TABLE I.
Effect of Ascarid Polysaccharide on the α and β Isoagglutinins in Various Anti-Rh Human Serums.

Serum No.	Agglutinins in serum	Conc. of polysaccharide in serum %	Untreated serums Erythrocytes tested Group A Rh+	Group A Rh—	Group B Rh+	Group B Rh—	Group AB Rh+	Group AB Rh—	Group O Rh+	Group O Rh—
1	β		+	—	+	+	+	+	+	—
2	β		+	—	+	+	+	+	+	—
3	α and β		+	+	+	+	+	+	+	—
4	α " β		+	+	+	+	+	+	+	—
5	α " β		+	+	+	+	+	+	+	—
6	α		+	+	+	—	+	+	+	—
			Serums treated with ascarid polysaccharide							
1	β	4	+	—	+	—	+	—	+	—
2	β	4	+	—	+	—	+	—	+	—
3	α and β	4	+	—	+	—	+	—	+	—
4	α " β	12	+	—	+	—	+	—	+	—
5	α " β	16	+	—	+	+	+	+	+	—
6	α	4	+	—	+	—	+	—	+	—

serum even after the addition of the polysaccharide to a concentration of 16%. Difficulty in removing the natural β isoagglutinins from some anti-Rh serums has been also encountered by Levine[5] when the specific substances A and B were added to serums. Work is in progress to determine the relations that might exist between these non-absorbable natural isoantibodies and those resulting from isoimmunization to the different Rh types.

The results of this study also confirm and extend the findings of Oliver-González and Montilla indicating that the polysaccharide from *Ascaris suum* may be used to inhibit the α and β isoagglutinins in human serums so that they can be used to detect Rh positive and Rh negative cells.

[5] Levine, Ph., *Arch. Path.*, 1944, **37**, 83.

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Characteristics of Diphtheria Bacilli Found in Baltimore Since November, 1942.*

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In Baltimore from time to time during the past 22 years systematic examinations have been made of diphtheria in its clinical, epidemiological, immunological and bacteriological aspects. Reports have been published concerning these various matters, including data on the distribution of diphtheria bacilli, fluctuations in ratio of virulent to avirulent strains, changes in carrier rate, the occurrence of various biological types such as the so-called gravis and mitis varieties, and miscellaneous facts relating to the natural history of diphtheria.[1,2] It is the purpose of the present paper to present new data accruing from

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† Of 169 virulent strains in this survey in which colony form was noted 92 formed minute colonies while 77 were large colony formers. Not all minute colony formers are minimus type organisms (Table II).

[1] Frobisher, M., Jr., *Am. J. Hyg.*, 1938, **28**, 13.

[2] Phair, J. J., and Smith, M. R., *Am. J. Hyg.*, 1942, **35**, 47.

TABLE I.
Fermentation Reactions of Various Corynebacteria, Baltimore, 1942-1944.

Nature of reaction	Cultures	Dextrose* Fr. Au.	St. Au.	Fr. Fil.	St. Fil.	Saccharose* Fr. Au.	St. Au.	Fr. Fil.	St. Fil.
Typical	13	+	+	+	+	—	—	—	—
Dextrose irregular, saccharose neg.	30†	+ —±	+ —±	+ —±	+ —±	—	—	—	—
Saccharose pos.	4	+	+	+	+	+	+	+	+
C. xerose		+	+	+	+	+	+	+	+
C. pseudodiph.		—	—	—	—	—	—	—	—

* Fr. Au. = Fresh, autoclaved tubes; St. Au. = Stored, autoclaved tubes; Fr. Fil. = Freshly prepared with filtered sugar; St. Fil. = prepared with filtered sugar but stored in refrigerator before inoculation.

† The presence of +, —, and ± signs indicates that some strains fermented strongly but not in every tube, others failed to ferment, while others fermented slightly or slowly in one or more tubes.

similar studies carried on during the period from 1942 to the close of 1944.

