Current Research Activities on Gonorrhea at the Center for Disease Control

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Gonorrhea—the phoenix of modern bacterial disease—is currently the number one reportable disease in the United States. Any disease which constitutes almost half of the incidence of notifiable diseases in the country and affects one in 330 persons can be considered an epidemic. The Venereal Disease Branch of the Center for Disease Control (CDC) has documented a steady rise in reported gonorrheal incidence during the years 1962–67 which has more than doubled in rate for the 1967–71 period. The enormity of the gonorrheal problem in our country is underlined by the fact that three-quarters of those persons seeking treatment do not attend public health clinics, and thus their cases are not reported (*1*). This recrudescence of gonorrhea has not been an overnight event, although recognition in a public sense has occurred rather abruptly.

Awareness or detection of the disease, particularly in the female, by patients and physicians is hampered by the lack of symptoms specific to gonorrhea, and myths about its superficiality have encouraged the "common

cold" concept of its severity. Complications of gonorrhea, while not generally life threatening nor frequent in occurrence, can be a severe physical problem for some persons. Fortunately, the gonococcus has not evidenced mutability toward a more severe form of infection in parallel with the decreasing susceptibility to penicillin, which has forced penicillin treatment schedules to the practicable limit.

The Health Services and Mental Health Administration has launched a multifaceted campaign to bring gonorrhea out of the shadows and into the public eye, to stimulate physician awareness, and to support an intensive broad-based research and development program to improve the diagnostic and treatment aspects of gonorrhea control. Currently, diagnosis of gonorrhea in a female requires cultivation of the gonococcus from a specimen acquired by a physician from an infected genitourinary, rectal, or other site. Control of gonorrhea would be more efficient and less expensive if an effective serologic test were available. Present research efforts by CDC and other agencies are directed not only toward developing a serologic test for gonorrhea but also an understanding of the long-neglected immunology of gonorrhea.

Gonorrhea Perspective

The current status of the research on serologic tests for gonorrhea reflects the history of research on *Neisseria gonorrhoeae,* the causative organism. Although *N. gonorrhoeae* (the gonococcus) was demonstrated to be the causative agent of gonorrhea in the 1880s, limited research progress was made until the late 1930s and early 1940s, when research on the gonococcus resulted in the development and subsequent widespread use of a complement fixation test for gonorrhea. Unfortunately, the test could not be used to distinguish between present and past infections. During the late 1940s, the introduction and widespread use of penicillin for treating gonorrhea led to a rapid decline in reported cases of gonorrhea. The decrease in incidence was paralleled by the decrease of both funds for and interest in gonorrheal research. The number of reported cases of gonorrhea rose steadily in the 1960s, however, and in the late 1960s the venereal disease research scientists at the Center for Disease Control began research toward the development of serologic tests for gonorrhea.

Serologic Tests

Insofar as the research at CDC is concerned, the development of serologic tests for gonorrhea naturally relates to the intended use of such tests. We are developing procedures which are either (*a*) rapid and simple enough to be used as epidemiologic tools for control purposes or (*b*) currently capable of being partially or wholly automated for use in examining the geographic or socioeconomic incidence of gonorrhea.

