Here all conjugates have been absorbed with intact R.t. and are again employed as in Block A. It will be noted that all cross-reactions previously observed have been removed. As expected in the homologous R.t. system, all reactivity has

*We are indebted to Dr. Edward G. Hampp, Nat. Inst. of Dental Research, N. I. H., Bethesda, Md., for furnishing 3 strains of "small mouth treponemes" (*Treponema microdentium*) for this study and to Dr. H. Veldkamp, Lab. Voor Microbiologie, Landbouwhogeschool, Wageningen, The Netherlands, for *Treponema zuelzerae* culture.

TABLE I. Differentiation of Treponemes by Means of Absorbed* Fluorescein-Labeled Antiserums.

	Fluorescein-labeled treponemal antiserums	Antigens			
		T. pallidum	Reiter treponeme	*T. zuelzerae*	*T. microdentium*
A	Unabsorbed serums				
	Treponema pallidum	R	R	R	N
	Reiter treponeme	R	R	R	N
	Treponema zuelzerae	R	R	R	N
	Treponema microdentium	R	R	R	R
B	Absorbed with Reiter treponeme				
	Treponema pallidum	R	N	N	N
	Reiter treponeme	N	N	N	N
	Treponema zuelzerae	N	N	R	N
	Treponema microdentium	N	N	N	R
C	Absorbed with *Treponema pallidum* or *Treponema zuelzerae*				
	Reiter treponeme	N	R	N	N

* or blocking antibody R—Reactive N—Nonreactive

also been eliminated. The removal of the common "Reiter" antibody component now reveals that specific staining of T.p., T.m., and T.z. has been accomplished. In that T.p. shares the common "Reiter" component as evidenced by the cross-reactions observed in Block A, the use of T.p. as the absorption agent should result in a specific R.t. staining reagent. That this is apparently true is shown in Block C (absorbed with *Treponema pallidum*). The common antibody contained in the R.t. antiserum has been removed by T.p. absorption leaving a specific identification fraction for the R.t. Thus, the identification of each treponeme has been accomplished by removal of a "single" shared antibody component that previously caused all of the antiserums to demonstrate a non-specific staining result. Since this antigen fraction could be associated with the "Reiter protein antigen" employed in the RPCF test and because there is no doubt that it operates in the FTA test as shown by T.p. reactivity with the R.t., T.m. and T.z. fluorescein-labeled antiserums, several carefully selected human serums from syphilitic individuals were tested in a variety of ways before and after absorption with the R.t. All of the serums were reactive in the several tests† prior to absorption.

The FTA test titers were invariably lowered by the R.t. absorption (Table II). For example, Serum No. C-68 originally presenting an FTA titer of 3200 dils. was reduced to 800 dils. after absorption. The FTA test performed with the R.t. as an antigen demonstrated 1600 dils. This was reduced to a nonreactive result (<10 dils.) following absorption with R.t. All qualitative tests following Reiter absorption remained reactive except the RPCF test. Similar results are demonstrated by Serums C-41, C-49 and C-42. Thus, 3 broad groups of antigens and antibodies presently operate in syphilis serology: First, those that react in lipid or cardiolipin-like test procedures; second, those that function in Reiter tests; and third, those that are concerned in T.p. procedures. The FTA test apparently operates with both the R.t. and T.p. fractions but can be made to operate in respect to the specific T.p. fraction if the proper absorption technic is utilized.

Discussion. The term 'T.p. fraction' is used in this study to indicate a closer relationship to the etiologic agent and therefore to syphilis. This has been done to distinguish the specific component of T.p. from the R.t. and other treponemes of the study related to T.p. and to each other through a common antigenic component unrelated to syphilis. Findings presented support DeBruijn's conclusions that the protein fraction of T.z. and R.t. is essentially the same(4). This does not necessarily invalidate the use of the "Reiter protein fraction" in tests for syphilis. Yet, the evidence

† Tests other than the FTA test were performed by Serology Section, Venereal Disease Research Lab.

TABLE II. Reactivity of Human Serums Following Reiter Treponeme Absorption.

	Unabsorbed		After absorption				
	Test results		Test results				
Serums	FTA using *T. pallidum*	FTA using Reiter treponeme	FTA using *T. pallidum*	FTA using Reiter treponeme	TPI	TPCF	KRP
C-68	3200	1600	800	<10	R	R	N
41	800	200	200	<10	WR	R	N
49	800	200	400	<10	Inc.	R	N
42	800	200	200	<10	R	R	N

Inc.—Inconclusive

strongly suggests that the R.t. antigen is not peculiar to T.p. and treponemal cultures purported to have once been T.p., but rather it appears to be an antigen common to many treponemes whether pathogens or free-living and is therefore a non-specific component.