Materials and Methods. The methods used throughout this work were the same as during the last 8 years in these laboratories and have been previously described.[1] From November 27, 1942 to June 23, 1944, 210 strains of bacteria morphologically typical of *Corynebacterium diphtheriae* were isolated from a total of about 750 throat cultures representing some 500 clinical cases, their contacts, and various healthy carriers. As in previous studies these bacteria were examined biologically either immediately on isolation or after varying periods of storage desiccated *in vacuo*. Virulence was tested by either the rabbit intradermal method of Fraser[3] or by the chick method,[4] or both. When both tests were used, no disagreements in result were observed. As a result of these tests 169 of the 210 strains were found to be virulent.† The present report deals principally with these virulent cultures. All of the cultures were also tested for 2 biochemical properties heretofore regarded as the *sine qua non* of identification as diphtheria bacilli, namely prompt, vigorous fermentation of dextrose and nonfermentation of saccharose. Most of the cultures were also examined for cultural properties characteristic of the gravis and mitis types. It has been pointed out previously[5] that in these publications the terms gravis and mitis are used to indicate diphtheria bacilli having certain cultural characteristics and have no reference to virulence or clinical details.

TABLE II.
Dextrose Fermentation by Strains of *C. diphtheriae* Producing Minute Colonies.

Animal test	Dextrose fermented promptly	Dextrose fermented slowly, slightly or irregularly	Totals
Virulent	4	22	26
Avirulent	2	8	10
Totals	6	30	36

Results. 1. *Gravis and Mitis Types.* Only 1 gravis-type culture was isolated during the entire survey. This was obtained during the convalescence of a patient who had yielded a mitis-like culture during his attack of diphtheria and whose sister, having diphtheria later on, also yielded a mitis-like strain on culturing. Thus the gravis-mitis situation in Baltimore seems at present to be not significantly different from what it was when similar surveys were made some 5 or 6 years ago.[1] That is, gravis type organisms are rare.

2. *Atypical fermentations of dextrose and*

TABLE III.
Dextrose Fermentation by Strains of *C. diphtheriae* Producing Large or "Ordinary" Size Colonies.

Animal test	Dextrose fermented promptly	Dextrose fermented slowly, slightly or irregularly	Totals
Virulent	12	3	15
Avirulent	4	2	6
Totals	16	5	21

[3] American Public Health Association, *Diagnostic Procedures and Reagents*, Chapter on Diphtheria. New York, 1941.

[4] Frobisher, M., Jr., Parsons, E. I., and Tung, T., *Am. J. Hyg.*, 1942, **35**, 381.

[5] Frobisher, M., Jr., *Am. J. Pub. Health*, 1943, **33**, 1244.

saccharose. Of primary importance to the laboratory bacteriologist is the fact that, during the study, numerous strains of diphtheria bacilli were encountered which gave atypical reactions in the fermentation of dextrose or saccharose, *i.e.,* some fermented saccharose, some failed to ferment dextrose regularly.

Because of the importance of these observations with respect to the laboratory diagnosis of diphtheria it was deemed advisable to test several times the dextrose and saccharose fermentation of a number of these cultures taken at random, arranging the tests as a comparative experiment in which the carbohydrates were prepared in various ways, some conducive to hydrolysis of saccharose. The methods of preparation were as follows:

1. The sugars were added to infusion broth with indicator, tubed, autoclaved and inoculated as soon as cool.

2. Tubes of broth were similarly prepared but stored several months in the icebox.

3. Tubes of broth were similarly stored but the sugars were prepared in 10% aqueous solution, sterilized by filtration and added aseptically to the broth before storage.

4. Tubes were prepared with filtered sugar solutions as in 3 but were inoculated immediately after preparation.

All tubes contained brom cresol purple. The inocula consisted of 1 or 2 drops of 48-hr broth culture per tube. All tests were inoculated within a period of 2 hours and were incubated simultaneously. Observations were made on the 7th and 14th days after inoculation. There were few significant changes after 7 days.