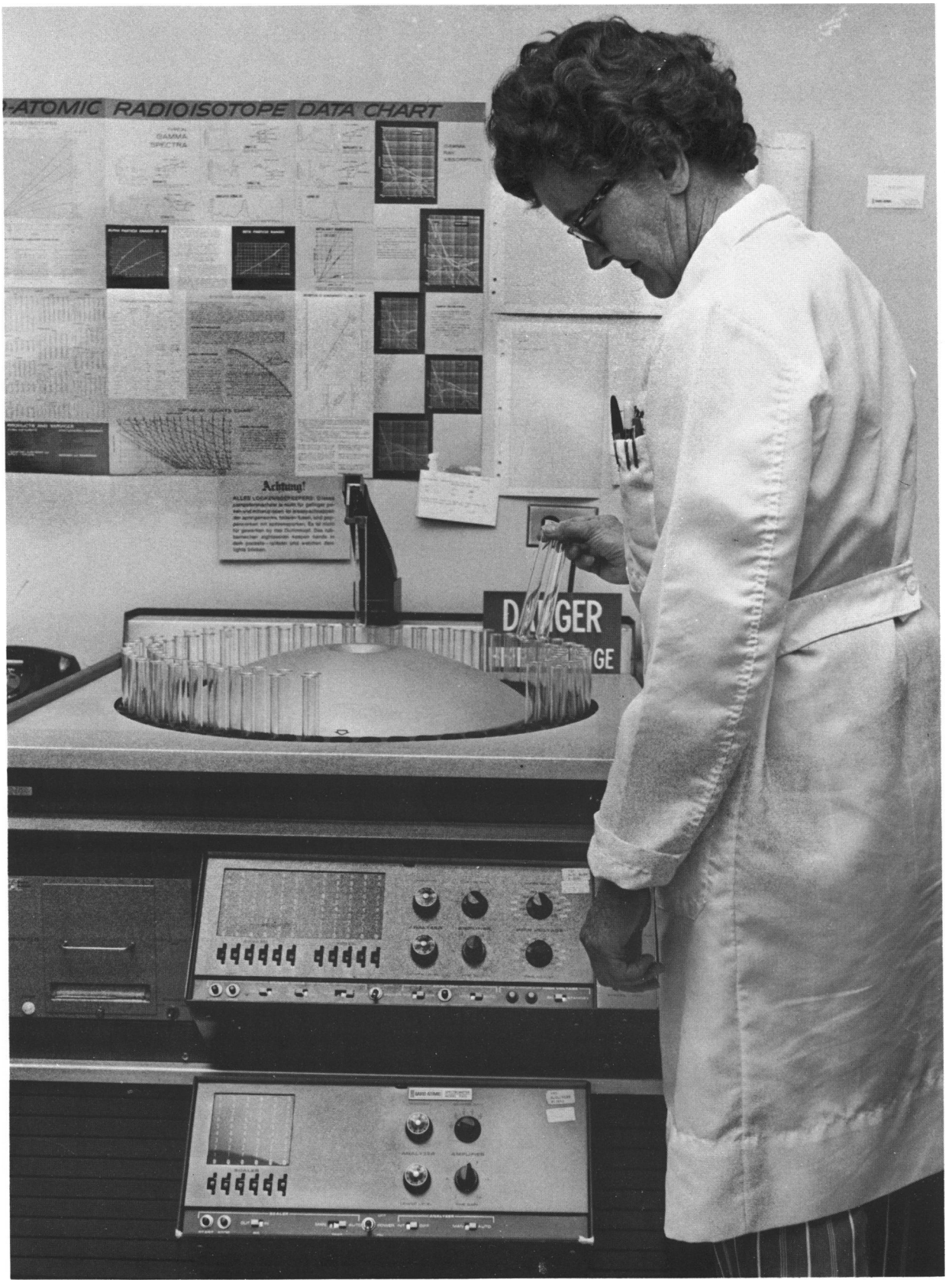
Two procedures in the rapid and simple group are the C-L (cholesterol-lecithin) flocculation test and the SP (selected particle) flocculation test, both of which are slide microflocculations. The C-L test uses soluble protoplasmic components from ultrasonically disrupted gonococci which are adsorbed onto cholesterol-lecithin particles (*2*). The SP test uses insoluble components of reconstituted lyophilized protoplasm which have been physically sized for optimal reactivity (*3*).

There are three procedures in the group for which equipment can be automated for use in examining the geographic or socioeconomic incidence of gonorrhea. Each of these procedures measures slightly different aspects of antibody-antigen interaction. One procedure is a modified version of the Laboratory Branch complement fixation (CF) test (50 percent hemolysis endpoint) in which soluble protoplasmic components are the antigen (*4*). A second procedure is a passive hemagglutination (PHA) test in which both soluble and insoluble components of ultrasonically disrupted gonococci can be used (*5*). The third procedure is an indirect fluorescent antibody (IFA) test, in which a selected strain of gonococci can be used, that exhibits a broad spectrum of response to a variety of reactive serums and is reasonably stable as a strain (*6, 7*).

