TYPHOID ENDOTOXOID VACCINE

A REVIEW OF THE RESULTS OF PREVENTIVE INOCULATION IN AN INOCULATED POPULATION OF 400,000

BY

E. GRASSET

*Officer in Charge of the Serum Department, South African
Institute for Medical Research, Johannesburg*

In a communication presented four years ago at the eleventh South African Medical Congress I gave (Grasset, 1935) an account of the principles and methods in the use of typhoid endotoxoid (endo-anatoxin) as an immunizing agent against typho-paratyphoid infections, and of the preliminary results of this new form of antigen in the prophylaxis of typhoid fever on the Rand gold-mines of the Transvaal. Since then a number of publications, concerned chiefly with technical points and progressive steps in the perfection of the method, have appeared in various scientific journals. The present account, however, deals with the development and application of this new form of prophylaxis as actually applied to over 400,000 persons. It also records the results and statistics relating to the use of typhoid endotoxoid in the prevention of typhoid fever, and provides a comparison with those obtained by other methods of anti-typhoid immunization.

Nature and Principles of Endotoxoid Vaccine

The vaccine, prepared according to the method I elaborated (Grasset, 1933; Grasset and Gory, 1927), consists of *B. typhosus* endotoxin, extracted from concentrated heated bacterial emulsions (30,000,000,000 organisms per c.cm., equivalent to about 10 mg. of dry bacilli per c.cm.) by means of an alternate freezing and thawing process. The toxic soluble antigen, separated by centrifugation, is subsequently transformed, after the addition of an equal volume of saline, or preferably of Martin's broth, which enhances the detoxicating action of the formol (0.5 per cent.) at incubator temperature for six weeks. This results in an atoxic product which nevertheless retains its original immunizing properties. Finally it is diluted for use to an antigenic concentration corresponding to that extracted from 5,000,000,000 to 6,000,000,000 organisms of the original emulsion—that is, 1.5 to 1.8 mg. of dry bacilli—as the standard concentration, and up to 8,000,000,000—that is, 2.4 to 2.5 mg.—when the single inoculation method is contemplated.

As has been shown by Felix (Felix and Pitt, 1934a, 1934b, 1935; Felix and Bhatnagar, 1935), Topley *et al.* (1937), and Perry *et al.* (1933a, 1933b, 1934) in the case of suspension anti-typhoid vaccines, so experimental investigations have also convinced us of the importance of selecting Vi typhoid strains in the preparation of endotoxoid. When injected into the mouse, typhoid endotoxoid protects this animal against the intraperitoneal injection of several fatal doses of both virulent Vi and O strains of *B. typhosus* and of their endotoxin (Grasset and Lewin, 1937). These higher immunizing powers, coupled with the tolerance of both animals and man to large doses of the vaccine, afford the practical advantage that endotoxoid can be safely injected in considerably higher concentration than would be possible, without risks of undue reaction, with other forms of suspension or detoxicated typhoid vaccine. On the same principle, endotoxoids have been prepared from paratyphosus A, B, and C bacilli, capable of producing similar specific protection against

the respective infective agent. By combining the different antigens a polyvalent typho-paratyphoid endotoxoid is obtained.

Nature of the Reaction

Preliminary applications carried out in 1933 on Europeans and natives, since confirmed by large-scale immunization, have shown that typhoid endotoxoid can be safely injected from infancy to old age. So far as children are concerned, observations on several thousand indicate that, apart from a certain hyperaemia at the point of inoculation, no appreciable reaction follows endotoxoid injection. The reactions observed in a small proportion of young subjects are of a mild and transitory nature, such as lassitude or slight malaise, accompanied on rare occasions by a rise in temperature of 0.5° to 1° F. during the day following the injection. The observations of de Villiers (1937), relating to systematic typhoid endotoxoid immunization in a closed European population of 5,499 persons (men, women, and children) on the Vaal-Hartz irrigation work, endorse our personal observations. Immunization consisted of two subcutaneous injections of 0.5 and 1 c.cm. of endotoxoid at eight days' interval for the adults, while in children proportional doses, varying from 0.1 to 0.8 c.cm., were used according to age. This system of dosage was employed throughout the whole series of immunizations recorded in this paper.

With regard to the temperature reaction following endotoxoid inoculation, Meyerstein (1938) has recorded his experiences on the same Vaal-Hartz works noted above in a group of 1,908 persons comprising men, women, and children—Europeans, natives, and Eurafricans. His findings are given in Table I below. The author states that so far as possible a record was obtained of any untoward generalized reaction towards the inoculation by the individual in the form of the persistence or not in over eighteen to twenty-four hours of any rise in temperature that might have been incurred due to the injection of the vaccine.