Table I shows the results obtained with 47 virulent strains tested in the manner indicated. It is seen that 4 cultures regularly fermented both dextrose and saccharose. Without unduly enlarging the table it is not possible to convey accurately the irregularity in dextrose fermentation exhibited by many of the other cultures. Some strains fermented slightly or slowly in all tubes; some fermented strongly in one or two tubes and little or not at all in others. There was nothing to suggest that method of preparation of fermentation tubes, either of dextrose or saccharose, affected the result. It is evident that a single dextrose fermentation test does not serve to demonstrate the full potentialities of diphtheria bacilli and that, in diagnostic laboratories, virulence tests had better be made with all morphologically suspicious organisms whether or not they give typical fermentation reactions.

Avirulent strains, morphologically indistinguishable from toxigenic *C. diphtheriae,* and having aberrant fermentative properties as described above, were encountered with the virulent ones but, since virulence is absent, differentiation of such strains from *C. xerose* and *C. hoffmanni* cannot be absolute, although fairly certain on morphological grounds.

3. *Small colony (minimus type) of C. diphtheriae.* A third observation made during these studies was the appearance of a variety of diphtheria bacilli characterized by the production of remarkably small colonies on either cystine-tellurite agar as regularly used for isolation, or on McLeod's tellurite agar used to bring out the distinctive properties of the large, flat gravis colonies. The minute colonies are either entirely black and opaque or have a dark center and light periphery. In diameter they may attain 1 mm, but are usually less and are often scarcely visible with the naked eye. The large colonies are about 2 to 3 mm in diameter. Interestingly enough the minute colonies may be either smooth and mitis-like in all respects but size, or rough and very much like gravis-type colonies except for their minuteness. It was especially interesting to observe that the majority of strains producing minute colonies are those described above as fermenting dextrose slowly, slightly or irregularly. The correlation between small colony production and defective dextrose fermentation is shown in Table II.

Such strains were found with increasing frequency from the beginning of this survey in 1942, onward. During this period of time "ordinary" cultures, *i.e.,* those not distinguished by minute colony formation, usually, though not invariably, reacted in the traditional manner in dextrose, *i.e.,* most of them fermented it promptly and regularly. For example, 21 ordinary cultures reacted as shown in Table III. Other large-colony strains studied at other times during this investigation gave similar results.

It appears that the type of diphtheria bacilli

characterized by minute colony formation and slow, slight or irregular dextrose fermentation is as distinct an entity as the gravis or mitis type and might, with equal or even greater propriety, be given the descriptive name *minimus* for convenience in discussing it. The variability of *C. diphtheriae* under laboratory, and probably natural, conditions is well known and none of the forms hitherto described, including gravis and mitis types, have been found stable; nor is it likely that the minimus type will prove an exception in this respect. Dextrose fermentation in these strains seems to be undergoing the actual process of changing from positive to negative or vice versa and very slight alterations in environment appear to affect the balance one way or the other. For example, strains tested for dextrose fermentation after subculture on blood agar acquired the property to ferment, especially in media containing casein hydrolysate. In routine pork-infusion broth, however, they remain defective for some time.

4. *Prevalence of minimus type.* Although the minimus type has not previously been noted as a distinctive variety it has nevertheless been observed in Baltimore a number of times during the past 8 years. Because of its rarity it had never been given special mention. In the interval between the last previous culture survey in Baltimore (Eastern Health District) in 1939 and the years covered by the present study it has become a relatively common† type. Furthermore, during these studies the minimus variety of *C. diphtheriae* was prevalent in an epidemic in Baltimore which is elsewhere described.[6] Since 1942 there has been a large influx into this city of population from other parts of the country, with whom many of the Baltimore children have mingled in school, and there has been a considerable increase in diphtheria morbidity and in the number of malignant cases in Baltimore. One wonders whether at least part of the increased diphtheria morbidity and fatality in Baltimore in the last year or so may not represent lack of experience of the human host with a hitherto strange variant of the parasite which, we know, readily alters itself in various respects.[5]

