in poultry houses, it was decided that a determination of the manner of absorption and persistence of the benzene hexachloride flavor should be made. To this end the following test was set up. Four chicken houses were selected. In one house, 5 birds were fed for 1 week with a mixture of 1 part of benzene hexachloride containing 6 per cent of the γ isomer in 200 parts of a mash-grain feed, and then transferred to the standard ration received by the other lots. In the second house, 5 birds were sprayed until wet with a benzene hexachloride suspension containing 0.25 per cent of the γ isomer. In the third, the walls were sprayed until wet with a benzene hexachloride suspension containing 0.25 per cent of the γ isomer and allowed to dry before the birds were placed inside. In the fourth house, which was isolated from the treated lots, 5 untreated birds were retained as controls. Sprays were applied with a 3-gallon compressed-air sprayer. The birds used were 2-pound White Leghorn cockerels.

One of the birds in the lot receiving the treated feed died at the end of the first week. The bird apparently died of starvation, since the entire lot ate less than a pound of the treated feed in the 7-day period. One of the birds in the sprayed lot also died. These deaths reduced the samples from the 5 originally planned to 4. The remaining birds remained healthy until sampled and killed. The test was begun on June 13, 1947, and the final sample was taken on August 22, 1947.

TABLE 1

COMPARATIVE ABSORPTION AND RETENTION OF BENZENE HEXACHLORIDE ODOR AND TASTE BY CHICKENS

Treatments		Comparative rating of odor and taste			
		2 weeks	4 weeks	6 weeks	10 weeks
Feed treated	Odor	Slight	Moderate	Strong	Strong
	Taste	Slight	Moderate	Strong	Strong
Birds sprayed	Odor	Moderate	Strong	Strong	Moderate
	Taste	Strong	Strong	Strong	Slight
House sprayed	Odor	Strong	Slight	Slight	Slight
	Taste	Moderate	Slight	Slight	Moderate
Control	Odor	Not checked	None	Slight	None
	Taste	Not checked	None	None	None

The first 3 samples were taken at intervals of 2 weeks and the last after a 4-week interval. At sampling time 1 bird from each of the lots was killed and cooled. The following day the birds were roasted in separate ovens at uniform temperatures, no salt or other condiment being used. Five tasters or samplers then checked each bird, which was identified only by a code letter. Checks were made by smelling each of the partially carved carcasses and then tasting small pieces of white and dark meat cut from each bird. Each sampler recorded his own reaction to the quality of the particular meat.

The terms used to describe odor were very strong, strong, sharp, bad, slight, questionable and good; those used to describe taste were very strong, strong, acidic, musty, medicinal, biting, objectional, and good. The terms were modified into those used in Table 1, which gives the results of the test. Statements concerning the presence and comparative degree of an off-flavor or odor are included in the table only when the reactions of 3 or more of the samplers were the same.

In Vitro Resistance of the Genus *Bacteroides* to Streptomycin

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There is little reference to the genus *Bacteroides* in the growing literature on the *in vitro* sensitivity of various gram-negative bacteria to streptomycin. Since these microorganisms, normally saprophytic inhabitants of the nasopharynx (*16*), gastrointestinal tract (*11*), and the female genitalia (*13*), frequently invade the deeper tissues of the body to produce serious and often fatal disease in man (*19, 20*), it seemed worth while to determine their sensitivity to streptomycin.

The obligate anaerobic, nonsporulating gram-negative bacilli comprising the genus *Bacteroides* are a heterologous group closely related to the genera *Dialister* and *Fusobacterium*. Bergey (*1*) lists 22 species of this genus, but since these have not yet been studied systematically, it is impossible to determine how many of them actually represent distinct species. Weiss and Rettger (*21*), for example, were able to classify 73 different strains into 4 serologic groups. Henthorne, Thompson, and Beaver (*14*) have reported that pleomorphic strains are serologically distinct from the nonpleomorphic strains. Therefore, following the convention adopted by Smith and Ropes (*20*), the strains reported in this study were classified as *B. funduliformis* if pleomorphic and *B. fragilis* if nonpleomorphic. Pleomorphism was determined by the formation of large bodies and filaments in artificial media and by a form of reproduction different than simple binary fission, associated with the production of anaerobic pleuropneumonia-like colonies (L-variation), as described by Dienes (*6*) and Dienes and Smith (*7–9*).