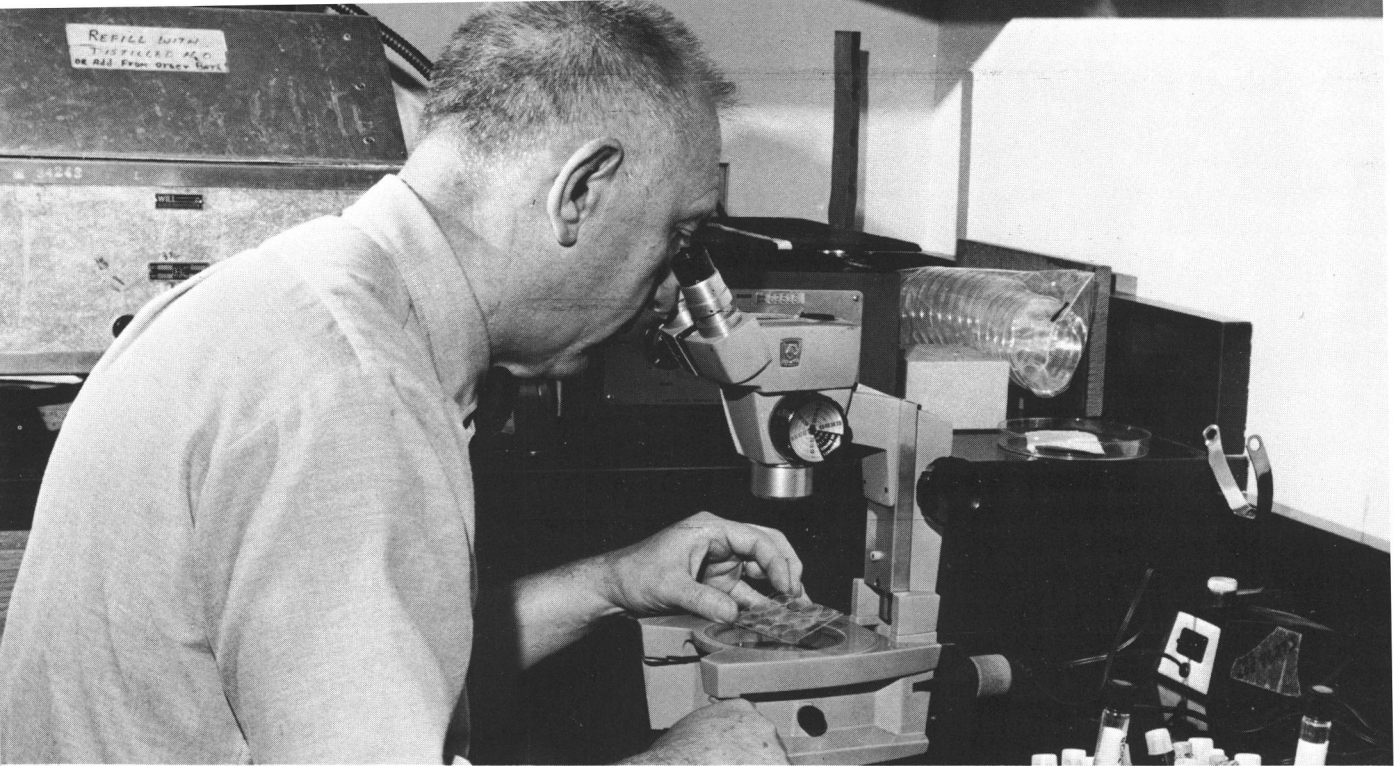
When the five tests described were used to examine groups of serums accumulated in our serum bank, antibodies were detected in 79–88 percent of the serums from culturally positive females and in 2–18 percent of the serums from culturally negative females, as shown below.

	Percent females	
Test	*Culturally positive*	*Culturally negative*
C-L flocculation	79	5
SP flocculation	85	12
PHA	88	18
IFA	80	2
CF	80	4