It is probable, as shown by Eller *et al.*,[6] that over 70% of the school-age children in the area where the largest number of cases of diphtheria occurred had received toxoid immunization and notable that 64% of the cases occurred in children who had received toxoid. Inquiries have been made as to whether the minimus type of organisms produces a toxin qualitatively different from that used in preparing the toxoid used in routine immunization. Complete studies have not yet been made but preliminary observations indicate that minimus toxin is not appreciably different from other diphtheria toxins. The above-mentioned lack of adaptation and consequent unfavorable reaction on the part of the host would, then, appear to be related to the bacillary part of the diphtheria organism and not to its exotoxin and would suggest the desirability of considering the use of antibacterial or anti-endotoxic substances, in addition to anti-exotoxin alone, in both the prophylaxis and therapy of diphtheria. Experimental work in animals has already shown that such bacillary endotoxic antigens have distinct protective value.[7]

Summary. In Baltimore during the past 3 years the gravis type of diphtheria bacillus has, as heretofore, been absent or rare. However, during this period there have been isolated, chiefly from clinical cases of diphtheria and contacts thereof, but also from casual carriers, many virulent and nonvirulent diphtheria bacilli characterized by (a) the production of minute colonies on blood-agar media containing potassium tellurite, and (b) slow, slight or irregular fermentation of dextrose. These characters are very frequently found together and for such organisms the designation *minimus* type is suggested as a convenience in discussion. Other virulent strains have been found which regularly ferment saccharose. The saccharose-fermenting strains studied do not appear to differ in other respects from ordinary diphtheria bacilli. A relationship between the minimus type of *C. diphtheriae,* and recent epidemiological and clinical observations in Baltimore, as well as the possible value of bacterial antigens in addition to routine toxoid

[6] Eller, C. H., and Frobisher, M., Jr., *Am. J. Hyg.*, 1945, in press.

[7] Frobisher, M., Jr., and Parsons, E. I., *Am. J. Hyg.*, 1943, **37**, 53.

in prophylaxis, are discussed. Authors' summary.

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Effect of Corn Grits on Nicotinic Acid Requirements of the Dog.*

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Pellagra has long been associated with the ingestion of relatively large amounts of corn. The early theories regarding this relationship included suggestions that corn contained toxic materials or acted as a carrier for pathogenic organisms. Goldberger and coworkers[1] clearly demonstrated that pellagra was caused by the deficiency of a dietary factor in certain foods commonly consumed by pellagrins, and in 1937[2] nicotinic acid was found to be the vitamin supplied in inadequate amounts. However, as analytical methods have been perfected, it has become apparent that on a dry weight basis, corn contains as much, or more, nicotinic acid as eggs, milk, polished rice, oats, and rye. Furthermore, Handler[3] has found that the blacktongue syndrome in dogs is more readily produced with diets containing corn than with purified rations which contain much less nicotinic acid. In addition, we have observed[4] that corn exerts a marked growth retardation in rats which is counteracted by nicotinic acid therapy. Thus there is some characteristic of corn aside from its low nicotinic acid content, which tends to increase the organism's requirement for this substance.

Corn grits were used in the present study since they comprise a large portion of the dietary of many people in certain sections of this country, and since corn grits as prepared and marketed, usually contain significantly less nicotinic acid than does whole corn. The effect of corn grits on the nicotinic acid requirement of the dog was determined by feeding a nicotinic acid-low synthetic ration so modified as to include a high percentage of corn grits.

Experimental. The experiments were conducted with growing dogs which had been kept since weaning on the nicotinic acid-low synthetic ration reported in previous studies.[5] All

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1 Goldberger, J., and Tanner, W. F., *Pub. Health Rep.*, 1925, **40**, 54.

2 Elvehjem, C. A., Madden, R. J., Strong, F. M., and Woolley, D. W., *J. Biol. Chem.*, 1938, **123**, 137.

3 Handler, P., PROC. SOC. EXP. BIOL. AND MED., 1943, **52**, 263.

4 Krehl, W. A., Teply, L. J., and Elvehjem, C. A., *Science*, 1945, **101**, 283.

5 Krehl, W. A., and Elvehjem, C. A., *J. Biol. Chem.*, 1944, **158**, 173.