AHH activity was determined by a modification of the method of Nebert and Gelboin (*12*). Homogenates were incubated for 30 minutes at 37°C in a final volume of 1.1 ml containing 1.0 mg of reduced nicotinamide adenine dinucleotide (NADPH) and 25 μg of BP. The reaction was stopped by addition of 4 ml of an acetone, hexane mixture (1 : 3) and vortex mixing for 1 minute. The phases were separated by centrifugation, and the organic phase was extracted with 0.5 ml of 1*N* NaOH for 1 minute. The aqueous and organic phases were separated by centrifugation and fluorescence of the hydroxylated BP in the aqueous phase was determined in an Aminco-Bowman spectrophotofluorometer with excitation at 396 nm and emission at 522 nm.

The AHH activity in leukocytes cultured for 72 hours in medium containing phytohemagglutinin was increased after treatment with 3MC, reaching a fourfold maximum by 12 hours (Fig. 1). In control leukocytes cultured under identical conditions the baseline levels of AHH activity was higher than that in fresh leukocytes. Fresh leukocytes were not induced by 24 hours of exposure to 3MC (Fig. 2). However, after 72 hours of preliminary incubation in phytohemagglutinin medium, the leukocytes responded to 3MC with a marked rise in AHH activity.

Activity of AHH in inducible experimental animals varies in response to environmental conditions, whereas the ability to be induced is expressed as a simple Mendelian characteristic (*9*). The AHH activity also varies in humans, notably, between cigarette smokers and nonsmokers (*6*). This cultured leukocyte system provides a simple method for studying the inducibility of AHH activity in the human population (*13*).

D. L. Busbee
C. R. Shaw
Section of Medical Genetics, University of Texas M. D. Anderson Hospital and Tumor Institute, Texas Medical Center, Houston 77025
E. T. Cantrell
Department of Pharmacology, Baylor College of Medicine, Texas Medical Center, Houston 77025

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Chronic Thyroiditis in the Rabbit Induced with Homologous Thyroid Microsomes

Abstract. *Rabbits immunized with freshly isolated homologous thyroid microsomes in complete Freund's adjuvant developed a severe chronic thyroiditis with the concomitant production of antimicrosomal antibodies. The antibody response was measured by indirect immunofluorescence and complement fixation, employing isolated microsomes as the antigen. Microsomes but not thyroglobulin absorbed out the immunologic activity of the rabbit serum.*

In view of the high incidence of antimicrosomal antibodies and the lack of correlation of disease with antithyroglobulin antibodies in Hashimoto's chronic thyroiditis, experiments designed to evaluate the role of microsomal antigen or antigens were indicated. Surprisingly, the number of investigators who have reported on this is small and the data conflicting. Jones and Roitt (*1*) attempted the experiment with homologous microsomes in the rat but reported failure, as did Roitt *et al.* (*2*) in the sheep. In contrast, Kite *et al.* (*3*) have been successful in producing transient mild thyroiditis in the monkey with human thyroid extract as well as obtaining an antimicrosomal antibody which was reactive with human target tissue as based upon cytotoxicity and complement fixation.

This report presents data on the production of a severe chronic thyroiditis in rabbits induced with homologous thyroid microsomes and the concomitant production of antimicrosomal antibody which appears to correlate with the severity of the disease.

Rabbit thyroid microsomes were isolated from an average pool size of no less than 200 thyroids (fresh iced, Pel-Freez, Rogers, Arkansas) by a modification of the method of Campbell and Kernot (*4*). After the thyroids were freed of superficial fat they were homogenized in a Sorvall Omni-Mixer at a ratio of 2.5 volumes of microsomal buffer (0.01*M* $MgCl_2$, 0.025*M* KCl, 0.035*M* tris buffer, *p*H 7.8, and 0.15*M* sucrose) to 1 gram weight of tissue. The homogenate was then filtered through gauze pads to remove much of the lipid material and centrifuged at 12,000*g* in a Sorvall RC-2 for 10 minutes to remove cell debris, nuclei, and mitochondria. The congealed upper lipid layer was removed with a spatula and the aqueous layer was decanted. This layer was then centrifuged in a Spinco model L at 105,000*g* for 50 minutes. The gelatinous button was homogenized in microsomal buffer with the aid of a hand-operated tissue grinder and recentrifuged at the same speed and time. This procedure was repeated twice and the pellet was finally suspended in microsomal buffer at a concentration of 10 mg of protein (as determined by micro-Kjeldahl) per milliliter. Microsomal preparations were declared as such and used only after evaluation by electron microscopy.

