tencies did not correlate with the narcotic antagonist potencies. In view of the fact that the narcotic antagonist analgesics are inactive on the usual analgesic tests in animals, it appears that the writhing test may be a valuable aid in attempting to predict the analgesic activity of narcotic antagonists in man.

1. Lasagna, L., Beecher, H. K., J. Pharmacol. Exp. Therap., 1954, v112, 356.
2. Isbell, H., Fed. Proc., 1956, v15, 442.
3. Archer, S., Albertson, N. F., Harris, L. S., Pierson, A. K., Bird, J. G., Keats, A. S., Telford, J., Papadopoulos, C. N., Science, 1962, v137, 541.
4. Harris, L. S., Pierson, A. K., J. Pharmacol. Exp. Therap., 1964, v143, 141.
5. Siegmund, E., Cadmus, R., Lu, G., Proc. Soc. Exp. Biol. and Med., 1957, v95, 729.
6. Bentley, K. W., Endeavor, 1964, v23, 97.
7. Gates, M., Montzka, T. A., J. Med. Chem., 1964, v7, 127.
8. Hendershot, L. C., Forsaith, J., J. Pharmacol. Exp. Therap., 1959, v125, 237.
9. Litchfield, J. T., Wilcoxon, F., ibid., 1949, v96, 99.
10. Lasagna, L., Pharmacol. Rev., 1964, v16, 47.
11. Keats, A. S., Telford, J., J. Pharmacol. Exp. Therap., 1964, v143, 157.
12. Lasagna, L., DeKornfeld, T. J., Pearson, J. W., ibid., 1964, v144, 12.
13. Foldes, F. F., Med. Clin. North Am., 1964, v48, 421.
14. Blumberg, H., Dayton, H. B., George, M., Rapaport, D. N., Fed. Proc., 1961, v20, 311.
15. Archer, S., Albertson, N. F., Harris, L. S., Pierson, A. K., Bird, J. G., J. Med. Chem., 1964, v7, 123.
16. Eddy, N. B., Lee, L. E., Jr., J. Pharmacol. Exp. Therap., 1959, v125, 116.

Received September 2, 1964. P.S.E.B.M., 1965, v118.

Experimental Caries Induced in Animals by Streptococci of Human Origin.* (29964)

DORAN D. ZINNER, JAMES M. JABLON, ANA P. ARAN AND MILTON S. SASLAW
(Introduced by M. Michael Sigel)
Department of Medical Research, National Children's Cardiac Hospital, Miami, Fla.

Traditionally, lactobacilli have been considered the principal cause of caries, but recent findings have questioned this concept. Orland(1) succeeded in producing experimental caries by infecting gnotobiotic rats with a strain of streptococcus. More recently, Fitzgerald *et al*(2) confirmed these findings, and Fitzgerald and Keyes(3) established the cariogenicity of another strain of streptococcus in hamsters. These 2 strains of streptococci were shown to be host-specific with regard to cariogenicity: the hamster strain (designated HS_1) produced caries only in the hamster and not in the rat, and the rat strain (designated FA_1) produced caries in the rat and not in the hamster. Fitzgerald and Keyes also demonstrated that experimental dental caries in animals is an infectious and contagious pathological state which could be transmitted from mother to young.

* Aided by research grant DE 01519, Nat. Inst. Dental Res., U.S.P.H.S.

Studies of cultures from human caries have brought out evidence for the presence of streptococci similar to the HS_1 and FA_1 strains. Preliminary findings indicate that the human streptococci similar to the HS_1 strain may be causally associated with human caries. Studies related to human streptococci similar to FA_1 strains are being investigated.

Materials and methods. Collection of specimens from human carious lesions. A sterile spoon excavator was used to remove carious material from the depths of lesions in both enamel and dentine from human teeth *in situ*. This material was inoculated in Todd-Hewitt broth, supplemented with 0.5% lactalbumin hydrolysate and incubated at 37°C for 18-24 hours.