TABLE I

Temperature	First Inoculation			Second Inoculation		
	E %	EA %	N %	E %	EA %	N %
Normal ..	98.13	99.40	99.88	99.40	98.84	99.95
<99° F. ..	1.70	0.60	0.12	0.48	1.16	0.05
<100° F. ..	0.17	0	0	0.06	0	0
>100° F. ..	0	0	0	0.06	0	0

E=European. EA=Eurafrican. N=Native.

The above figures show that the temperature reaction following the endotoxoid inoculation is practically negligible.

Typhoid Endotoxoid Immunization on the Rand Gold-mines

The numerous data relating to the immunization of over 300,000 native workers on the gold-mines emphasize the high protection conferred by typhoid endotoxoid, and the exceptionally low incidence of reactions, varying from 0.3 to 1 per cent., an advantage which contributes to its easy acceptance, since there is no interruption of work. Analysis of the occasional reactions observed leads to the opinion that among the responsible factors the individual susceptibility plays a much more important part than the nature or concentration of the antigen.

Orenstein (1937), referring to the results of typhoid endotoxoid inoculation on the Rand mines, states: "There

are no cases on record among those inoculated with typhoid endotoxoid of any reaction which incapacitated the inoculated person in any way whatsoever for even a day." Van Blommestein (1936), in his paper on the control of typhoid fever by systematic immunization with endotoxoid on the Brakpan Mine, comes to the same conclusion. Referring to the efficacy of endotoxoid during the two years of observation, he concludes: "60,000 natives have been inoculated with this vaccine, and the incidence of enteric fever among those inoculated is one case of enteric and two of paratyphoid."

These results are particularly interesting if one considers the appreciable incidence of typhoid on this mine during the years preceding the use of endotoxoid, in spite of systematic T.A.B. immunization. The incidence was for the period 1932-4 an average of 5 per 1,000, with a mortality of 1 per 1,000, compared with 0.2 per 1,000 and no mortality for the two years 1935-6 since the use of typhoid endotoxoid. T.A.B. immunization had been carried out for some years on other Rand mine groups, but, according to Orenstein, the number of reactions was so high that mass protection with this vaccine was a practice difficult to carry out. It is for this reason that, prior to endotoxoid inoculation, oral immunization according to Besredka's method had been used on a large scale for some years. A preliminary comparative immunization with this last method and with endotoxoid, carried out on one of the Rand mines (E.R.P.M.) by Frew in the years 1934-5 on 14,000 natives and reported by Orenstein (1937), demonstrated both the much milder type of infection following the use of endotoxoid and the much lower mortality. Immunization by endotoxoid showed a mortality of 0.3 per 1,000, against 1.8 per 1,000 with oral immunization; and a case mortality of 4.48 per cent., as against 40.42 per cent. Unfortunately the full benefit of the endotoxoid immunization could not be deduced from these experiences, particularly in relation to the incidence rates, as in most of the doubtful typhoid cases final diagnosis was based on agglutination tests alone, which introduced the following fallacy. Further serological investigations showed, in about 90 per cent. of the endotoxoid-immunized persons, the presence of typhoid H and O agglutinin to high titre, with an average of 1/800 H and 1/220 O, and in many cases much higher—1/8,000 H and 1/5,000 O—these titres being considerably above those normally accepted as diagnostic of typhoid (Grasset and Lewin, 1936).

Following on these preliminary experiences a very rapid application of endotoxoid was witnessed. In the gold-mines, while 2,000 persons were immunized in 1933, 21,704 were inoculated in 1934, 45,849 in 1935, 84,837 in 1936, and over 160,000 in 1937, which represents approximately 50 per cent. of the permanent labour personnel. The total number of workers inoculated was 314,000, from thirty-six different mines. Thanks to the statistical department of the Transvaal Chamber of Mines, and to the co-operation of the mine medical officers, we were enabled to obtain general statistics for the whole of those employed in the Rand gold-mines for the period 1934-6, relating to the respective morbidity and mortality rates for the three following sections: (1) those immunized with endotoxoid; (2) those submitted to oral immunization; (3) non-immunized subjects. As will be seen from Table II, giving the comparative data relating to typhoid endotoxoid and oral immunization, a gradual fall in typhoid incidence from 1.8 to 0.89 per 1,000 occurred during the three years among the workers inoculated with endotoxoid, with a pronounced drop in the death rate, which was respectively 0.64, 0.11, and 0.19 per 1,000,

compared with the considerably higher and irregular incidence and death rates among natives submitted to oral immunization.