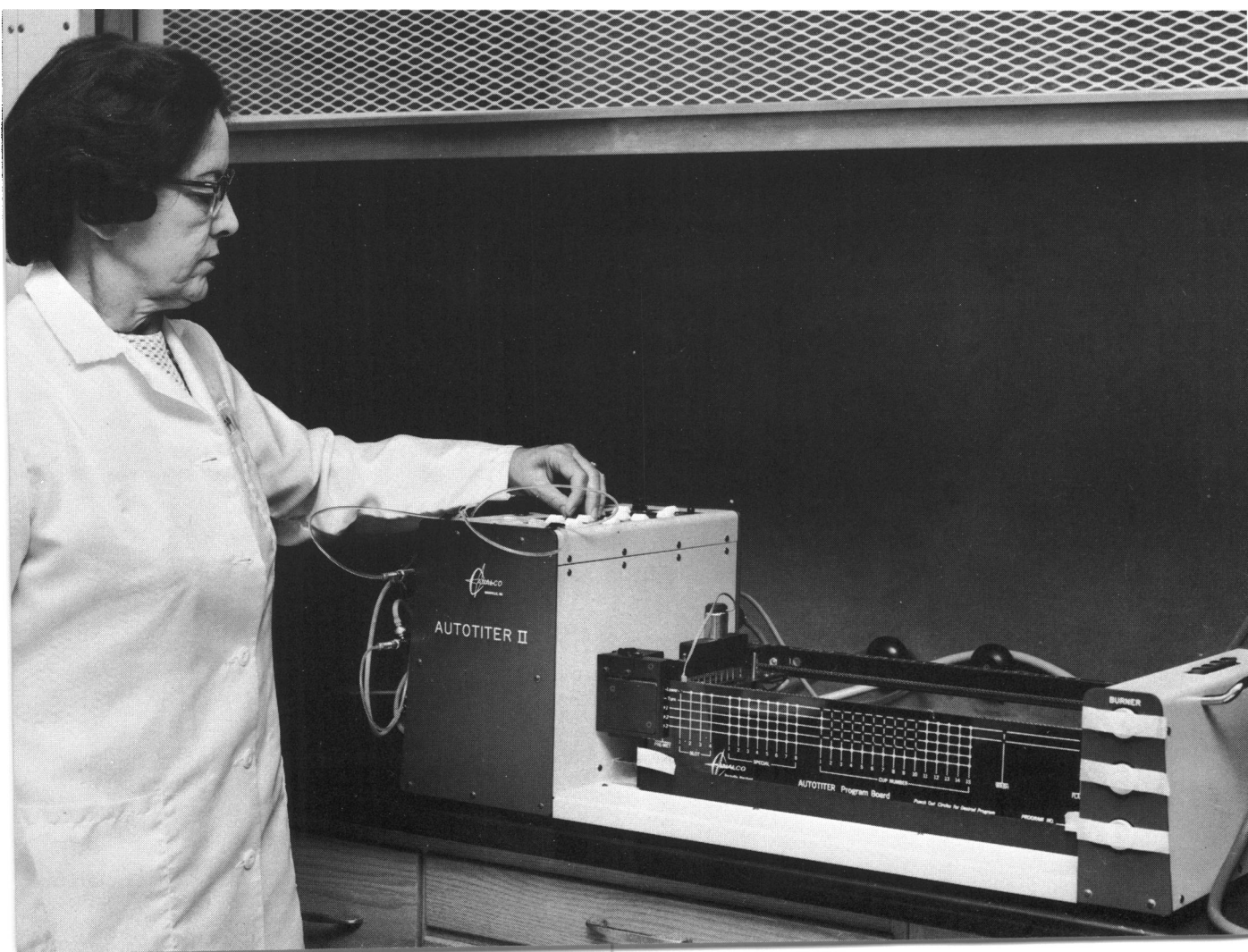
When the PHA, IFA, and CF tests were given a pilot evaluation with fresh serums acquired from patients attending a venereal disease clinic in a large metropolitan area, antibodies were



Detecting radioactively labeled antibodies



Flocculation test reading (above); automated dilution technique for serologic tests (below)