Rabbit liver microsomes were prepared in an identical manner from a pool of five normal rabbit livers.

Twelve female New Zealand white rabbits (2 to 2.5 kg) were each injected with 5 mg of microsomal protein incorporated in complete Freund's adjuvant (CFA) distributed among the four footpads. Fourteen days later they received 2.5 mg of freshly isolated microsomal protein antigen in CFA intramuscularly and another 2.5 mg of freshly isolated microsomal protein antigen in CFA intramuscularly 7 days

later. One week after the last injection the animals were exsanguinated and the thyroids and livers were removed for inspection and testing.

Four rabbits, of similar weight, sex, and breed, were immunized in an identical fashion with homologous liver microsomes.

The rabbits immunized with thyroid microsomes had thyroids which were diffusely enlarged from one- to fivefold, pale gray, and heavily indurated. In some rabbits they were firmly adherent to the trachea and to the surrounding musculature of the neck. Microscopically, an inflammatory process involving the thyroid and often extending to the perithyroidal tissue was seen. There was extensive destruction of follicles with, in severe cases, total effacement of the architecture of the organ. More commonly, a few colloid-containing follicles were present, surrounded by numerous inflammatory cells. Regeneration of the epithelium of the thyroid was conspicuous in most animals and resulted in formation of tubular structures without colloid and formed by several layers of cells. Solid nests of epithelial cells were seen at times, but no giant epithelial cells. The inflammatory infiltrate was made up of large mononuclear cells, lymphocytes, plasma cells, eosinophiles, and histiocytes. Eosinophiles and neutrophiles were particularly numerous around small foci of necrosis present in the most severe cases. Histiocytes with abundant foamy cytoplasm often completely filled the lumen of degenerating follicles. Fibroblast-like spindle cells and collagen fibers of variable thickness were intermixed with inflammatory cells and were more abundant in the cases of greater severity.

The histologic picture of the microsome-induced disease differed from that of thyroglobulin-induced disease (produced in our laboratory) only in magnitude, being much more severe. The disease evolves from a moderate histiocytic infiltration at 7 days to moderately severe disease in 14 days, with maximal disease between 21 and 28 days (*5*). The classification of 4+ simply means that few if any follicles can be discerned and that the entire architecture of the gland has been disrupted by cellular invasion.

Animals immunized with liver microsome demonstrated no gross histologic or immunofluorescence abnormalities in either thyroid or liver.

Assay of antimicrosomal antibody was conducted by both complement fixation and indirect immunofluorescence employing normal rabbit thyroid as target tissue for the latter. The complement-fixation reaction was conducted on serial dilutions of the rabbit serums, titered guinea pig complement, and a microsomal antigen suspension that was neither hemolytic nor anticomplementary. The end point was taken as that dilution which produced complete lack of lysis. An interesting aspect of the reaction was that in those tubes that demonstrated complement binding in the presence of rabbit serum, visual fine aggregation of the microsomal suspension occurred after incubation overnight in the cold.

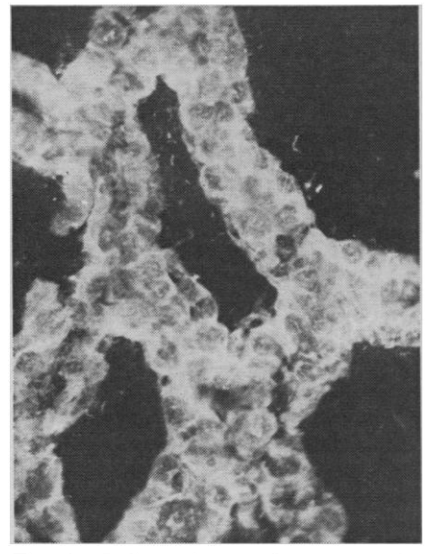

Fig. 1. Indirect immunofluorescence localization of antimicrosomal antibody on normal rabbit thyroid.

