than in the male. Acute degeneration of the whole gland followed inoculation with the non-lipoid cortical constituents, together with adrenaline.

Thyroid.—The gland was apparently stimulated by the cortex, but adrenaline over-stimulated it to the point of exhaustion.

Haemolymph Glands.—The most striking change observed was in the pigment. Three types could be distinguished. First, the yellow-brown amorphous pigment due to breakdown of haemoglobin; this was always increased, showing that the suprarenal stimulates blood destruction. Secondly, bright yellow pigment in fatty droplets, probably representing some of the inoculated lipid material laid down in altered form in these glands. Thirdly, brown pigment found in special cells of varying size and shape in varying amounts; these are melanoblast cells, and were found most frequently in the haemolymph glands, occasionally in the true lymph glands, and rarely in almost any situation, distributed by the lymph channels. The suggestive point about these cells is that they were only found when the preparation contained adrenaline, and never under any other conditions. Excessive or perverted activity of the suprarenal medulla, therefore, may be directly responsible for the pigment formation of Addison's disease and may also play a part in the formation of pigmented tumours.

Conclusions.

Although it is not possible to apply results obtained with certain animals to another species, nor possible always to distinguish between glandular hyperactivity and perverted activity, nor between purely structural hyperplasia and functional hyperplasia, yet the following conclusions seem worthy of record:

1. The suprarenal cortex plays a part in fat metabolism and in calcium metabolism.
2. The suprarenal cortex stimulates the thymus to involution.
3. The suprarenal cortex controls the gonads in both male and female with respect to both reproductive and interstitial cells.
4. The suprarenal gland acts as a whole in controlling general health, in controlling haemoglobin breakdown, in regulating the production of lymphocytes, in regulating the production of mast cells, and in stimulating the thyroid.
5. The suprarenal medulla is connected with the formation of melanoblast cells.
6. Inoculation with various suprarenal extracts appears to lower the resistance of experimental animals, as is indicated by the condition of the lymph and haemolymph glands.

I should like to thank Professor Cullis for her continued interest and helpful criticism throughout this work. The expenses of this research are being defrayed by a grant from the Royal Society.

THE VIRULENCE OF DIPHTHERIA-LIKE ORGANISMS.

(Further Note.)

BY

A. J. EAGLETON, M.D.LOND., M.R.C.P.LOND.,

AND

E. M. BAXTER, M.Sc.SHEFFIELD.

(From the Wellcome Physiological Research Laboratories.)

THE bacteriological diagnosis of diphtheria, though based generally on the morphology and cultural characteristics of the *Bacillus diphtheriae*, must depend ultimately on the animal test, whereby the pathogenicity of the infecting organism can be definitely established. While the animal test is advisable, even in clinical work, as a periodical check on the routine methods of diagnosis, in dealing with the carrier problem and prevention of diphtheria it is indispensable. The time may come when some other test may replace it; this is, however, very improbable.

By the intracutaneous method (Eagleton and Baxter, 1921) a large number of pathogenicity tests can be carried out speedily and economically. Further experience of this method has served to confirm our belief in its reliability, evidence of which is given in the second part of this paper. The first section deals with 161 cultures of diphtheria-like organisms, all of which were tested for pathogenicity, and the majority for fermentative power. Slopes of Loeffler's

serum medium were employed for primary cultures, and plates of the same medium for subsequent purification.

The intracutaneous method was used for testing pathogenicity, checked frequently, especially when the findings were negative, by subcutaneous injections, as will be described later.

I. EXAMINATION OF CULTURES.

The cultures were derived from three sources, and each group will be considered separately.

(a) Thirty-seven cultures from cases of clinical diphtheria gave the following results:

Morphology.	Glucose.	Saccharose.	Virulence.
<i>B. diphtheriae</i>	Acid	Negative	+ 33
<i>B. diphtheriae</i>	Not done	Not done	+ 3
Hoffmann's bacillus ...	Not done	Not done	— 1
			37

Of the 33 virulent cultures of *B. diphtheriae* the "sugar" reactions of which were tested, 2 fermented saccharose as well as glucose. These cultures, after plating, had been maintained on Loeffler medium for over ten weeks, and were seemingly pure, judging by smear preparations and appearance of growth. On replating, however, a very slight contamination was found—in one case a coccus and in the other a Gram-positive bacillus which produced acid in saccharose. When freed from these impurities the two strains failed to ferment saccharose.

(b) A single throat swab was taken from 112 patients who had had diphtheria and had completely recovered at the time of examination. Nineteen strains of *B. diphtheriae* and Hoffmann's bacillus were obtained, and gave the following results:

Morphology.	Glucose.	Saccharose.	Virulence.
<i>B. diphtheriae</i>	Acid	Negative	+ 9
<i>B. diphtheriae</i>	Not done	Not done	+ 1
<i>B. diphtheriae</i>	Acid	Negative	— 1
Hoffmann's bacillus ...	Negative	Negative	— 5
Hoffmann's bacillus ...	Not done	Not done	— 3
			19

Five of the nine virulent strains, when first tested in sugars, produced acid in saccharose; this was, however, due to a very slight degree of contamination. The fermentation of saccharose in these cases was generally slight, and might not take place till after the third day of incubation.