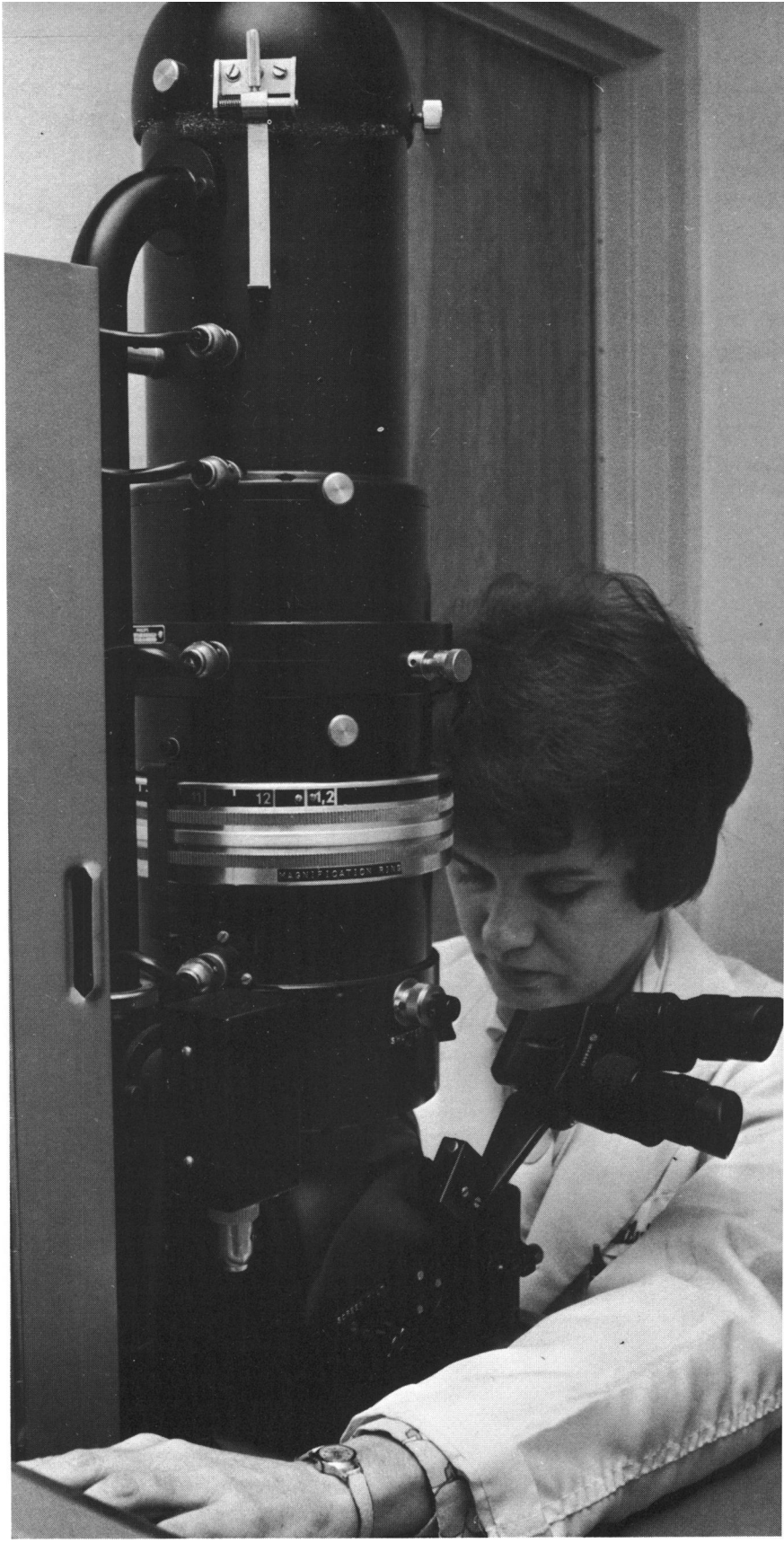


detected in an unexpectedly low percentage (39–63 percent) of serums from culturally positive patients and in an unexpectedly high percentage (13–37 percent) of serums from culturally negative patients. An intensive review of the technical aspects of the tests did not reveal any reasons for the differences in reactivities between fresh clinic serums and the banked serums used in the intramural study. When serums from our serum bank were subsequently reexamined, no change in the test sensitivities and specificities was demonstrated.

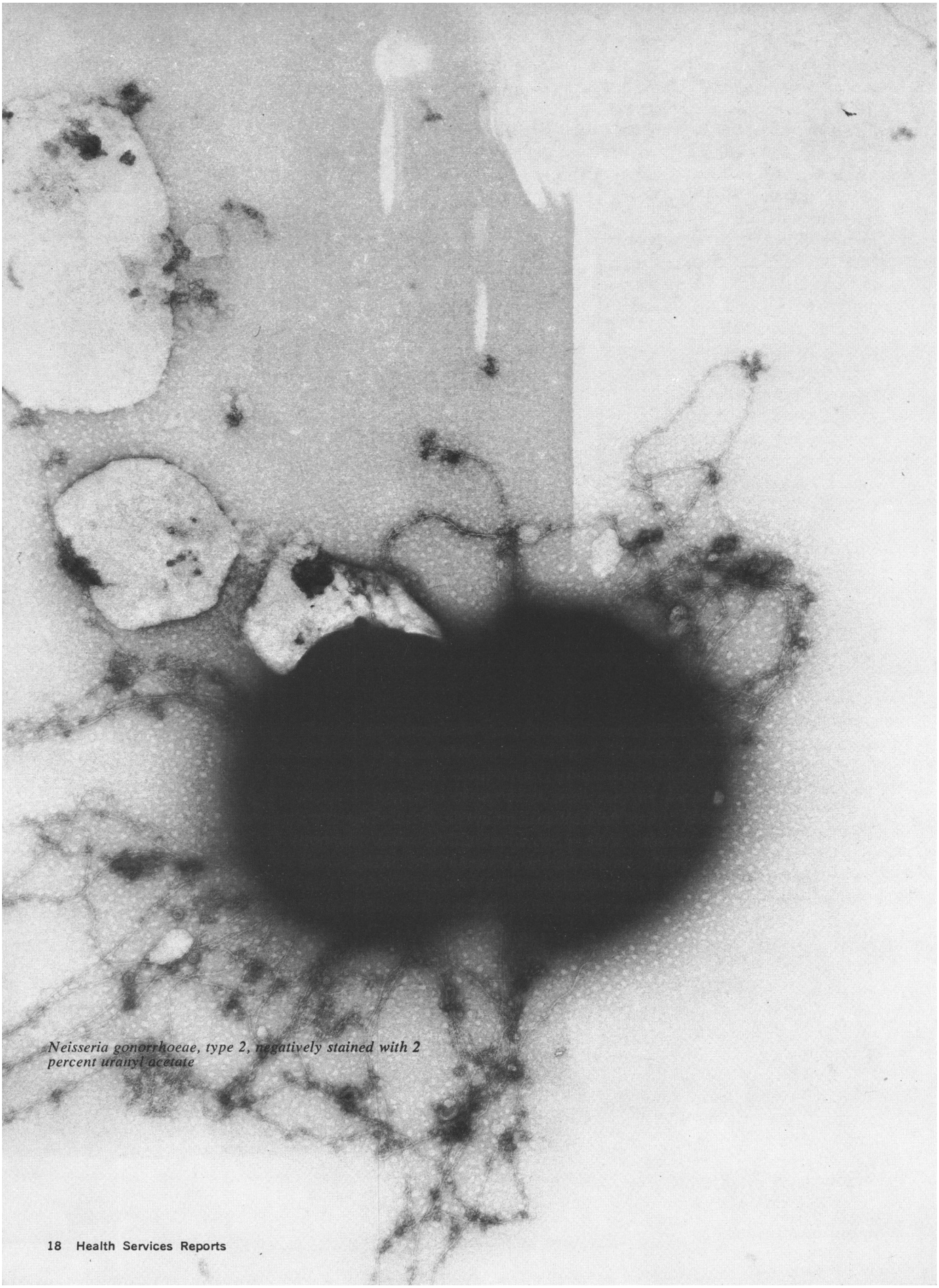
Fresh serums from celibate nuns and virginal college students showed an acceptably low degree of reactivity in the PHA, IFA, and CF tests—less than 5 percent. Recent studies on serums collected sequentially from human patients after treatment for gonorrhea and from chimpanzees after experimental infection indicate that the tests have their original sensitivities and specificities.

