interference of aureomycin with penicillin occurs not only *in vitro* but also in mice inoculated with either *S. pyogenes* or *K. pneumoniae*(7). Mice treated with both penicillin and aureomycin showed a higher death rate than did those treated with penicillin alone.

It is of interest that not only chloramphenicol but all 3 of the "newer" antibiotics that are widely used clinically, interfere with the bactericidal action of penicillin. While these agents have certain features in common there is no evidence that they resemble one another chemically. To date no evidence has appeared suggesting that the interference of these drugs with penicillin action is due to a direct physical or chemical interaction. Other possible mechanisms for this "antibiotic antagonism" have been previously suggested(4,6). It might be that the 2 interfering drugs compete with one another for "receptors" on the bacterial cell. No evidence has been produced directly favoring this possibility. One drug might alter the developmental characteristics of the microorganism in such a way as to diminish their susceptibility to other antimicrobial agents. All instances of antibiotic antagonism demonstrated thus far in this laboratory have dealt with an interference of bacteriostatic drugs with the bactericidal action of penicillin. Most of the data are compatible with the hypothesis that the presence of a bacteriostatic concentration of chloramphenicol, aureomycin, or terramycin may interfere with the multiplication of bacteria. Since penicillin principally affects dividing microorganisms such an interference with multiplication might result in interference with penicillin action. Future experiments will have to explore this hypothesis.

While striking manifestations of antibiotic antagonism have been produced experimentally in mice it cannot be inferred that the phenomenon may be of importance in the antibiotic therapy of human patients. It should be noted, however, that the drug levels showing antibiotic antagonism *in vitro* and in experimental animals fall in the range commonly obtained in human body fluids and tissues with ordinary therapeutic doses.

Summary. Aureomycin and terramycin interfered *in vitro* with the bactericidal action of penicillin against *S. pyogenes* and *K. pneumoniae*. The early bactericidal rate was slower with mixtures of aureomycin or terramycin with penicillin than with penicillin by itself. Ultimately, however, the mixtures were more likely to destroy all exposed bacteria than were the single antibiotics. Interference was most marked when bacteriostatic concentrations of aureomycin or terramycin were mixed with actively bactericidal concentrations of penicillin.

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Received October 13, 1950. P.S.E.B.M., 1950, v75.

A Serologic Study of Chorionic Gonadotrophin. (18263)

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The antigenicity of chorionic gonadotrophin* has been suggested by Leathem(1), Leathem and Rakoff(2) and others. Chase(3) has demonstrated that the serum of rabbits immunized with gonadotrophic hormones reacted with these hormones in a variety of immunological test procedures. CG in urine

* Hereafter designated as CG.

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2. Leathem, J. H. and Rakoff, A. E., *Am. J. Obstet. and Gynec.*, 1948, v56, 521.

3. Chase, J., *Yale J. Biol. and Med.*, 1945, v17, 517.

is readily detected by various biological methods. If this hormone be antigenic it is conceivable that it might be demonstrated serologically as are certain soluble bacterial antigens when present in body fluids or excreta(4-6). In order to investigate this possibility, studies were conducted in which the urine of pregnant and nonpregnant women were tested for CG by both biologic and precipitin tests.

Methods. A total of 17 rabbits, each weighing 2½-3½ kg, were injected with "APL"†. This biologic preparation is a combined extract of human placental tissue and pregnancy urine which contains 1000 International Units (I.U.) or CG per ml. The animals were approximately equally distributed with respect to sex. After a serological base line was established, the rabbits were injected intravenously with 10 I.U. of CG for 3 successive days, followed by 20 I.U. on the fourth day. After 3 days rest the dosage was increased to 25, 50, 100 and 200 I.U. on successive days, respectively. Blood was obtained 5-6 days after the last injection. Following this, the animals were injected again on the first 4 days of alternate weeks with dosages increasing up to 2000 I.U. weekly. They were bled 5-6 days following the last injection of each series. The sera from the bleedings were tested by the precipitin method according to the following procedure: the serum dilutions used were undilute, 1-2, 1-4, 1-8, and 1-16; 0.1 ml of the serum was placed in 3 mm precipitin tubes, and overlayed with "APL" in concentrations of 62.5, 125, 250, 500 and 1000 I.U./ml. The tubes were incubated at 37°C for 1 hour and then read as a ring test.