Production of antiserum. Rabbits were immunized with a vaccine prepared from heat-killed streptococcal cultures (60°C for 1 hr). The bacterial suspensions were washed twice in saline and adjusted to the density of the

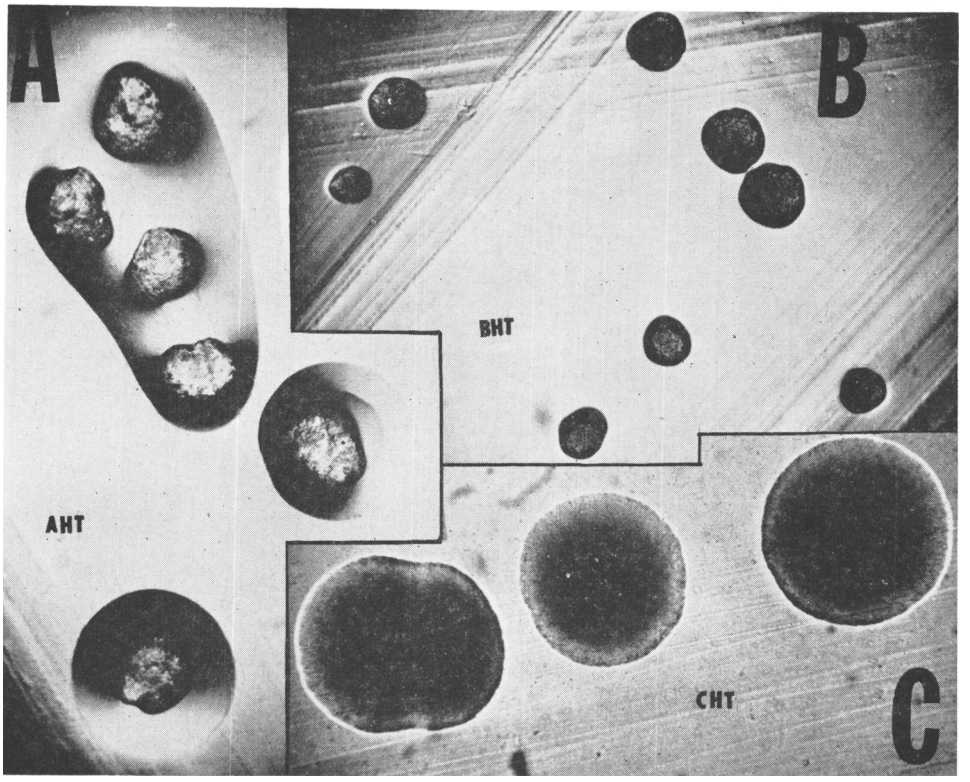

FIG. 1. Colonial morphology of streptococci isolated from human carious lesions. AHT, BHT, and CHT represent the 3 different strains isolated.

#4 tube of the McFarland nephelometer series. The rabbits were injected intravenously on alternate days for 4 weeks, 0.5 ml each injection during the first week and 1.0 ml per injection during the remaining 3 weeks. After one week of rest, trial bleedings showed that the antisera were specific for any one of the strains used, HS_1, FA_1 or any one of the 3 human strains (designated AHT, BHT, or CHT).

Preparation of fluorescein-tagged antiserum. The gamma globulin fraction of each of the prepared antisera was precipitated with ammonium sulfate at one-half saturation and tagged with fluorescein-isothiocynate according to the method outlined by Cherry *et al*(4).

Micro-precipitin tests. Acid-extracts of the streptococcal cultures were prepared by the Lancefield method(5) and tested with appropriate antisera by the micro-precipitin technique(6).

Antigenic analysis. The identity of the somatic antigens of the cultures was determined by the agar-gel diffusion technique of Ouchterlony(7).

Identification of streptococci in human carious material by means of fluorescent microscopy. Smears of carious material were stained with fluorescein-tagged specific gamma globulin and examined by fluorescence microscopy. Carious material was examined by direct smear as well as after 3 and 24 hours' incubation(8). A Leitz Ortholux microscope equipped with a darkfield condenser and BG 12 and OG 1 filters was used. The light source was an Osram HBO 200 mercury vapor lamp.

Isolation of streptococci from human carious material. Material from 71 carious lesions or plaques of 48 individuals was cultured in Todd-Hewitt broth. Samples of specimens which fluoresced with HS_1, FA_1 or both antisera were streaked on mitis salivarius agar supplemented with 0.1% tellurite. Colony morphology was studied.