Each of the three tests is currently being modified or recalibrated, or both, where necessary, to further improve its sensitivity and specificity. The present data indicate that there is no geographic distinctiveness in the reactivities of serums in our serum bank. Knowledge of the circumstances concerning the individual patient's reason for appearing at the clinic indicates that the tests may be exposing our lack of knowledge concerning the duration of the present infection and the recency of past infections.

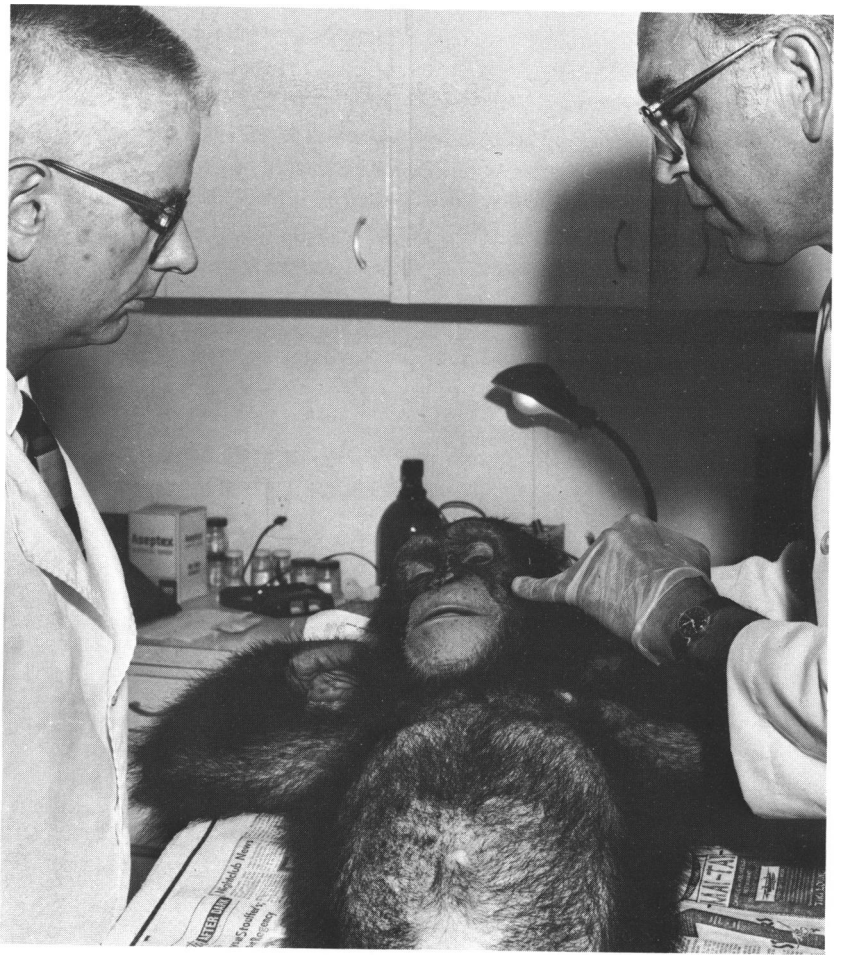
Two other procedures under examination are a bentonite flocculation procedure developed in Canada and a radioimmunoassay (RIA) procedure developed at CDC. The sensitive RIA proce-