Four strains of *B. funduliformis* and 8 of *B. fragilis* have been encountered in this laboratory during the past year. These were isolated in primary culture on anaerobic horse blood or ascitic fluid agar plates, or in sodium thioglycollate broth. Subcultures were carried in chopped meat or sodium thioglycollate enriched with 30 per cent ascitic fluid and sealed with vaseline. All strains produced varying amounts of gas in these media and were characterized by a sharp, acrid odor. Strains were examined for pleomorphism and the production of anaerobic pleuropneumonia-like colonies by the cultivation and in situ agar block staining methods previously described by Dienes (*5*).

Streptomycin sensitivity was determined in unsealed 0.1 per cent sodium thioglycollate broth containing 0.1 per cent agar-agar, according to a titration based on the methods described by Price, Nielsen, and Welch (*17*). Streptomycin sulfate was diluted so that 4 times the desired doses were contained in 0.5-ml. volumes of broth. To these 0.5-ml. volumes was added 1.5 ml. of a 1:100 dilution of an 18- to 24-hour culture of the strain to be tested, giving a titration with final concentrations of streptomycin ranging from 4 0 mg. to 2.0 μg./ml. in a total volume of 2.0 ml. At the same time, each strain was tested for its ability to grow in the presence of 100 Oxford units of penicillin/ml. in the same medium. All titrations were examined after 48 hours incubation at 37°C. The first tube in which growth was inhibited was taken as the minimal inhibiting dose.

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All strains grew in the presence of 100 Oxford units of penicillin/ml. As may be seen in Table 1, *B. funduliformis* seemed to be somewhat more sensitive to streptomycin than did *B. fragilis*, although in either case the minimal doses of streptomycin required for inhibition were well beyond those usually obtainable *in vivo*.

TABLE I

In vitro RESISTANCE OF *Bacteroides* TO STREPTOMYCIN

Species	Strain No.	Source of culture	Streptomycin resistance	
			Inhibited by μg./ml.	Minimal inhibiting dose corrected for suppressive effect of thioglycollate and anaerobiosis
B. funduliformis	1	Subphrenic abscess	1,500	750
	2	Liver "	1,500	750
	3	Brain "	3,000	1,500
	4	Brain " , chronic otitis media	1,500	750
B. fragilis	5	Multiple buttocks, ischiorectal abscess	3,500	1,750
	6	Breast abscess	2,500	1,250
	7	Brain " , chronic otitis media	3,000	1,500
	8	Brain abscess, chronic otitis media	2,500	1,250
	9	Infected rectal tumor	3,000	1,500
	10	Stool	3,500	1,750
	11	"	2,500	1,250
	12	"	4,000	2,000

It has been reported that the presence of reducing substances in the medium interferes with the inhibiting action of streptomycin (*2, 4, 10, 12*). Opinion differs, however, on the inhibiting effect of sodium thioglycollate. Geiger, Green, and Waksman (*12*) found that thioglycollate agar did not inhibit streptomycin, while Donovick and Rake (*10*) reported that thioglycollate broth increased the minimal concentration of streptomycin necessary for the inhibition of *Klebsiella pneumoniae*. If streptomycin reacts with the sulfhydryl compounds containing basic amino groups in the vicinity of the —SH, as has been suggested by Cavallito (*3*), its inactivation could be brought about by the active —SH groups contained in sodium thioglycollate. However, the observations of Geiger, Green, and Waksman (*12*) with facultative anaerobes suggest that simple anaerobiosis, with the consequent accumulation of acid resulting from bacterial metabolism, may suppress the inhibiting action of streptomycin by as much as 50 per cent. However, as suggested by these same authors, as well as by Bondi and Dietz (*2*), the resistance exhibited to streptomycin by anaerobic microorganisms may be the result of differences in the metabolism of the anaerobes—streptomycin may more effectively block an enzyme system essential to the growth of aerobic bacteria.

