tial for the appearance of the d.i. in its maximal form which results from derangement of structures in the region of the hypophysis, and that the pars anterior plays its rôle through the thyroid by way of the thyrotropic principle. It also seems justifiable to say that the experiment further establishes d.i. as being due to a deprivation of the antidiuretic principle.[9] Certainly the nature of the experiment and particularly the time that elapsed between operations and extract injections rules out any possibility of the polydipsia being due to irritative phenomena.

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Pathogenic Staphylococci in the Anterior Nares: Their Incidence and Differentiation.*

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I. INCIDENCE. Because there is no recorded incidence of pathogenic staphylococci in the normal nose a study of their frequency was undertaken in order to establish a standard with which the incidence in infectious conditions might be compared. Such a standard will aid in the interpretation of the reports of Dolman,[1] Danbolt,[2] and Valentine,[3] on the rôle of the nares as a source of infection in recurrent furunculosis.

Cultures were made from the nares of 468 persons without evidence of upper respiratory infection or of staphylococcal infection elsewhere. The staphylococci isolated from these cultures were differentiated into pathogenic and non-pathogenic forms by means of the plasma-coagulation test. This test has been found by various investigators to be a reliable method for such differentiation.[4-8]

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9 Fisher, C., and Ingram, W. R., *Endocrinology*, 1936, **20**, 762.

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1 Dolman, C. E., *Lancet*, 1935, **1**, 306.

2 Danbolt, N., *Skrift. Norske Vidensk. Oslo Mat. Natur. Kl.*, 1931, Monograph, 1932; *Biol. Abst.*, 1933, **7**, 1925.

3 Valentine, F. C. O., *Lancet*, 1936, **1**, 526.

4 von Daranyi, J., *Cent. f. Bakt.*, 1926, **99**, 74.

5 Gross, H., *Klin. Wochensh.*, 1933, **12**, 304.

6 Kemkes, B., *Cent. f. Bakt.*, 1928, **109**, 11.

7 Chapman, G. H., Berens, C., Peters, A., and Curcio, L., *J. Bact.*, 1934, **28**, 343.

8 Fisher, A. M., *Bull. Johns Hopkins Hosp.*, 1936, **54**, 393.

Using the results of this cultural study of uninfected persons as a normal standard for comparison, the incidence of pathogenic staphylococci was likewise determined in a group of 51 persons with chronic staphylococcal osteomyelitis, in whom the nares might be considered as a possible focus of infection.

Subjects. The subjects who were cultured, with the exception of a group of 109 adult college students, were patients in the Hospital for Joint Diseases. As previously stated, the group which is considered as uninfected consisted of persons who had no evidence of infection in the nares and who were without evidence of staphylococcal infection elsewhere. The group with chronic osteomyelitis were all proved by culture of the lesion to be infected with staphylococci. If environmental influence is to be considered it should be emphasized that all of the subjects in this series were residents of New York City.

Methods. Swabbings were made from both sides of the nasal cavity and were streaked on plates of beef-extract agar adjusted to pH 7.2. After 24 hours' incubation smears were made of individual colonies and were stained by Gram's method. Staphylococci thus identified were fished and streaked on dextrose-brain-heart-infusion (Difco) agar slants for maintenance of the cultures. The cultures from the children of this series were subcultures from the routine nasal cultures which are taken with throat cultures on all children admitted to the hospital. These were plated on beef-extract agar and were treated as above.

The plasma-coagulation reaction as described by Gross[5] was used as an index of pathogenicity of the staphylococci. To 0.5 cc. of fresh citrated rabbit's blood was added one loopful of a 24-hour agar culture of each strain tested. This was incubated in a water-bath at 37°C. for 3 hours. The reaction was considered positive if a solid plasma-clot was formed within this time. Testing was done on each strain within 4 or 5 days after isolation. The cultural results are shown in Table I.

Comparison of incidence by ages. On comparing the figures shown in Table I it is noted that in all age-groups there is a comparable percentage harboring staphylococci of some kind in the nares. Nevertheless, there is a 20% greater incidence of *pathogenic* staphylococci in the persons under 20 years of age than in those who are older. The age or ages at which this change occurs was not determined because of insufficient data. However, it was found that there is no chronologic decrease (or increase) in the incidence of pathogenic staphylococci in the ages of one through 12 years, but rather a sharp variation around the mean.