Antisera which were positive in a dilution of 1-4 or greater when tested with 62.5 I.U./ml were used for the detection of CG in urine from pregnant and nonpregnant women. These urines were simultaneously assayed by standard biological pregnancy tests. The pH and specific gravity of each urine was determined prior to testing. In a preliminary study of the effect of the pH of urine upon the precipitin reaction, it was found that at or below pH 4.0 and above pH 7.0 it is quite possible to obtain false or interfering reactions. Consequently, the pH of the urines used in the precipitin test was adjusted, where necessary, to fall into the range of pH 4.0 to 7.0.

In order to remove from immune rabbit sera any factors which reacted with urines from normal nonpregnant women, the following absorption procedure was conducted. Each serum showing detectable antibodies was tested with a group of urines from pregnant and nonpregnant women. Known nonpregnancy urines which reacted with the CG antisera were used in absorption. These urines were sterilized by filtration and added to the serum in the ratio of 0.2 cc of urine to 5 cc of serum. Then the mixture was incubated at 37°C for 1 hour and stored overnight in the refrigerator. The mixture was centrifuged, the supernatant restored to the original volume and again tested with known pregnancy and nonpregnancy urines. This procedure was continued until the serum no longer reacted with the test group of nonpregnancy urines, but continued to react with the known pregnancy urines.

Results. Seven of the 17 immunized rabbits showed detectable antibodies when tested with "APL." Six of the 7 were females. The sera from these rabbits then were used for the detection of CG in urine by the precipitin method. A total of 285 urines submitted for pregnancy tests were studied serologically. The results of the precipitin tests in a group of 85 urines as compared with those obtained with the Salmon(7) rat test‡ are shown in Table I.

On the basis of the large number of "false

4. Bacterial and Mycotic Infections of Man, edited by R. J. Dubos, p. 392.

5. Alexander, H. E. and Rake, G., *J. Exp. Med.*, 1937, v65, 317.

6. Dochez, A. R. and Avery, O. T., *J. Exp. Med.*, 1917, v26, 477.

† The material for this study was kindly supplied by Ayerst, McKenna and Harrison.

7. Salmon, U. J., Geist, S. H., Salmon, A. A. and Frank, I. L., *J. Clin. Endocrinol.*, 1942, v2, 167

‡ These urines were assayed in the Endocrine Laboratory of the Department of Obstetrics and Gynecology, Duke Hospital, under the direction of Mr. Austin A. Salmon.

TABLE I. Comparison of Urines Tested by Salmon Rat Test and Precipitin Tests.

Precipitin test*	Salmon test: Positive urines	Salmon test: Negative urines	Total No. of urines
Positive	42	12	
Negative	6	25	
Totals	48	37	85

* Anti-CG rabbit serum + patient's urine.

TABLE II. Precipitin Test of Chorionic Gonadotrophin Rabbit Serum with Urines from 15 Normal Nonpregnant Women.

Sera	No. of urines giving positive reaction	No. of urines giving negative reaction	"APL" antigen control
Crude anti-CG #18,5/25	8	7	Positive
Anti-CG absorbed serum #18,5/25	0	15	"
Normal rabbit serum	0	15	Negative

TABLE III. Comparison of Urines Tested by Frog and Precipitin Tests.

Precipitin test	Frog test: Positive urines	Frog test: Negative urines	Total No. of urines
Positive	103	6	
Negative	6	85	
Totals	109	91	200

positives" in this preliminary study, absorbed serum was considered necessary for use. The serum was absorbed as described in the section under "Methods." The result of one such absorption is seen in Table II, where it is shown that although the crude rabbit antiserum reacted with 8 of 15 urines from nonpregnant females, the absorbed serum was significantly more specific. Absorbed serum was then used in the serological testing of an additional 200 urine specimens which had been assayed by the frog test(8)§. The results of this comparison are shown in Table III.

