

tive. Acid production was high, varying from pH 4.5 to 4.8. Five of 11 strains killed mice injected intraperitoneally in doses of 0.5 cc. of broth culture.

Conclusions. (1) Hemolytic streptococci are constantly present in the tonsils of apparently normal dogs (92.3%). They occur in predominant numbers in acute tonsillitis of dogs and are secondary invaders in the bronchopneumonia of distemper. (2) Experimentally acute tonsillitis is produced by swabbing freshly isolated canine strains on the surface of the tonsils of dogs. A human strain (*S. epidemicus*) failed to produce a reaction. (3) The streptococci from dogs conform to the animal type and differ from the human type.

8676 P

Combined Staphylococcal Endo- and Exo-Antigen.

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The pathogenic activity of microorganisms may be due to toxic substances of either cellular or extracellular nature or both. These substances are usually designated endotoxin and exotoxin, although it is likely that no sharp division line exists.

An ideal antigen for active immunization in the case of invasive microorganisms, which produce both endo- and exotoxin, should contain both endo- and exotoxic principles. Likewise, an ideal antibody-solution when administered therapeutically should be able to neutralize both the endo- and exotoxin of the homologous organism.

Staphylococcus pyogenes produces a soluble exotoxin¹ which is readily converted into formol-toxoid, and is used for active immunization of laboratory animals and human beings. The staphylococcus contains endotoxin,² and vaccines have been used for a long time in the prophylaxis and treatment of localized staphylococcal infections. Bacteriophage-solution is also used therapeutically, both on account of its lytic activity towards the organisms and be-

¹Burnet, F. M., *J. Path. and Bact.*, 1929, **32**, 714; 1931, **34**, 471; Leonard, G. F., and Holm, A., *J. Immunol.*, 1935, **29**, 209.

²von Lingelsheim, *Aetiologie u. Therapie d. Staphylokokken Infektion*, Berlin-Wien, 1900; Borrisow, P., *Beitr. z. path. Anat. u. z. Allgem. Path.*, 1894, **16**, 432.

³Larkum, N. W., *Am. J. Pub. Health*, 1933, **23**, 1155; *Proc. Soc. Exp. Biol. and Med.*, 1933, **30**, 1395.

cause bacteriophagic solution of the lysed staphylococcus is antigenic.³ The toxoid, the vaccine, and the bacteriophagic solution may be considered as representing different antigenic groups.

If an antigen embodied all these antigenic principles, the immunity would presumably be more effective than specific antitoxic immunity or a strict antibacterial immunity, and the administration of an antitoxin which in addition contained antibacterial antibodies would better match the toxin-producing and invading characteristics of *S. pyogenes*. Prompted by these considerations we prepared a multivalent staphylococcal toxoid which contains endotoxic as well as exotoxic principles of staphylococci.

Lysis by bacteriophage was preferred to other methods in order to avoid denaturation and because the antigenic activity of bacteriophage solution is well established.³

A detailed description of the method of preparation will be published later but the general outline is as follows: Inoculation of a Berkefelded staphylococcal toxin with pooled cultures of the different strains used in the preparation of the toxin; addition of an adapted bacteriophage; addition of formalin and merthiolate after clearing of the culture by the bacteriophage; incubation for conversion of the toxins into toxoid; Berkefeld-filtration and testing for innocuity and antigenicity in the usual manner.

In 3 experiments on 54 rabbits, we compared the antigenic potency of the staphylococcal bacteriophage-toxoid with a toxoid derived from the same staphylococcal toxin, and with a fresh bacteriophagic solution prepared in the same medium and with the same strains of staphylococci that had been used for the bacteriophage-toxin.

When the data for the 3 experiments were combined, it was found that the mean antitoxin-titers after inoculation with the 3 antigens were: Bacteriophage-toxoid, 7.3 units; staphylococcal toxoid, 4.3 units; and bacteriophage-solution, 0.56 units.

It has been claimed that resistance in bacteremias caused by the staphylococcus depends upon the titer of serum-antitoxin, but other factors may also be of importance as indicated by the results of the intravenous injections of 5 billion live virulent staphylococci into 2 groups of the immunized animals.

Of 15 rabbits which were injected with living organisms after immunization with staphylococcal toxoid, none survived more than 9 days, while 11 died within 48 hours. Of 14 rabbits immunized with bacteriophage-toxoid, 8, or 55%, survived the later injection of staphylococci. Finally, of 12 rabbits inoculated with

bacteriophage-solution, 3 or 25%, withstood the injection of live staphylococci. Horses have also been immunized with staphylococcal bacteriophage-toxoid for the production of a combined antiserum.

The procedure outlined may with minor changes be utilized with other microorganisms for the preparation of bacteriophage-toxoid, especially with those organisms that yield endo- as well as exotoxin and which may be lysed by bacteriophage. Thus it should be possible to obtain more effective antigens both for active immunization of human beings and for the production of more potent antibodies in the horse or in other animals.

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Determination of Ethyl Alcohol in Saliva.

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The concentration of alcohol in the blood and cerebrospinal fluid of man has for many years been accepted as an index of the state of his intoxication. There is often, however, difficulty in obtaining blood or cerebrospinal fluid from individuals under the influence of alcohol; and it is therefore more advisable to use, when possible, a more accessible and equally trustworthy index.

Friedmann and Brook¹ have recently shown that saliva also contains ethyl alcohol. We have performed a series of determinations on this fluid to see whether or not the concentration of EtOH found in it has some simple relationship to that found in a corresponding blood sample. The methods (macro and micro) used in this study have been described;² we should like to point out that in the following cases the results of the two methods agreed within $\pm 3\%$.

In 30 cases of normal individuals the saliva EtOH concentration was 0 mg. %, while the blood alcohol values of this group were from 0 to 7 mg. %.

Similar determinations in 50 cases of individuals in various states of intoxication showed satisfactory correlation of the EtOH concentrations in the saliva specimens with those in the corresponding

¹ Friedmann, T., and Brook, T., *Proc. Am. Soc. Biol. Chem.*, 1936, **30**, xxxvii; *J. Biol. Chem.*, 114.

² Abels, J. C., *Proc. Soc. Exp. Biol. and Med.*, 1936, **34**, 4.