THE HEAT-STABILITY AND "TENACITY"
OF TUBERCULIN

BY

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It has been realized for some years that tuberculin is an extremely heat-stable substance. Parish and Okell (1929) found that undiluted old tuberculin might be autoclaved for half an hour without detectable loss, and after two hours might retain 60 per cent. of its original value. Dilutions were slightly less heat-stable, but retained 40 per cent. of their original value after autoclaving for two hours. Douglas and Hartley (1934) stated that "after autoclaving and prolonged heating at high temperatures, both of undiluted tuberculin and of dilutions thereof, apparently not less than one-half of the original activity remained." We have recently found that tuberculo-protein, prepared by Seibert's method, has a similar high degree of heat-stability.

In view of these results it appeared to be advisable to find out to what extent minute amounts of tuberculin might remain adherent to "clean" glassware and other apparatus. It is already well known in serological laboratories, but not universally appreciated, that biological products other than tuberculin—for example, concentrated diphtheria antitoxin—adhere with considerable tenacity to glassware; if a syringe is filled with antitoxin and washed out several times afterwards, the presence of antitoxin can be demonstrated in the final washings by delicate tests. (In laboratory practice all apparatus used for work with antitoxins is boiled for ten minutes after washing to destroy the last traces of antitoxin. Syringes used clinically for the injection of diphtheria antitoxin should be boiled after washing before they are employed for the withdrawal of blood samples intended for estimations of circulating antitoxin.)

Description of Experiments

In the experiments about to be described it has been shown that tuberculin adheres to glassware, filter candles, etc., with even greater tenacity than antitoxin. In each experiment the apparatus was deliberately "contaminated" with undiluted old tuberculin, equal in potency to the international standard old tuberculin. The apparatus was then washed out thoroughly, and in many of the experiments also autoclaved. All test samples of washings made thereafter were injected intracutaneously into tuberculous guinea-pigs. The limit of sensitivity of the guinea-pigs was usually 1 in 8,000 of the international standard old tuberculin; in a few experiments the guinea-pigs were sensitive to 1 in 25,000. The following summaries indicate the type of results we obtained:

1. A 250 c.cm. bottle was washed out thoroughly three times with tap water and autoclaved for half an hour at 15 lb.; 100 c.cm. borate buffer solution (B.B.S.) was introduced, and a sample tested in very sensitive guinea-pigs. Slight but definite reactions were produced, equivalent to 1 in 25,000 tuberculin.

2. *Pipette*.—Tap water was drawn up twice. The pipette was autoclaved for half an hour at 15 lb. B.B.S. was then drawn up and subsequently injected into guinea-pigs. Tuberculin equivalent to 1 in 250 was found. Another pipette was similarly treated, but not autoclaved, and 1 in 250 tuberculin was again detected in the B.B.S.

In a later experiment thorough washing through from a tap for two minutes, with and without subsequent autoclaving, reduced the amount of tuberculin in the final washings to 1 in 25,000. Again autoclaving had no effect.

3. A *Berkefeld N Filter Candle*, 10 by 2 inches, was treated as follows. Cold tap water was passed through for three hours, followed by hot tap water in the reverse direction for half an hour; 1,500 c.cm. of B.B.S. were passed through. The first 10 c.cm. of B.B.S. contained 1 in 500 tuberculin, and the last 10 c.cm. contained 1 in 2,000 tuberculin. The candle was now autoclaved for half an hour at 15 lb.; 1,500 c.cm. of B.B.S. were again passed through. The first 10 c.cm. of B.B.S. contained 1 in 250 tuberculin, and the last 10 c.cm. contained 1 in 4,000 tuberculin.

This experiment was repeated several times with various modifications (for example, longer period of washing and substitution of saline for B.B.S.), and always the tuberculin content appeared greater immediately after autoclaving. We thought this was possibly due to the heat "releasing" the tuberculin from the walls of the candles, but a similarly treated candle standing overnight without autoclaving gave the same result. The first few cubic centimetres passed through candles which have been washed thoroughly and then autoclaved or rested have shown a higher tuberculin content than could be demonstrated in the previous washings. The "drippings" from such candles are often definitely straw-coloured. This is probably due to elution of the tuberculin which was adsorbed on to the candles; the tuberculin collects in the moisture held by the candles during the resting periods, and is subsequently washed out when water, saline, or B.B.S. is passed through.