Additional studies. The fermentation re-

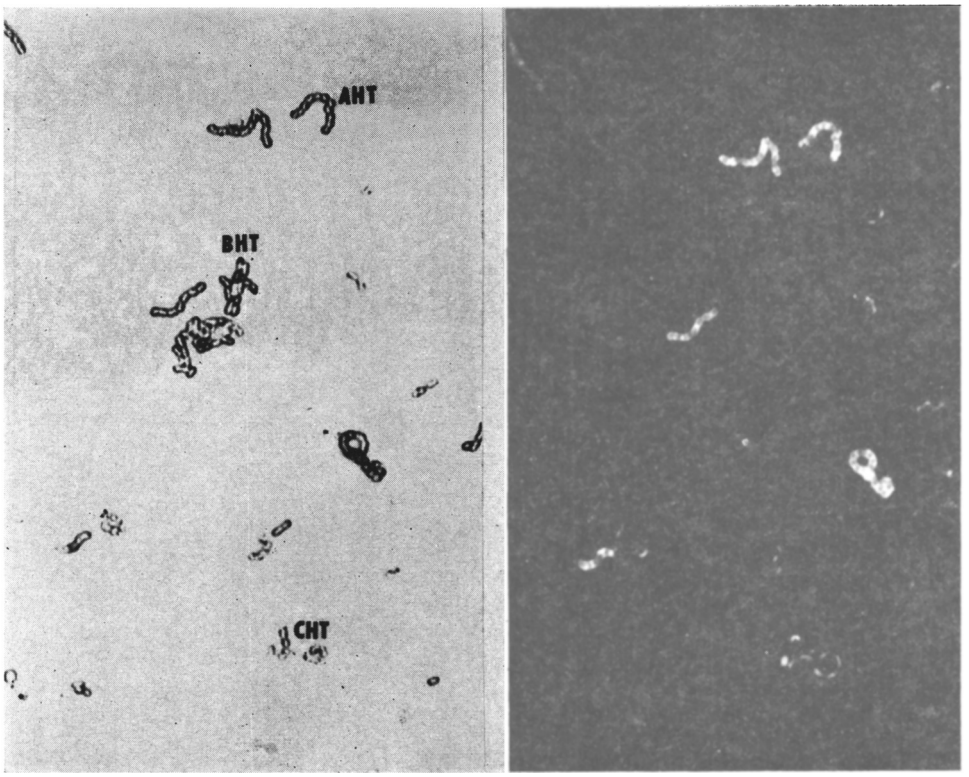

FIG. 2. Comparison of darkfield microscopy with fluorescent microscopy of identical fields, each containing streptococci belonging to AHT, BHT, and CHT strains. Left side of photograph is the dark field, right side is the fluorescent. Note that BHT strains fail to fluoresce.

actions of human and animal strains on 10 different sugars were observed. Growth curves and pH determinations were recorded.

Cariogenic activity of the human strains of streptococci in the hamster. Nineteen-day-old 30 g Syrian hamsters were infected orally with the various strains of streptococci isolated from human caries. The method of infection was that described by Keyes(9). A week later the animals were reinfected with the same organisms.

Results. Fluorescence of streptococci from human caries was observed after 24 hours' incubation followed by staining with fluorescein-tagged specific gamma globulin. Shorter incubation time, 3 hours, yielded only a few cells which showed fluorescence and direct smears failed to reveal the presence of fluorescent cells with either HS_1 or FA_1 globulins. These streptococci constitute only a small number of all the organisms seen in darkfield examination of the above culture.

Cultures from 32 of the 48 individuals studied, showed fluorescence with the HS_1 and FA_1 or both antisera. Of the remaining 16 subjects, 3 manifested superficial caries, 2 deep, and 11 no caries at all.

Colonial morphology, as observed on the mitis salivarius agar, was of 3 distinct types, designated AHT, BHT, and CHT (Fig. 1). Colonies of AHT are indistinguishable from the hamster cariogenic strain of HS_1. They are raised and dark with irregular margins and present a surface appearance resembling chips or crumbs. In addition, there is a gelatin-like emanation from the periphery of the colony. BHT colonies resemble Fitzgerald's rat strain FA_1. These colonies are small, raised, with circular margins and dark rough centers with no gelatinous emanations.