TABLE II

Year	Typhoid Endotoxoid					Oral Immunization				
	No.	Cases	Dths.	Rates per 1,000		No.	Cases	Dths.	Rates per 1,000	
				Inc.	Dths.				Inc.	Dths.
1934	21,704	39	14	1.80	0.64	21,573	179	36	8.23	1.67
1935	45,849	59	5	1.29	0.11	26,403	122	30	4.62	1.14
1936	84,837	76	16	0.89	0.19	29,595	337	70	11.39	2.36
Total	152,390	174	35	1.14	0.23	77,571	638	136	8.24	1.75

General statistics on the total of 152,390 workers immunized with endotoxoid during these three years show 174 cases, twenty-nine of these after one inoculation, with thirty-five deaths; while among 77,571 subjects submitted to oral immunization during the same period there were 638 cases with 136 deaths. These comparative figures show that the morbidity and mortality rates were seven times lower in the endotoxoid-immunized than in the oral-immunized group.

Graph showing comparative results of endotoxoid and oral tablet immunization.

Finally, 2,231 cases of typhoid and 477 deaths were observed during the same period in 580,514 non-immunized workers, representing a mortality of 0.82 and a morbidity of 3.84 per 1,000, being respectively 3.5 and 3 times higher than among the endotoxoid-immunized subjects. These comparative figures are particularly interesting if one considers that most of these uninoculated workers belong to mines where inoculation was not introduced because epidemiological conditions were considered satisfactory. They reflect the influence of typhoid endotoxoid immunization on herd immunity.

In consequence of the rotation policy adopted with regard to workers on the mines, with an average period of contract of about twelve months, periodical introduction of carriers is inevitable. Sporadic cases or small epidemics are most likely to occur in spite of adequate hygienic measures unless systematic immunization of all new workers is carried out. The importance of such a policy being realized, the following resolution was passed by the members of the Transvaal Mine Medical Officers' Association (1937) at their general meeting in October, 1937: "That this association recommends the prophylactic inoculation against typhoid fever with endotoxoid vaccine

of all native labourers arriving on the mines, and that this inoculation be started immediately."

The effect of the wide application of inoculation, carried out on the thirty-six most important mines of a total of fifty-six, is already evidenced by the substantial drop in the general typhoid incidence and mortality on the whole of the Rand mines during 1937. Statistical figures of the Chamber of Mines relating to the whole of the workmen employed on the Rand mines during that year, and irrespective of the percentage inoculated, show, among 299,786 workers, a typhoid morbidity of 2.44 and a mortality of 0.54 per 1,000—that is, respectively, 28 per cent. and 30 per cent. lower than the corresponding figures for 1936. These are the lowest morbidity and mortality rates since 1929.

Application of Typhoid Endotoxoid Immunization by Other Public Health Organizations

Apart from the gold-mines and collieries, there has been a wide and increasing application of typhoid endotoxoid immunization, largely in place of other typhoid prophylactics previously in use, by public health organizations in both urban and rural areas—on several large irrigation schemes, in various towns and municipalities, in gaols, medical centres, native townships, and for hospital staffs, etc. The Union Department of Public Health has also advocated its use on the strength of a recommendation from the South African Council of Public Health. A typical example of the results of systematic endotoxoid immunization in a mixed population is that realized at the Vaal-Hartz irrigation scheme quoted above. As shown in the reports of de Villiers and Meyerstein—medical officers in charge of the works—not a single case of typhoid was reported in a population of 9,329, composed of workers and their families, inoculated from 1933 to 1937 with two doses of typhoid endotoxoid.

The information obtained from the various sources shows that wherever endotoxoid immunization has been introduced typhoid morbidity and mortality have dropped in impressive proportions. Evidence of these experiences soon resulted in endotoxoid being employed outside South Africa. Extended use has already been made in various British and foreign colonies. Investigations are now proceeding in several European countries especially affected by typhoid, such as Hungary and Russia. Recently, after my report to the sixth Conference of the International Association for Preventive Paediatrics, held at Rome in September, 1937, the recommendation of the use of typhoid endotoxoid as an immunizing agent against typhoid was incorporated among its conclusions (Grasset, 1937).