Assay by immunofluorescence was conducted on 4-μm-thick, air fixed, normal rabbit thyroid sections. The serums from immunized rabbits were employed undiluted, reacted for 1 hour, washed three times with buffered saline, *p*H 7.4, with gentle shaking, and then the fluorescein-conjugated goat anti-7*S* rabbit γ-globulin was treated similarily.

Absorption studies were conducted on the rabbit serums by reacting equal volumes of microsomal suspension (10 mg of protein per milliliter) with serum at room temperature for 30 minutes and then removing the microsomes by centrifugation at 105,000*g* for 50 minutes. Control for the procedure consisted of equal volumes of rabbit serums and microsomal buffer treated in an identical fashion. Additionally, absorption was carried out on the antiserums with equal volumes of thyroglobulin solution (10 mg of protein per milliliter).

Subjective grading of the fluorescence was based upon the intensity of the staining as compared to the controls. Figure 1 depicts the usual microsomal localization of the rabbit antibody, which was completely removed by prior treatment with isolated thyroid microsomes but not with thyroglobulin. Absorption of the antiserum with isolated rabbit liver microsomes removed a portion of the staining capacity, leaving an antiserum which was organ specific as based upon evaluation with liver, kidney, and spleen tissue. No colloid localization was observed with either unabsorbed or absorbed serums.

Table 1 is a compilation of the graded histologic data, complement-fixation titers (expressed as the reciprocal of the dilution), and the immunofluorescence grading. Inasmuch as all animals demonstrated severe thyroid abnormalities, the minor serologic variations, in particular the complement-fixing titers, are not considered as detracting from the apparent correlation between severity of disease and antimicrosomal antibody content (*5*).

Table 1. Induction of chronic thyroiditis with thyroid microsomal immunization.

Rabbit No.	Severity of chronic thyroiditis	Epithelial cell regeneration	Fibrosis	Complement-fixing titer*	Immunofluorescence†
740	4+	4+	1+	512	4+
741	2+			512	4+
742	1+			512	3+
787	4+	2+	2+	512	4+
788	4+	2+	2+	512	4+
789	4+	2+	2+	1024	4+
790	4+	1+	1+	512	3+
791	4+	1+	1+	512	4+
793	4+	4+	1+	128	3+
794	4+		1+	512	3+
795	4+		4+	256	3+
796	4+	4+	4+	512	4+

* Reciprocal value of serum dilution. † Subjective grading as measured against controls.

In order that the biologic activities which have been indicated for the antimicrosomal antibody not be assigned to an antithyroglobulin response, the following statements as based upon experimental data can be made. Although the well-washed microsomes can be shown to have a thyroglobulin contaminant with guinea pig antithyroglobulin antiserum, rabbits immunized with the microsomes initially developed low levels of antithyroglobulin titers as measured by passive hemagglutination, with a subsequent rise in titer late in the disease (maximum, 1 : 256), whereas the antimicrosomal antibody appeared early and in high titer. Moreover, as indicated in the absorption studies, thyroglobulin did not remove the stated properties of the antibody. Finally, 20 mg of thyroglobulin protein was required to induce a milder form of thyroiditis as compared to 10 mg of microsomal protein which induced the described severe disease.

The foregoing data establish two main features of experimental thyroiditis never before reported: (i) the induction of severe chronic thyroiditis in nonprimate mammals with homologous microsomal immunization, and (ii) the concomitant production of a circulating antimicrosomal antibody.