No experiments have been done with Pasteur-Chamberland candles, as they are quite unsuitable for use with old tuberculin.

Discussion

These experiments show that thorough washing may not be effective in removing the last traces of tuberculin from glassware and filter candles, and that subsequent autoclaving may not produce any further detectable reduction.

Since human subjects are sometimes sensitive to 1 in 100,000 or even a higher dilution of tuberculin, injected intracutaneously, a sample reported as negative on the guinea-pig test would have to pass a Mantoux test on a highly sensitive individual before it could be regarded for practical purposes as tuberculin-free; actually such tests have been unnecessary in the above series of experiments, since all samples tested gave positive reactions on injection into guinea-pigs. In another series, syringes were used for tuberculin diluted 1 in 50,000 to 1 in 100,000, they were washed out ten times under the tap and then steamed; 0.2 c.cm. of saline was taken up into each and injected into a Mantoux positive volunteer who, rather surprisingly, gave faint but definite reactions. This experiment was repeated with similar results.

Experiments were then made with syringes from different makers and with pipettes of different types of glass. Various dilutions of tuberculin were used. The syringes were subsequently washed out thoroughly, followed by immersion in water or saline for periods up to twenty-four hours. Although the final washings contained no detectable tuberculin in many instances, in other experiments the results were positive. Our data suggest that special syringes should be reserved for use not only with tuberculin, but also with particular dilutions; these syringes may conveniently be ringed with rubber bands of various colours for purposes of identification.

A pathologist recently reported to us the occurrence of reactions of an unusual type after the use of Schick control fluid which had been found to be satisfactory by all other workers. We suggested that the explanation was that the syringe employed at his hospital for the injections had previously been in contact with tuberculin. This possibility was not readily accepted by him because "the syringe was only used for tuberculin at 1 in 1,000—then thoroughly rinsed." The incident adds point to the present communication.

We have been unable to find a simple but reliable method of removing tuberculin or tuberculo-protein from

(a) syringes intended for human use or (b) filter candles. We have carried out a few experiments with trypsin, but, even after prolonged digestion, traces of tuberculin or tuberculo-protein were frequently detected in subsequent washings with saline or B.B.S. The difficulties encountered in removing tuberculo-protein, which contains no glycerin, from syringes and pipettes, suggests that the "tenacity" of old tuberculin to glassware is not a function of its high glycerin content.

Since this work was carried out, Professor E. R. Long, who has recently visited this country, has informed us that he and Dr. F. B. Seibert had also noticed the property of tuberculin of adhering to glassware, and insist on the use of separate syringes for tuberculin and control fluids. More recently our attention has been drawn to a paper by Zieler (1911), who anticipated us by many years in describing this dangerous "tenacity" of tuberculin. This publication seems to have also escaped the attention of the writers of textbooks.

Summary

Tuberculin adheres to apparatus with exceptional tenacity and is extraordinarily heat-stable; every trace of tuberculin is not removed by thorough washing and autoclaving. A simple but reliable method of removing traces of tuberculin, applicable to the ward side-room, has yet to be found.

All glassware and filter candles used for handling tuberculin should be clearly marked and restricted to that purpose. Tuberculin should, in fact, be kept most rigorously apart from all other products, including control fluids, in all stages of preparation and injection.

Separate syringes should be reserved for different dilutions of tuberculin.

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BIBLIOGRAPHY

- Douglas, S. R., and Hartley, P.: *Tubercle*, 1934, xvi, 105.
 Parish, H. J., and Okell, C. C.: *Journ. Path. and Bact.*, 1929, xxxii, 51.
 Zieler, K.: *Deut. med. Woch.*, 1911, xxxvii, 2075.