A third type of colony of streptococci which does not fit the morphology of either HS_1 or FA_1 is designated as CHT. These colonies are large, round, flat, gray, and smooth, and have no gelatinous emanation.

The fermentation reactions on 10 different

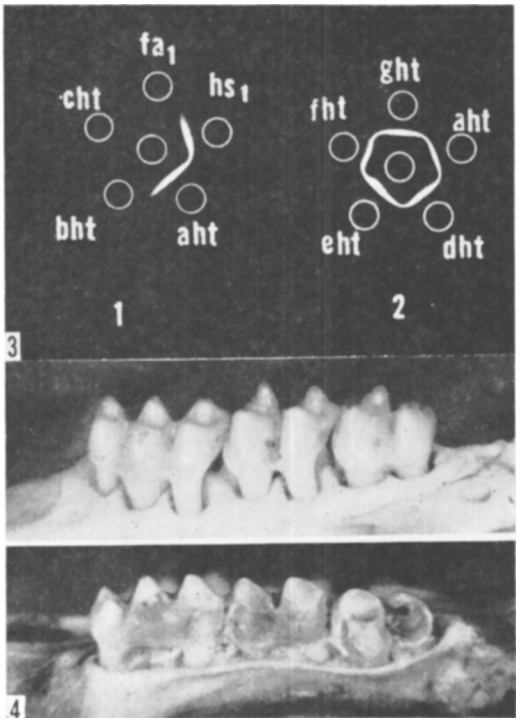

FIG. 3. Results of Ouchterlony gel diffusion studies with cariogenic streptococci. Center well in each instance contains antiserum prepared against the AHT strain.

FIG. 4. Maxilla of the normal uninfected hamster (above) and of the infected hamster (below).

sugars indicate that all 3 types human strains gave the same reactions as both animal strains. They all fermented dextrose, maltose, lactose, sucrose, mannose, raffinose and galactose; and failed to ferment arabinose, rhamnose, and xylose. Growth curves and pH studies in varying dilutions of dextrose disclosed the terminal pH of 4.7.

Fig. 2 compares darkfield microscopy with fluorescent microscopy in the recognition of 3 kinds of reactions. Fig. 2A, under darkfield microscopy, contains all 3 types of human streptococci; Fig. 2B, the same field under fluorescence microscopy stained with HS_1 antiserum, differentiates the 3 types.

In addition to morphologic similarity between human and animal strains, antigenic determinations employing fluorescent microscopy revealed close relationships between AHT and HS_1, and between BHT and FA_1. The relationship of the CHT organisms is not as definite; the reaction with fluorescein-conjugated HS, FA, or both antisera results in an aberrant stain in which the fluorescence is limited to one side of the organisms leaving much of the cell wall unstained. Preliminary indications of serologic similarity between the human AHT and hamster HS_1 as well as the human BHT and rat FA_1 were obtained from the tube precipitation reaction and the Ouchterlony agar-gell diffusion procedures. The gel diffusion results are shown in Fig. 3. In the pattern on the left the antiserum of the human isolate, AHT, was placed in the center well. Acid extracts of the streptococcal strains indicated were placed in the peripheral wells. The only reactions were against AHT and HS_1 extracts. In the pattern on the right, the same antiserum, AHT, was placed in the center well. In the peripheral wells were placed acid extracts of 5 other isolates from carious lesions of 5 different people. These extracts reacted identically with AHT.

Hamsters infected with human streptococcal strains of the AHT type (20 strains of AHT, 20 of DHT, 4 of EHT and 11 FHT, total 55 strains) developed rampant carious lesions within 65 days. No caries were produced in the hamsters by the human BHT or CHT strains. Fig. 4 illustrates caries of the hamster teeth. Uninfected control Syrian hamsters maintained on Keyes cariogenic diet did not develop caries. The hamster HS_1 streptococci caused caries in the Syrian hamster whereas the rat FA_1 did not.

Discussion. These data confirm the observations of Fitzgerald and Keyes that streptococcus HS_1 can cause caries in the hamster. In addition they provide the first evidence that similar streptococci are present in association with human caries and that these streptococci can reproduce this disease in hamsters. Isolation of these organisms was facilitated by use of the fluorescent antibody technique using specific, fluorescein-tagged antiserum. Other streptococci, including BHT, similar to the rat cariogenic strain, did not produce caries in hamsters. This BHT strain is being studied for cariogenicity in germfree rats. Several strains of lactobacilli, 3 from human mouths and 2 from the mouths of orangutans, also were tested for cariogenic activity in the hamster and the results proved negative. These findings provide a tentative hypothesis that the strain of streptococci,

designated AHT, may be related etiologically to human caries. Thus far no attempt has been made to reproduce caries in the human mouth using this agent.