The most difficult contamination to eliminate is a Gram-positive sporing bacillus, which may lie dormant for weeks in a culture on solid medium. This organism ferments glucose and saccharose, and, when abundant or in pure culture, liquefies Loeffler medium.

(c) Comprises 105 cultures from 67 carriers, none of whom had had diphtheria, but most of whom had been in contact with a case. The results found in this group were as follows:

Morphology.	Glucose.	Saccharose.	Virulence.
<i>B. diphtheriae</i>	Acid	Negative	+ 30
<i>B. diphtheriae</i>	Not done	Not done	+ 3
<i>B. diphtheriae</i>	Acid	Negative	— 52
<i>B. diphtheriae</i>	Acid	Acid	— 8
<i>B. diphtheriae</i>	Not done	Not done	— 1
Hoffmann's bacillus ...	Negative	Negative	— 11
			105

Of the 30 virulent cultures, one produced acid in saccharose, but as with similar cases in the other groups, a very slight contamination was found to be responsible.

When we compare these three groups we notice that in Group A only one culture was avirulent and that was Hoffmann's bacillus. All the remainder were virulent, and, except for three not tested, gave typical "sugar" reactions. In Group B we find 10 virulent and one avirulent *B. diphtheriae*, the remainder being Hoffmann's bacillus; whereas in Group C, of 94 bacilli morphologically resembling *B. diphtheriae*, only 33 were virulent; excluding the eight strains which fermented saccharose (*B. xerosis*) and the one avirulent

strain, the "sugar" reactions of which were not tested, we find 33 virulent and 52 avirulent.

Of the cultures of *B. diphtheriae* from clinical cases we find 100 per cent. virulent; of those from convalescents 90.9 per cent.; while of the cultures from carriers which were typical on morphology and "sugar" reactions we find only 38.8 per cent. virulent.

These results agree with the findings of most other workers in so far as they show that avirulent strains are rarely found except in people who have never, or at all events not recently, suffered from diphtheria (Graham Smith, 1908, Arkwright, 1912).

Fermentation Reactions.—Of the 161 cultures in the three groups the sugar reactions were tested in all except twelve instances. We used as a routine only glucose and saccharose. Thirty strains were also examined for action on dextrin, salicin, and litmus milk. We found, however, that by using glucose and saccharose alone we obtained as much information as we could from the more extended set of reagents. All the virulent *B. diphtheriae* which we have tested hitherto produce acid in glucose but not in saccharose. The same reaction was, however, given by 53 out of 61 avirulent cultures, though the amount of acid produced by two of them was very slight. We have not so far met with a virulent strain which, when absolutely pure, produces acid in saccharose. Graham Smith (1908) has reported several from one epidemic. Our difficulties in getting rid of contamination by saccharose-fermenting organisms have been noticed. We succeeded only by repeated plating, and in more than one instance after picking off several colonies from the same plate and testing the sugar reactions of each, we found that some of the "single" colonies failed to ferment saccharose, whilst others did so.

We have so far found no evidence that a culture may "lose its virulence" on being kept. It need only be mentioned that smears must be made from every culture before it is used for inoculating animals. One culture in our series appeared to be a pure culture of Hoffmann's bacillus and was avirulent by intracutaneous test. Two months later, when examined for morphology, sugar reactions and virulence, it was found to be indistinguishable from *B. diphtheriae* on morphology and "sugar" reactions, and to be virulent. The culture was plated, and from the plate a number of colonies were picked off. Some proved to be pure cultures of Hoffmann's bacillus, others *B. diphtheriae*. Hoffmann's bacillus and *B. diphtheriae* may therefore live in the same tube for months and neither be destroyed. A culture originally avirulent may, when next examined, be found virulent because the *B. diphtheriae* are in enormous excess, and vice versa.

II. RELIABILITY OF INTRACUTANEOUS TEST.

We have compared the results of determination of virulence by the intracutaneous method with those obtained by the methods hitherto mostly used—that is, the subcutaneous injection of a forty-eight hour broth culture or of the growth from a Loeffler slope.

We chose for comparative testing by the subcutaneous method 83 cultures which had been tested and classified by the intracutaneous method; of these, 57 were avirulent and 26 virulent. All these cultures were subsequently tested by the subcutaneous injection into guinea-pigs of either an emulsion of the whole growth on an eighteen-hour Loeffler slope culture or of 2 c.cm. of a forty-eight or ninety-six-hour broth culture. Twenty-one of the cultures were tested in two ways by the subcutaneous method, the emulsion of a Loeffler slope culture and 2 c.cm. of a forty-eight or ninety-six-hour broth culture being injected subcutaneously into separate guinea-pigs.

As a control a known virulent culture, a Park 8 strain, was used throughout. A small fraction of an eighteen-hour Loeffler slope culture of this organism killed, while of a forty-eight-hour broth culture 0.01 c.cm. killed; smaller doses were not tested.