PALATAL PARALYSIS IN EXTRA-FAUCIAL DIPHTHERIA

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The theory that diphtheritic paralysis is the result of an ascending neuritis has several exponents among the leading French authorities. One of the arguments advanced in its favour is the assertion that paralysis of the palate is not met with in extra-faucial diphtheria. "Comme l'avait remarqué Trousseau, en pareil cas, la paralysie débute sur le membre, ou sur la partie du corps primitivement touchée, et les paralysies vélopalatines font défaut" (Lereboullet). This supposition concerning the palate is also a premise of Walshe's interesting conception of the pathogenesis of the condition. In 1918 and 1919 he described a number of cases of faucial and extra-faucial diphtheria, but unfortunately only a few of them could be followed throughout their entire course. As he himself pointed out, some of his observations "remain inconclusive." Nevertheless, his contribution is a valuable one, and although his views have not secured any general acceptance by fever clinicians, they are frequently quoted in neurological works.

A full discussion of the whole subject of diphtherial paresis would be outside the scope of the present paper. We merely seek to demonstrate that the occurrence of palatal palsy is certainly not restricted to the faucial variety of the disease. The establishment of this point is important, because it would refute the hypothesis that the impairment of palatal function is necessarily the result of an involvement of the medullary nuclei by toxin which travels from the fauces via the perineural lymphatics.

Instances of extra-faucial diphtheria are common enough, but the site of infection is usually the nose, larynx, ear, or conjunctiva. Unless faucial infection co-exists, paresis is very exceptional in this group. On the other hand, paresis may sometimes be associated with wound diphtheria, and, as might be expected, the war period furnished a number of examples. Modern surgical methods have almost completely banished such cases from hospital practice. In recent years medical literature has made few references to their occurrence; the very occasional examples which have been recorded seem to have responded well to serum therapy and to have had no parietic sequelae. A case of this kind was under treatment in the South-Eastern Hospital in January, 1933, and mention might be made of the cases reported by Dixon (1921) and Anderson (1931). In June, 1933, we were fortunate enough to admit a second case, which was complicated by extensive paresis. The following are the clinical details.

Case Report of Wound Diphtheria

A male, aged 25, was transferred to the South-Eastern Hospital on May 18th, 1933. He had a history of an abscess of the right thigh, which was opened on February 26th at his first hospital; the resulting wound broke down extensively, leaving a large indolent ulcer. About the middle of April he found himself unable to read on account of blurring of the print. His vision for distant objects was normal. This paresis of accommodation lasted for some two weeks, and in the first week of May it was noted that his voice was distinctly nasal in character. He now complained of numbness of the fingers and toes, and shortly before transfer to the South-Eastern Hospital there was an increasing weakness of both arms and legs, but the palatal paresis was improving. This sequence aroused suspicion of a diphtheritic origin of the paresis, and on May 15th a culture of the wound showed the presence of Klebs-Loeffler bacilli. Two cultures of the throat gave negative results.

On admission the patient was thin and cachectic. There was a large granulating ulcer with shelving edges on the outer surface of the upper aspect of the right thigh, which in its largest diameter measured $3\frac{1}{2}$ inches and extended towards the gluteal region. The surface was covered by a thin layer of purulent debris, but no membranous exudate was present at this stage. There was marked wasting of the muscles of the right leg, and tenderness of the calves of both legs. The tendon reflexes were absent. The throat was clean and uninjected; there was no evidence of rhinitis. Movement of the right side of the palate was impaired and the voice was slightly nasal. The right lower facial segment was parietic. No ocular symptoms were discovered. Muscular power of the limbs was poor; the grip of the right hand was weaker than that of the left. Sensation was impaired; below the elbows and knees light touch was not perceptible, and appreciation of a pin-prick was diminished. Formication was complained of over the fingers and toes. Astereognosis was marked, and ataxy was a notable feature. Neck and trunk muscles were then unaffected. The heart sounds were indicative of myocardial weakness; the first sound was abrupt and occasionally reduplicated, and there were frequent extrasystoles. Blood pressure was 110/80. Cultures were taken from the throat, nose, and wound. The throat and nose were negative for diphtheria, but polar staining organisms were found in the wound culture. Subsequent laboratory investigations showed them to be saccharose fermenters, and animal inoculation tests proved them to be avirulent. A Schick test was negative. An intramuscular injection of