Eleven caries-free human mouths have been cultured and in no instances were streptococci similar to the AHT, BHT, or CHT observed.

Summary. Streptococci were isolated from human dental carious lesions which reacted with fluorescein-tagged antisera against rat or hamster "cariogenic" streptococci. The human strains fell into 3 different categories, in fluorescence, morphology, microprecipitin and gel diffusion studies. One category, similar to the hamster strain, produced caries when used to infect hamsters. This occurred in 55 animals of 55 tested, and in no controls. The other 2 categories of human isolates failed to produce hamster caries; one of these is being studied for possible cariogenicity in germfree rats.

1. Orland, F. J., Blayney, J. R., Harrison, R. W., Reyniers, J. A., Trexlar, P. C., Ervin, R. F., Gordon, H. A., Wagner, M., J. Am. Dent. Assn., 1955, v50, 259.

2. Fitzgerald, R. J., Jordon, H. V., Stanley, H. R., J. Dent. Res., 1960, v39, 923.

3. Fitzgerald, R. J., Keyes, P. H., J. Am. Dent. Assn., 1960, v61, 9.

4. Cherry, W. B., Goldman, M., Carski, T. R., Moody, M. D., Public Health Service Pub. 729, Wash., D. C., U. S. Govt. Printing Office, 1960, p48.

5. Lancefield, R. C., J. Exp. Med., 1928, v47, 91.

6. Swift, H. F., Wilson, A. T., Lancefield, R. C., ibid., 1943, v78, 127.

7. Ouchterlony, O., Acta Path. Microbiol. Scand., 1949, v26, 507.

8. Zinner, D. D., Jablon, J. M., Haddox, C. H., Jr., Aran, A., Saslaw, M. S., J. Dent. Res., accepted for publication.

9. Keyes, P. H., ibid., 1944, v23, 439; 1959, v38, 525.

Received September 2, 1964. P.S.E.B.M., 1965, v118.

Growth Stimulating Effects of Acid Mucopolysaccharides.* (29965)

L. M. MORRISON, K. MURATA, J. J. QUILLIGAN, JR., O. A. SCHJEIDE AND L. FREEMAN

Loma Linda University School of Medicine, Los Angeles County General Hospital, Laboratory of Nuclear Medicine and Radiation Biology, Department of Biophysics and Nuclear Medicine, School of Medicine, University of California, Los Angeles

A preliminary report from this laboratory (1) has shown that certain acid mucopolysaccharides (AMPS) and calf aorta extracts (containing a mixture of AMPS) have growth stimulating effects and lipid reducing activity by biochemical determinations in lipid saturated chick aorta cells and HeLa cells. However, the reduction of cellular lipid was accompanied by cell growth as stimulated by AMPS or crude calf aorta extracts rich in AMPS(1). Other investigators employing histochemical techniques(2,3) have also described the acid mucopolysaccharide heparin as having a lipid-clearing effect upon lipid-loaded aortic cells.

This communication describes the effects of certain AMPS, calf aorta extracts, and phospholipid on HeLa cell growth. Studies of large volumes of HeLa cells have made it possible to relate cytocrits, cell counts, cell volumes, and dry weights on a quantitative basis.

Materials and methods. The HeLa cells were diluted 1:1000 v/v in Eagle's Minimum Essential Medium with 5% calf serum, planted 50 ml in 1-liter and 100 ml in 2-liter bottles, and grown for a total of 13 days. After initial growth of the cell cultures for 7 days, human lipemic serum (HLS) was added in 10% concentrations and incubated for an additional 3-day period. The medium was then replaced with fresh Eagle's Minimum Essential Medium containing the substances to be tested, and this was renewed daily for 3 days. The following test substances were employed(1): calf aorta extracts (Extract #1

* Studies supported by grants from John A. Hartford Foundation, Nat. Inst. Health, and Crenshaw Research Foundation.