As to why this laboratory has been successful in achieving microsome-induced thyroiditis and antimicrosomal antibody in a nonprimate mammal whereas others have failed is not too easily dissected. However, as possible reasons the following are presented: (i) break in tolerance by the extremely large pool size of thyroids for microsomal isolation; (ii) the use of freshly isolated microsomal material for each injection (frozen thyroids were ineffectual for these experiments); and (iii) electron microscopic evaluation of each preparation prior to use and discard of unsatisfactory ones.

MARAYART MANGKORNKANOK
A. S. MARKOWITZ
HECTOR A. BATTIFORA
Department of Microbiology, University of Illinois at the Medical Center, Chicago, Hektoen Institute for Medical Research of Cook County Hospital, Chicago, and *Department of Pathology, Wesley Memorial Hospital, Chicago, Illinois*

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7. Presented in part for the degree of doctor of philosophy, University of Illinois at the Medical Center, by M.M.

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Herpesvirus Type 2 in the Male Genitourinary Tract

Abstract. *A population study of 190 randomly selected male patients with no history of genital herpesvirus infection revealed a high incidence of herpesvirus type 2 in genitourinary specimens. This indicates that men serve as a reservoir of genital herpesvirus.*

Epidemiologic studies indicate that genital herpes (herpesvirus type 2) is venereally transmitted (*1*). Antibodies to herpesvirus type 2 are associated with early and vigorous sexual activity and promiscuity, and are more common in prostitutes than in a control population (*2*).

A strong association exists between antibodies to herpesvirus type 2 and cervical neoplasia. The evidence, although circumstantial and sometimes conflicting, is based on the higher frequency and titer of antibodies to herpesvirus type 2 found in patients with carcinoma of the cervix than in matched populations without evidence of cervical cancer (*3*). In addition, Aurelian (*4*) reported the isolation of herpesvirus type 2 from a cervical carcinoma grown in culture, and Duff *et al.* (*5*) reported the oncogenic transformation of hamster cells exposed to a strain of irradiated herpesvirus type 2. Such cells were able to produce tumors in newborn hamsters.

A previous survey of a population of upper-middle-class women revealed an extremely low incidence of recovery of virus from the genital tract (*6*). The present study was undertaken to determine whether the male genitourinary tract could be a reservoir of herpesvirus.

Over a period of 5 months 190 male patients were randomly selected from the University of Florida Urology Clinic; they ranged in age from 15 to 85 years, represented virtually the entire gamut of socioeconomic classes, and were of mixed racial composition. Subjects had no previous history of genital herpesvirus infection.

Specimens collected included urethral swabs, prostate fluid, sections of vas deferens removed during procedures for sterilization, prostate biopsies, and miscellaneous samples such as testicular biopsies and foreskin tissue. As controls, swabs were opened in the room in which the patient cultures were taken and were then put in coded vials. These and the other coded specimens were collected in basal minimal media, inoculated into tube cultures of human embryonic kidney, and examined daily for evidence of viral cytopathic effect.

Those cultures showing cytopathic effect within 7 days were passed to new tube cultures of human embryonic kidney. All cultures that showed evidence of viral growth in the second passage were proved to be those of herpesvirus by neutralization with antiserums to herpesvirus. In addition, in a random selection of positive samples herpesvirus type 2 was demonstrated by indirect immunofluorescence.

The compiled data revealed that, of the 190 men studied, 15 percent were positive for herpesvirus. There was no significant difference between age groups

Table 1. Distribution, according to age groups, of cultures positive for herpesvirus.

Age group (years)	Positive cultures (No.)	Men (No.)	Positive for age group (%)
70+	7	46	15.2
60 to 70	5	28	17.9
45 to 60	6	41	14.6
15 to 45	9	68	13.2

Table 2. Distribution of herpesvirus-positive cultures in genitourinary specimens. The difference between urethral swabs and all other specimens was found to be statistically significant as determined by paired comparisons ($.02 < P < .05$).

Source	Positive cultures (No.)	Men (No.)	Positive for type of sample (%)
Urethral swabs	11	144	7.6
Prostate fluid	3	13	23.1
Prostate biopsy	4	20	20
Vas deferens	9	31	29
Other samples	4	21	19