TABLE I.
Incidence of Staphylococci in Nares.

Subjects	Total No. cultured	No. and % with staphylococci	No. and % with pathogenic staphylococci	No. and % with non-pathogenic staphylococci	No. and % with no staphylococci
Uninfected		%	%	%	%
Children (2 mo.-12 yr.)	272	201 (73.9)	159 (58.4)	42 (15.4)	71 (26.1)
"Teen" ages (13-19 yr.)	38	30 (78.9)	23 (60.5)	7 (18.4)	8 (21.0)
Adults (over 19 yr.)					
Hospital	49	37 (75.5)	22 (44.9)	15 (30.6)	12 (24.5)
Student	109	87 (79.8)	40 (36.7)	47 (43.1)	22 (20.2)
Total adult	158	123 (78.4)	62 (39.2)	62 (39.2)	34 (21.6)
Staphylococcal Osteomyelitis					
Children	9	9 (100)	4 (44.4)	5 (55.6)	
"Teen" Ages	13	11 (84.6)	7 (53.8)	4 (30.8)	2 (15.4)
Adults	29	22 (75.8)	13 (44.8)	9 (31.0)	7 (24.1)

Incidence in staphylococcal osteomyelitis. In persons under 20 years of age with staphylococcal osteomyelitis there appears to be a tendency to a lower incidence of pathogenic staphylococci in the nares than in uninfected persons of the same ages; whereas the incidence of pathogenic staphylococci in the nares of adults with chronic osteomyelitis tends to be slightly higher than in uninfected persons of the same ages.

Comparison of incidence in children by sexes. There is no difference in the percentage of young boys and girls whose nasal cultures show pathogenic staphylococci, there being 58% of the boys and 56% of the girls having pathogenic forms in the nares. However, in the boys of the "teen" ages there is an incidence of 68% as against 47% in the girls of the same ages.

Effect of hospitalization. In spite of the differences in environment and vigor which existed between the adult subjects of the student and hospital-ward groups, it may be noted that the incidence of all staphylococci as well as the incidence of pathogenic staphylococci are similar in each group.

II. DIFFERENTIATION. Several tests were used to check the results of the coagulase-test of pathogenicity. Sufficient material was gathered to make a comparative evaluation of these tests as means of differentiation. The supplementary tests included chromogenesis, plate-hemolysis, fermentation of mannite, the crystal-violet-agar reaction, and the production of hemotoxin and dermonecrotoxin. The results of these tests, together with their correlation with the plasma-coagulation test, are given below.

The constancy of the coagulase-test. The testing of 233 strains at intervals from one week to one month after isolation showed the reaction to be constant in 94.4% of the strains. The few variable strains more frequently gave a falsely negative than a falsely positive reaction on the first test.

Chromogenesis. Heretofore the differentiation of staphylococci isolated from the nares has been chiefly between the *albus* and *aureus* forms.[9, 10, 11] No further differentiation into pathogenic and non-pathogenic strains was made. In this study the color-chart and arbitrary color-groupings of Winslow and Winlow[12] were used as aids in classifying the colonial color of 215 strains of staphylococci which were isolated from the nares. Of these strains 156 fell into

[9] Bloomfield, A. L., *Bull. Johns Hopkins Hosp.*, 1921, **32**, 290.

[10] Mackey, L., *Brit. Med. J.*, 1919, **2**, 159.

[11] Williams, A., Nevin, M., and Gurley, C. R., *J. Immunol.*, 1921, **32**, 297.

[12] Winslow, C.-E. A., and Winslow, A. R., *The Systematic Relationships of the Coccaceæ*, John Wiley and Sons, 1908.

the "orange" group. The coagulase-test showed 81% of these to be pathogenic. Of the 53 strains which were in the "yellow" group, 17% were pathogens. Except for 6 "white" strains, all of which were non-pathogenic by the coagulase-test, the pathogenic and non-pathogenic staphylococci covered an identical color-range. Because of this and because such a relatively large percent of the "orange" strains are non-pathogenic we feel that too much reliance should not be placed upon chromogenesis as a means of differentiation.