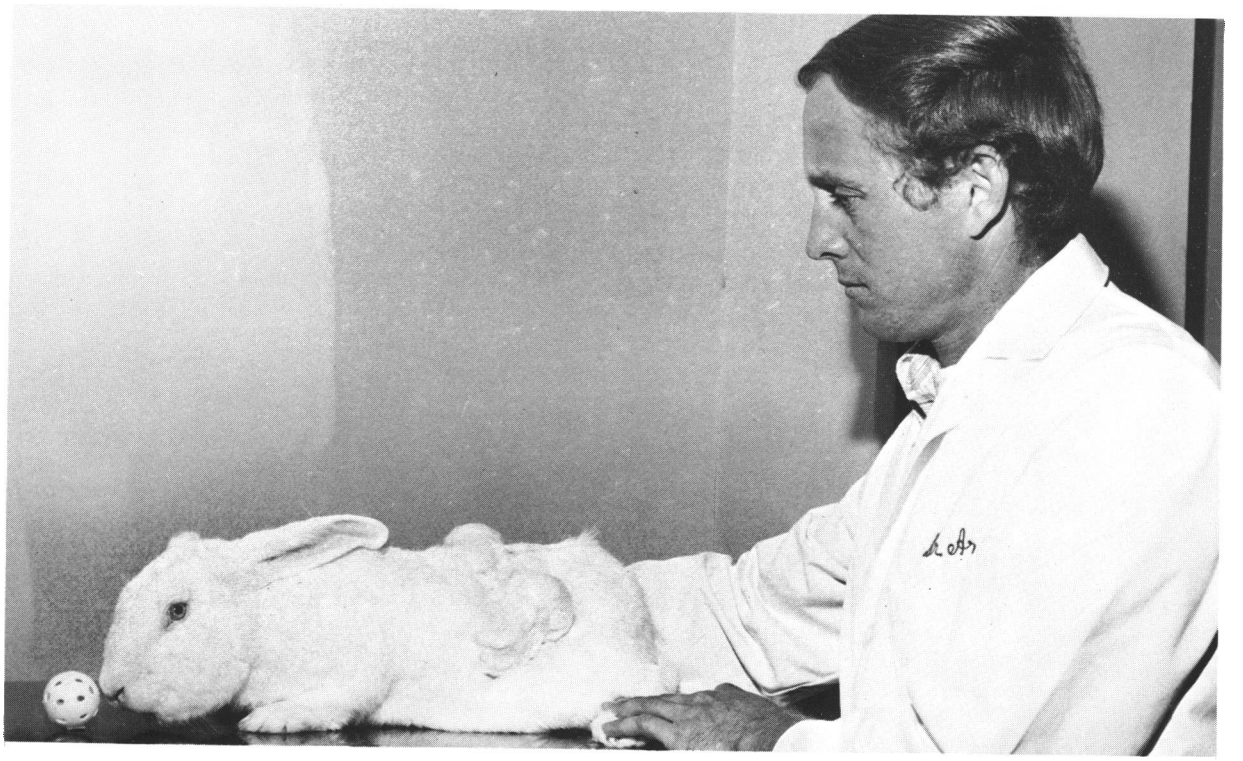
Electron microscopy of gonococcal pili



Neisseria gonorrhoeae, type 2, negatively stained with 2 percent uranyl acetate



Chimpanzee with experimental gonorrhea (right); subcutaneously implanted "golf ball' chambers for in vivo cultivation of N. gonorrhoeae (below)



Separating gonococcal antigens by ultracentrifugation

dure is becoming increasingly important for the evaluation of the small amounts of purified antigens that result from the separation procedures.

Antigen Analysis

Besides their development as potential serologic tests, each procedure is being used to assess the utility of various antigen preparations isolated by other members of the research team. Several types of antigens, including endotoxins, proteins, and polysaccharides, are available in semipurified states and are being examined with panels of serums. Two of these antigens improve the sensitivity and specificity of the serologic procedures over those obtained with the cruder antigen preparations. Two researchers at the Rockefeller Institute and one at CDC recently described pili or fimbrae (hairs) on virulent, but not avirulent, gonococcal cells (*8*). These pili are being analyzed for antigens which might have applicability in the serologic tests. Their association with only the virulent types of gonococcal cells is stimulating an examination of their role in pathogenicity and their possible use in potential vaccines.