In order to determine the effect of the presence of sodium thioglycollate on the *in vitro* streptomycin resistance of *Bacteroides*, representative strains of *B. funduliformis* and *B. fragilis* were tested for sensitivity to streptomycin in tryptic digest broth containing 0.1 per cent dextrose. The medium was boiled in order to remove dissolved oxygen and streptomycin sulfate titrated as previously described. Each tube of the titration was then sealed with a thick layer of vaseline and incubated at 37°C. for 48 hours. There was no significant difference between the results obtained by this method and those obtained in sodium thioglycollate with the same strains of *Bacteroides*. It might be mentioned also that in connection with another study (*15*), successive urine cultures collected from patients receiving streptomycin therapy were cultivated in sodium thioglycollate broth as well as on the usual aerobic media. In no instance was growth obtained in sodium thioglycollate, which theoretically should have inactivated the streptomycin present in the specimen, when growth was not obtained on aerobic media as well.

With the strains reported here, even if the observed minimal inhibiting dose is reduced by 50 per cent to allow for the possible suppressive effects of sodium thioglycollate and anaerobiosis, the concentrations of streptomycin necessary to inhibit the growth of *Bacteroides* still place them in the category of highly streptomycin-resistant microorganisms. The 3 strains of *B. fragilis* isolated from stools (Table 1) were recovered from patients receiving oral streptomycin. These apparently survived exposure to the very high concentrations of streptomycin obtained in the feces following oral therapy (*18*).

In view of the resistance of these microorganisms to streptomycin, together with the possible beneficial effect of sulfadiazine in one of the cases reported by Smith and Ropes (*20*), 2 strains of *B. funduliformis* and 5 of *B. fragilis* were examined for sensitivity to sulfathiazole and sulfadiazine. These tests were done in sterile, buffered ascitic fluid under vaseline seal. With the exception of a single strain, all 7 cultures so tested grew in the presence of 10 mg. per cent of either drug. The single exception, a strain of *B. fragilis* (Strain 4, Table 1), isolated from a brain abscess secondary to chronic otitis media, was inhibited by 10 mg. per cent of sulfadiazine but not by a similar concentration of sulfathiazole.

References

1. BERGEY, D. H., *et al.* *Determinative bacteriology.* (5th ed.) Baltimore: Williams & Wilkins, 1939. P. 556.
2. BONDI, A., JR., DIETZ, C. C., and SPAULDING, E. H. *Science*, 1946, **103**, 399.
3. CAVALLITO, C. J. *J. biol. Chem.*, 1946, **164**, 29.
4. DENKLEWATER, R., COOK, M. A., and TISHLER, M. *Science*, 1945, **102**, 12.
5. DIENES, L. *J. infect. Dis.*, 1939, **69**, 24.
6. DIENES, L. *J. Bact.*, 1942, **44**, 37.
7. DIENES, L., and SMITH, W. E. *Proc. Soc. exp. Biol. Med.*, 1942, **51**, 297.
8. DIENES, L., and SMITH, W. E. *Proc. Soc. exp. Biol. Med.*, 1943, **53**, 195.
9. DIENES, L., and SMITH, W. E. *J. Bact.*, 1944, **48**, 125.
10. DONOVICK, R., and RAKE, G. *Proc. Soc. exp. Biol. Med.*, 1946, **61**, 224.
11. EGGERTH, A. H., and GAGNON, B. H. *J. Bact.*, 1933, **25**, 389.
12. GEIGER, W. B., GREEN, S. R., and WAKSMAN, S. A. *Proc. Soc. exp. Biol. Med.*, 1946, **61**, 187.
13. HALLÉ, J. Recherches sur le bactérologie du canal génital de la femme. Paris thesis, 1898.
14. HENTHORNE, J. C., THOMPSON, L., and BEAVER, D. C. *J. Bact.*, 1936, **31**, 255.
15. KANE, L. W., and FOLEY, G. E. (To be published.)
16. OLITSKY, P. K. *Medicine*, 1930, **9**, 387.
17. PRICE, C. W., NIELSEN, J. K., and WELCH, H. *Science*, 1946, **103**, 56.
18. REIMANN, H. A., PRICE, A. H., and ELIAS, W. F. *Arch. int. Med.*, 1945, **76**, 269.
19. SMITH, W. E., MCCALL, R. E., and BLAKE, T. J. *Ann. int. Med.*, 1944, **20**, 920.
20. SMITH, W. E., and ROPES, M. W. *New Engl. J. Med.*, 1945, **232**, 31.
21. WEISS, J. E., and RETTGER, L. F. *J. Bact.*, 1937, **33**, 423.