Endotoxoid Immunization among Children

Particularly interesting are the results obtained in campaigns carried out in several native townships in Johannesburg. In the Alexandra township of 20,000 persons, where typhoid had been particularly prevalent among children during the last few years (5.1 morbidity and 2.2 mortality per 1,000 in 1934), 4,351 natives, mostly children, were immunized by endotoxoid during 1935–7. Among the group of 3,208 immunized in 1935–6 only one case of typhoid fever, not fatal, was observed. During the same period 131 cases of typhoid were notified among the non-immunized population, the incidence rate being 3.4 per 1,000, compared with 0.31 among the inoculated persons—that is, ten times higher; the death rate of *nil* among inoculated subjects was a contrast to the 1.05 per 1,000 among the non-inoculated, representing forty deaths.

Statistics relating particularly to children in 1936 show the presence of only one case among 2,052 inoculated (0.48 per 1,000), compared with forty-seven cases among 3,000 non-inoculated children (15.6 per 1,000)—a ratio about thirty-two times higher. Not a single case of typhoid was observed during 1937 among the 4,351 immunized persons. Inoculation consisted of two injections of endotoxoid at eight days' interval: 0.2 and 0.3 c.cm. for children of 1 to 5 years; 0.3 and 0.4 c.cm. for those of 5 to 10 years, and 0.4 and 0.8 c.cm. for those from 10 to 15 years; and 0.5 and 1 c.cm. for the adults.

Another example which may be quoted is that of the campaign carried out against typhoid at Sophiatown, a native township of some 10,000 persons, where typhoid endotoxoid immunization was introduced among school children in the year 1936. This leads to the consideration of a special aspect of the subject.

Combined Anti-typhoid-pneumonia Immunization

The susceptibility of the black races to pulmonary infections is well known. The work of Lister in South Africa has shown the importance of the types of pneumococci in the epidemiology of this infection and in the preparation of anti-pneumonia vaccine, used, on a large scale and with success, on the Transvaal gold-mines during the last twenty-two years (Lister, 1917; Lister and Ordman, 1935). The frequent occurrence of both pneumonia and typhoid in these native communities, and the difficulties experienced in carrying out multiple inoculations, have led us to devise a combined immunization by means of a mixed anti-typhoid-pneumonia vaccine, consisting of a suspension of 10,000,000,000 pneumococci per c.cm. in typhoid endotoxoid. Injected into mice this produced an anti-typhoid protection as strong as that from the endotoxoid alone. The simplification of technique resulting from this combined procedure has been followed by its rapid application among both children and adult native members of the population. Immunization consists of three inoculations at eight-day intervals, the third dose being necessitated by the pneumonia immunization.

For the sake of comparison, of a total of 1,357 children from the age of 2 to 17 years inoculated in the schools of Sophiatown, 824 were submitted to the combined immunization and 535 received typhoid endotoxoid alone. No cases of typhoid were observed in either of these two groups during the following sixteen months, including two typhoid seasons, while typhoid fever continued to occur among the non-immunized section of the population, including three cases in a neighbouring school, where no vaccination had been performed. Among adults, since the beginning of 1937, 48,000 native labourers on the gold-mines have been submitted to inoculation with this combined vaccine, with similarly good results and no considerable increase in reaction.

Anti-typhoid Immunization by Means of a Single Typhoid Endotoxoid Inoculation

Serological investigations having shown that a single injection of typhoid endotoxoid of high antigenic powers is sufficient to produce in man an agglutinin titre as high as 1/10,000 H and 1/5,000 O without undue reaction, a series of single inoculation immunizations was instituted among children and adults. On the Van Ryn Deep Mines 5,445 workers of a total of 6,652 were submitted to this single inoculation during the period September, 1936, to July, 1937. Only one non-fatal case of typhoid has been noted among these inoculated subjects during the year

following the introduction of the immunization, while eight cases with two deaths were recorded among the small proportion of non-inoculated workers (Grasset, Lewin, and van der Merwe, 1937).

With a view to the possibility of reinforcing the immunity so produced investigations were undertaken with an alum-precipitated endotoxoid prepared on lines similar to the principle elaborated by Glenny for diphtheria immunization. Such precipitated endotoxoid confers upon mice a protection against *Vi B. typhosus* at least as strong as that conferred by unmodified endotoxoid; in man, without causing undue reaction, it is followed by an agglutinin formation to titres such as 1/800 of H and 1/200 of O during the month following the inoculation.

Recent investigations on the nature of typhoid endotoxoid carried out in collaboration with H. D. Barnes—to be incorporated in a separate publication—have shown that by means of alcoholic precipitation a purified antigen is obtained. On being redissolved in saline the precipitated material confers on the mice and rabbits a degree of immunity and an agglutinin titre equal to that afforded by the untreated endotoxoid. These results are in agreement with those obtained by Topley, and Boivin in France, with the lipide-polysaccharide complex, isolated by various methods from the *B. typhosus*. Immunization of man has been undertaken with this purified endotoxoid, and this procedure also results in the formation of H and O agglutinins to considerable titre.