To provide larger quantities of all antigenic gonococcal components under optimally controlled conditions, we are growing gonococci in broth fermentor cultures. Under such conditions the proportions of the antigenic components can be varied by the environment of the cultures, within the limits of the genetically controlled metabolism.

Since the production of antibodies to antigens requires first an exposure of the body to the antigens, research is currently in progress on detection of antigens in body fluids. If circulating antigens can be detected in body fluids, then gonorrhea can be detected before an antibody response occurs or in the absence of such a response. Success in this area has been limited by the small amounts of antigenic material in such fluids; however, by the application of new procedures for the concentration of antigens and by sensitive detection techniques, this situation is being improved.

Antigens that are valuable in serologic tests may or may not be efficacious as vaccine constituents; therefore, all antigens separated from gonococci are being examined for a variety of characteristics which might indicate their potential value in vaccines. In addition to collaborating with governmental and academic research activities, we also provide assistance to commercial organizations working on the development of serologic tests for gonorrhea. At least one of these companies has a functional test under evaluation at this time.

Gonorrheal Immunology

The immunology of gonorrhea has received relatively little attention. Since antibodies in serums are the third component of a serologic test along with the procedure and the antigens, venereal disease researchers at CDC have been examining the antibody responses to *N. gonorrhoeae*. In studies on volunteers a few years ago, it was observed that antibody responses to the same gonococcal strain varied considerably from person to person (*9*).

Evidence is emerging from several sources which indicates that the antibody response to a gonorrheal infection is detectable with present methodology 1 to 2 weeks after exposure and initiation of infection. Of equal importance is information that the decline in antibody titer after successful treatment takes place slowly (one doubling dilution per 2 to 3 week period) over a period of weeks or months. Additionally, some serums differ qualitatively in their reactivities in different serologic procedures over and above the quantitatively different levels of sensitivity of the procedures. Thus, information about the types of immunoglobulins reacting with our antigen preparations may be necessary to the development not only of sensitive and specific serologic tests but also to the assessment of potential vaccine materials.

Recently CDC-VD researchers demonstrated for the first time that both females and males produce specific antibodies within their genital tracts (*10*). The appearance of both circulating and secretory antibodies against the gonococcus without evidence of immunity to gonorrhea has raised a question as to the probability of developing a vaccine for the disease.

The development of partial or complete immunity to gonorrhea in previously infected persons would either reduce the frequency of their clinic visits or eliminate them entirely. A study of the reasons for former gonorrhea patients' failure to become reinfected with gonorrhea should determine whether they have indeed developed some immunity or instead have altered their contact pattern, moved, or were using preventive measures. The number of gonorrhea repeaters seen in VD clinics has led researchers to believe that little, if any, immunity to the disease does develop.

What influence strain variation has on gonococcal virulence is

unknown, but it may be a factor in determining the possibility of or extent of immunity. We have substantial evidence of antigenic differences between gonococcal strains and hope to be able to use this information toward understanding strain variation and immunity in gonorrhea.

An additional factor for investigation is the development of an asymptomatic state in 80 percent or more of females (*11*) and upwards of 12 percent of male contacts of females known to be infected (*12*). Such a condition is more indicative of a carrier state than an infection and may require a new approach to the development of a protective response in the host.

Animal Models

Experiments with laboratory animals have produced two significant advancements during the past 2 years that are helping to understand the immunology of gonorrhea. One was the transfer of gonococcal urethritis from man to chimpanzees—the first time this has been achieved in an animal (*13*). Since the infection in chimpanzees is similar to that in man, and chimpanzees can be more closely controlled than human beings, their immunological responses when first infected and when reinfected are being systematically observed. The current problems with documentation of human cases of gonorrhea are circumvented in chimpanzee studies by our extensive knowledge of their history.

The second advancement was the demonstration of transmissible in vivo cultivation of *N. gonorrhoeae* in chambers implanted in small laboratory animals such as rabbits and guinea pigs (*14*). The potential of this work, which was presented at recent meetings of both the American Society of Microbiology (April 23–28, 1972) and American Federation for Clinical Research (April 29 and 30, 1972), is being explored extensively for immunological and other host response information.

Future Needs

When the utility of a serologic test is assessed, well-documented information must be available on the serums; the information should include the duration of the patient's present infection, and the number, type, and recency of past infections. Without adequate documentation on the serums obtained from the population on which the tests are to be used, good tests can give poor results and be discarded. Alternatively poor tests can appear to perform well, and a large amount of the available money, personnel, and time are wasted in eliminating them from consideration.

Closer liaison and expanding collaborative efforts between gonorrhea researchers on both national and international levels will significantly increase the pace of development of serologic tests for gonorrhea.

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