Plate-hemolysis. In order to determine the relationship of the aerobic hemolytic activity of staphylococci on human blood-agar plates to pathogenicity as indicated by the coagulase-test, 480 staphylococci consecutively isolated from the nasal passages were plated on blood agar. There were 439 strains which showed hemolysis. Among these strains 144 (32.8%) gave negative coagulase-reactions indicating them to be non-pathogenic. Of the 41 strains showing no hemolysis, 20 were pathogenic and 21 were non-pathogenic by the coagulase-reaction.

From these results it would seem that plate-hemolysis *per se* is of little significance. We feel that this reaction as a supplementary means of differentiation has been overemphasized. This is contrary to the opinion of Chapman and his coworkers,[7] who include plate-hemolysis among the important supplementary methods for differentiation.

Fermentation of mannite. The opinion of Hine,[13] Dudgeon and Simpson,[14] and others that the fermentation of mannite with acid-production is indicative of pathogenic staphylococci, and not of non-pathogenic staphylococci, was confirmed in this study. There was agreement with the results of the coagulase-test in 90.97% of the 487 strains which were tested for mannite-fermentation. While the majority of these strains fermented mannite in 24 hours many of them were incubated from 2 to 7 days before fermentation occurred.

Crystal-violet reaction. The crystal-violet-agar method of differentiation of Chapman and Berens[15] was used for the testing of 142 strains of staphylococci of this series. There was agreement in the results of the crystal-violet reaction and the coagulase test in 88.03% of the strains tested.

Production of hemotoxin and dermonecrotoxin. One hundred and thirteen strains of staphylococci were tested for their ability to

[13] Hine, T. G. M., *Lancet*, 1922, **2**, 1380.

[14] Dudgeon, L. S., and Simpson, J. W. H., *J. Hyg.*, 1928, **27**, 160.

[15] Chapman, G. H., and Berens, C., *J. Bact.*, 1935, **29**, 437.

produce hemotoxin (*alpha* hemolysin) and dermonecrotoxin. Toxins were prepared by a modification of the technic of Parker, Hopkins, and Gunther.[16] Cultures were grown in semisolid agar in an atmosphere of 30% CO_2. After incubation at 37°C. for 48 hours the cultures were centrifugalized at high speed and the supernatants were tested for hemotoxin and dermonecrotoxin. The hemolysis of a 1% suspension of washed rabbit-erythrocytes after incubation for one hour at 37°C. by the supernatants was taken to indicate the presence of hemotoxin. The production of an area of necrosis in the skin of an albino guinea pig following intradermal injection of 0.2 cc. of the supernatants was taken to indicate the presence of dermonecrotoxin.

There was no correlation between the production of hemotoxin and the hemolysis of human blood-agar plates.

There were 12 strains which showed the production of strong hemolysin but which showed no dermonecrotoxin. These were equally divided between coagulase-positive and coagulase-negative strains. The remaining 101 strains showed good correlation between the production of hemotoxin and dermonecrotoxin. These also checked with the results of the coagulase-test. This confirms the work of Burnet[17] who stated, "although filtrates having no skin-necrosing action may be active hemolysins, no skin-active filtrates lacking hemolysin have been reported."

Summary. (1) Forty percent of persons over 19 years of age who have no evidence of infection in the nares, or of staphylococcal infection elsewhere, harbor pathogenic staphylococci in the nares. There is a tendency to a slightly higher incidence in persons of the same ages with staphylococcal osteomyelitis. (2) Sixty percent of persons under 20 years of age who have no evidence of infection in the nares, or of staphylococcal infection elsewhere, harbor pathogenic staphylococci in the nares. There is a tendency to a slightly lower incidence in persons of the same ages with staphylococcal osteomyelitis. (3) While the plasma-coagulation reaction was the preferred method of differentiating between pathogenic and non-pathogenic strains of staphylococci, the fermentation of mannite, the crystal-violet reaction and the production of dermonecrotoxin are also reliable confirmatory methods for such differentiation.†

16 Parker, J. T., Hopkins, J. G., and Gunther, A., PROC. SOC. EXP. BIOL. AND MED., 1926, **23**, 344.

17 Burnet, F. M., *J. Path. and Bact.*, 1929, **32**, 717.

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