The batch of broth employed was in constant use for the routine production of high grade toxin; we were, therefore, certain that the broth used in testing our cultures was one suitable for the production of toxin.

In every instance the results obtained by the intracutaneous and subcutaneous methods agreed—that is, no culture proved to be virulent by one method but avirulent by the other.

We think it may be justly claimed that this method of testing virulence is not only rapid and economical but simple and reliable.

Results of Repeated Swabbing.

We had the opportunity of examining swabs taken from seven notified "carriers" who were under observation for two to four months. Twenty-six cultures of organisms morphologically indistinguishable from *B. diphtheriae* were obtained

from these carriers and examined by the intracutaneous method. One child gave four "virulent" cultures over a period of two months. One gave a "virulent" followed by an "avirulent" culture. In one case an "avirulent" *B. diphtheriae* and a Hoffmann's bacillus were found on different dates over a period of three months. Cultures indistinguishable from *B. diphtheriae* but, when isolated, found to ferment glucose and saccharose, were repeatedly obtained from the throat swab taken from two children.

Results and Conclusions.

Of 161 strains cultivated from cases of diphtheria, convalescents and carriers, 79 were virulent *B. diphtheriae*, 62 were avirulent *B. diphtheriae* (including 8 *B. xerosis*), and 20 were Hoffmann's bacillus (all avirulent).

Cultures of *B. diphtheriae* from cases of diphtheria were virulent in 100 per cent. of those examined; from convalescents in 90.9 per cent.; from carriers in only 38.8 per cent.

Sugar reactions were carried out with 149 cultures; glucose and saccharose were the only reagents used as a routine. The value of "sugar" tests may be summed up as follows:

If glucose is not fermented, the culture is not virulent *B. diphtheriae*.

If glucose is fermented but not saccharose, the organism may be virulent or may not.

If both glucose and saccharose are fermented, the organism may be *B. xerosis*, may be contaminated, or (Graham Smith) may be virulent.

We have found no evidence that virulent cultures, if isolated in a pure condition, become avirulent.

For absolute diagnosis, a culture morphologically resembling *B. diphtheriae* and capable of producing acid in glucose must be submitted to a virulence test.

The intracutaneous method is simple, economical, and apparently reliable.

Our thanks are due to those who have provided us with material for this research, especially Dr. F. Foord-Caiger and Dr. Graham Forbes. Without the help of Dr. Okell and our other colleagues and the guidance of Dr. O'Brien, our work would have been still less complete than we have left it. Finally, to Mr. Tottem and the rest of the laboratory staff we hereby offer acknowledgement of their large share in whatever success we may have obtained.

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¹ Eagleton and Baxter. "The Virulence of Diphtheria-like Organisms," BRITISH MEDICAL JOURNAL, May 28th, 1921. ² Nuttall and Graham Smith. *The Bacteriology of Diphtheria*, Cambridge, 1903, pp. 160-161, 194, 185, and 200. ³ Ledingham and Arkwright. *The Carrier Problem in Infectious Diseases*, 1912, chap. iv. (Summaries of the work done up to that date and full references.)

Memoranda:

MEDICAL, SURGICAL, OBSTETRICAL.

AN EMERGENCY METHOD IN PRESBYOPIA AND HYPERMETROPIA.

THE following cases are recorded as examples of the use of the stenopaic disc in presbyopia and hypermetropia:

Case 1.—Aged 58. Vision: R. = J. 6; L. = J. 6. With disc, R. = J. 1; L. = J. 1.

Case 2.—Aged 61. Hypermetropic, wearing for distance + 4.5 D. sphere in each eye. Vision: R. = 6/60; L. = 4/60. With distant glasses, R. = 6/12; L. = 6/12 partly. With disc only, R. = 6/9 partly; L. = 6/12 partly. Without disc, R. = J. 18; L. = J. 16. With disc only, R. = J. 10; L. = J. 6. With disc and correcting lens for distance, R. = J. 1; L. = J. 1. Retinoscopy revealed astigmatism in the left eye.

Case 3.—Aged 47. Vision: R. = 6/6 H.M., with + 1.0 D. sphere, R. = 6/6; L. = 6/6 H.M., with + 1.0 D. sphere, L. = 6/6. Without disc, R. = J. 2 with some difficulty; L. = J. 2 with some difficulty. With disc only, R. = J. 1; L. = J. 1.

We have thus a simple substitute, in emergency, for the reading glasses of presbyopes.

The presbyope, even an early presbyope, without the suitable lens, is in a most helpless condition when trying to decipher the small print of a telephone directory, time table, newspaper, exhibition catalogue, etc. This small print is easily, though slowly, read by means of an improvised stenopaic disc, which can quickly be made with a piece of paper or cardboard, through which a hole 1 to 2 mm. in diameter is pierced by a pin, the point of a pencil, or knife. Such an emergency measure would be of real benefit to the presbyope, stranded without reading glasses, specially in the country or on a railway journey. A hole, about the same size, through a piece of bread forms an excellent emergency substitute for a convex lens! The tunnel formed by the fingers and palm of a partially closed fist acts in the same way, though not so clearly.