One might inquire whether treponemal antigens other than those of the well-defined pathogens causing syphilis, Yaws and Pinta are capable of producing antibodies in humans and, if so, are such antibodies detected by syphilis tests. This problem arose in connection with an increased sensitivity of the initially described FTA test(3). Apparently as the test became more efficient in terms of antibody detection, an increasing number of reactions were noted in serums obtained from a "Normal" population group. Finally it was determined that approximately 25% of the so-called "Normal" group demonstrated reactive results above 5 dils. and in a few cases as high as 100 dils.(8). Subsequent absorption studies indicated that the reactive components in these serums were true treponemal antibodies since they could be removed by T.p. and R.t. absorptions but not by lipid antigens or other similar treatment. That there was a difference between the treponemal antibodies in "Normal" and syphilitic individuals was determined by comparing serums giving identical FTA titers (100 dils.). After absorption with the R.t., "Normal" serum titers were reduced to less than 5 dils., the dilution set for the qualitative test. The reactivity of the syphilis serums under identical circumstances was reduced to 50 dils. This means that syphilis serums would remain strongly reactive if the 5-dil. qualitative limitation had been employed. These findings suggest that the treponemal antibodies from "Normal" individuals contain "anti-Reiter antigen" antibodies but do not contain antibodies to the specific 'T.p. fraction' and therefore are unrelated serologically to syphilis.

Data presented here suggest that any of the treponemes in the study other than T.p. could cause this serological behavior since all contain the Reiter or common fraction. It seems unlikely that the R.t. would be involved and T.z., being a free-living mud treponeme, can probably be ruled out. Mouth treponemes on the other hand, being a part of the normal human flora, may be placed on our list of suspects. If organisms of this kind are capable of producing detectable "Reiter antibody" in about 25% of the "Normal" population as indicated by the FTA test, the use of the Reiter Complement Fixation tests can be expected to have very definite limitations in terms of sensitivity and specificity. A similar problem was encountered and resolved by modification of the FTA test from a 1-5 to a 1-200 test dilution(8). This not only greatly reduced non-specific reactions, but also reduced the number of reactions that might be expected in syphilis categories.

The data reported here should make possible greater sensitivity of the FTA test without loss of specificity. This can be obtained through absorption or by the use of blocking antibody. It should be possible to eliminate non-specific reactions at 5 dils. so as to obtain the more specific T.p. reaction with syphilis serums of low treponemal antibody titer. An estimated 50% increase in sensitivity can be expected in the Primary Syphilis category, and an increase can be expected in other syphilis categories. Such a modification of the FTA test is being studied.

Our findings are also important in relation to treponeme identification and their future taxonomy based on antigenic analysis. This investigation has shown that treponemes may be differentiated rather easily by fluorescent antibody (FA) methods and suggests an expanded study.

Statements relative to the inhibition or blocking technic have been mostly of a reference nature. Where absorptions have been indicated in the present work the blocking antibody technic has been shown to demonstrate equal results. Because of its simplicity, this technic would be preferred in future FTA test modifications.

Summary. 1. The antigens of intact T.p., the R.t., T.m. and the free-living mud organism, T.z., are compared. 2. An antigen appearing to be identical to the Reiter Protein antigen was found to be a common or shared component in all of the treponemes studied. 3. By means of absorption or blocking procedures and employing FA methods, all treponemes could be identified. 4. It is suggested that FA methods can play an important part in future taxonomic studies of treponemes and that the FTA test can be modified so as to increase both sensitivity and specificity.

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Received February 12, 1962. P.S.E.B.M., 1962, v110.

Acetylcholine and Iodine Metabolism in Thyroid Slices.* (27516)

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For many years the thyrotrophic hormone (TSH) has been known to control the metabolism of iodine in the thyroid gland *in vivo*. More recently *in vitro* TSH effects have been observed in the iodine metabolism of thyroid slices(1,2,3). In addition to *in vitro* iodine effects, Field and his colleagues (4,5) have also demonstrated that TSH stimulates oxidation of glucose $-C^{14}$ to $C^{14}O_2$, and production of TPN from DPN in thyroid slices as well. Further work by the Field group(6) has suggested that the neurohormone acetylcholine has a similar specific function in the thyroid gland in the control of glucose oxidation. Some differences exist, however, between the actions of TSH and acetylcholine in glucose metabolism. In particular, TSH preferentially increases the oxidation of glucose *via* the hexose monophosphate pathway (*i.e.*, C_1 carbon oxidation) whereas acetylcholine stimulates both C_1 and C_6 carbon oxidation equally. In view of these similarities and differences in control of carbohydrate oxidation, it is of interest to establish whether acetylcholine, like TSH, has a controlling influence on iodine metabolism in thyroid slices, and if so whether the effects observed are similar to those observed with TSH. This paper reports the experiments designed to answer these questions.

Experimental. Thyroid glands obtained from nembutal-anesthetized dogs were kept at ice-water temperature, trimmed of all ex-

* This investigation was supported by research grant from Division of Arthritis and Metab. Dis., U.S.P.H.S.

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