It was desired to determine whether the antibodies which gave a positive reaction with "APL" were the same as those which reacted with the urines from pregnant women. Serum was absorbed with "APL" using the method described above. This absorbed serum no longer reacted with "APL" or with the 10 pregnancy urines tested. Similarly, serum absorbed with pregnancy urine failed to react with the 10 pregnancy urines used or with "APL."

Discussion. The specificity of chorionic gonadotrophin antiserum is of paramount importance if that serum is to be used to detect the presence of the hormone. The first series of 85 urines were tested serologically with unabsorbed antiserum. Positive precipitin tests were obtained with 42 of the 48 urines positive by the rat test; and false positive tests were found with 12 of 37 urines negative by the biologic method. In the next series, 200 urine specimens were tested with absorbed serum and the results were compared with those of the frog test. One hundred and three of the 109 pregnancy urines gave positive precipitin results, while 6 of the 91 urines negative by the frog test reacted serologically, thus constituting 6 false positives. These results indicate that the correlation between the biologic and serologic methods of detecting CG in urine is greater when absorbed antiserum is used. Consequently, before the antiserum is used for test purposes, it is necessary to determine the potency and especially the specificity of the serum. It is not to be implied that the antigen used in preparing this serum was pure. It is conceivable that the antiserum prepared with a more highly purified hormone preparation might give serum of higher potency and greater specificity.

The precipitin test was compared with the Salmon rat test and the frog test. It has been found that the Salmon test as performed at Duke Hospital has an accuracy of 98%. At the present time, it is not possible to state exactly the accuracy of the frog test. For our purposes in this study we have used

8. Cutler, J. N., *J. Lab. and Clin. Med.*, 1949, v34, 554.

§ Veterinary Division, Army Medical Department Research and Graduate School, and by Dr. Cornelius Burns, Department of Pathology, Georgetown University Medical School.

the biologic test as a basis of comparison; clinical histories can be later used to further assess accuracy.

Included in the series of urines studied were specimens from 3 patients with hydatidiform moles, 1 with chorionepithelioma and 3 with testicular tumors. These specimens were tested either by the rat or frog test, and also by the Aschheim-Zondek method. The serological results with these specimens coincided with the biological assays.

Summary. 1. Seventeen rabbits were immunized with "APL," which is a combined extract of human placental tissue and pregnancy urine. Seven rabbits (6 females) produced detectable antibodies. 2. Two hundred eighty-five urines submitted for pregnancy tests were tested for the presence of CG by biological and serological methods. 3. Native rabbit CG antiserum tends to give many cross-reactions. Therefore, it is necessary to absorb the serum and test it for potency and specificity prior to use in testing urines for the presence of increased amounts of CG. 4. It is suggested that it may be possible to use this serologic method for the detection of pregnancy; the development of an antiserum of somewhat greater specificity would be advantageous.

Received September 25, 1950. P.S.E.B.M., 1950, v75.

Relation of the Adrenal to Glycogen Content and Respiration of Lymphoid Organs. (18264)

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The effects produced by adrenal cortical factors and by epinephrine upon the storage, breakdown and utilization of carbohydrate have been described at length(1-5). Although studies have been made of the influence of the adrenal gland upon the quantities of carbohydrate substances, principally glycogen, in the liver, muscle and certain other organs(6-8), no attention has been paid to the part played by this gland in the carbohydrate metabolism of lymphoid tissue. This is surprising in view of recent work which has shown the importance of the lymphoid organs in a variety of defense reactions within the body and which has stressed the influence of adrenal cortical factors upon this function (9-11).

A knowledge of carbohydrate changes within the lymphoid organs, induced by alterations in adrenal hormone levels, might help to elucidate further the role of this endocrine gland in defense mechanisms. This report describes experiments dealing with the chronic effects of adrenalectomy and cortical extract or epinephrine administration upon the glycogen content of lymphoid organs, the liver glycogen and the respiratory activity of these tissues.

Materials and methods. 1. *Animals.* Female rats of a hardy, closely inbred strain, weighing 140-170 g at the time of operation, were used. The 36 adrenalectomized rats employed were divided into three groups, the first of which consisted of 10 rats, to

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10. Dougherty, T. F, and White, A., *J. Lab. and Clin. Med.,* 1947, v32, 584.

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