With regard to the duration of the immunity produced by endotoxoid, serological investigations show that a very appreciable H and O agglutinin titre is still present at the end of the first year in the blood of the persons inoculated. Epidemiological data indicate that there is present in the second year a degree of protection practically equivalent to that observed during the first year.

The introduction of typhoid endotoxoid is too recent for an actual limit of protection to be fixed. That information can only be accumulated over a period of years by epidemiological data and by a close study of the greatest possible number of subjects who have been immunized by this new form of vaccine.

REFERENCES

- De Villiers, J. J. (1937). *S. Afr. med. J.*, **11**, 221.
 Felix, A., and Bhatnagar, S. S. (1935). *Brit. J. exp. Path.*, **16**, 422.
 — and Pitt, R. M. (1934a). *J. Path. Bact.*, **38**, 409.
 — (1934b). *Lancet*, **2**, 186.
 — (1935). *J. Hyg., Camb.*, **35**, 428.
 Grasset, E. (1933). *C. r. Soc. Biol., Paris*, **113**, 20.
 — (1935). *S. Afr. med. J.*, **9**, 208.
 — (1937). *Int. Ass. prev. Pédiat.*, 6th Conference, Rome, 69.
 — and Gory, M. (1927). *C. r. Soc. Biol., Paris*, **97**, 1211.
 — and Lewin, W. (1936). *Brit. J. exp. Path.*, **17**, 179.
 — (1937). *C. r. Soc. Biol., Paris*, **124**, 331.
 — and van der Merwe, T. (1937). *S. Afr. med. J.*, **11**, 660.
 Lister, F. S. (1917). *Publ. S. Afr. Inst. med. Res.*, **1** (No. 10), 303.
 Lister, Sir Spencer, and Ordman, D. (1935). *Ibid.*, **7** (No. 37), 1.
 Meyerstein, R. (1938). *S. Afr. med. J.*, **12**, 324.
 Orenstein, A. J. (1937). *Ibid.*, **11**, 401.
 Perry, H. M., Findlay, H. T., and Bensted, H. J. (1933a). *J. R.A.M.C.*, **60**, 241.
 — (1933b). *Ibid.*, **61**, 81.
 — (1934). *Ibid.*, **62**, 161.
 Topley, W. W. C., et al. (1937). *Lancet*, **1**, 252.
 Transvaal Mine Medical Officers' Association. Report of Meeting (1937). *Proc. Transv. Mine med. Off. Ass.*, **17**, 44.
 Van Blommestein, J. H. G. (1936). *Ibid.*, **16**, 9.

Dr. Nathan Bristol Van Etten, medical director of New York City's Morrisania Hospital, has been chosen president-elect of the American Medical Association, and will be inducted into office in June, 1940, when the Association meets in New York. At the recent annual meeting in St. Louis the attendance was 7,412.

SUDDEN BLINDNESS

BY

JOSEPH MINTON, F.R.C.S.

Ophthalmic Surgeon to Out-patients, Hampstead General Hospital; Ophthalmic Surgeon, Queen's Hospital for Children; Assistant Ophthalmic Surgeon, West End Hospital for Nervous Diseases; late Research Scholar, Royal Eye Hospital, London

Of sudden loss of sight of one or both eyes vascular lesions are the most common cause. Von Graefe reported the first cases of embolism of the central artery of the retina in 1859, and for many years this was accepted as the only possible cause of obstruction of the central retinal artery. The great advance in the study of ophthalmoscopic appearances of the retinal arteries and veins has enabled us to understand more fully the pathological changes which underlie the occlusion or obstruction of the retinal artery and the sudden loss of sight that ensues. George Coats in 1906, as a result of pathological research, came to the conclusion that obliterative endarteritis, with or without thrombosis, can produce a sudden obstruction of the central artery or of any of its branches, and that this form of obstruction is a much more likely event than embolism.

Spasm of the retinal arteries was first observed in 1874 by Raynaud in a patient suffering from the disease which now bears Raynaud's name. Since this date angiospasm of the retinal arteries with associated temporary blindness has been studied by many observers and proved to occur in lead and quinine poisoning, epilepsy, and migraine. In recent years Mylius has given an account of ophthalmoscopic observations in eclampsia and pre-eclampsia, in which conditions temporary visual disturbances due to spasmodic contractions of the retinal arteries are fairly common and may be one of the earliest diagnostic features. The observations of Mylius and Wagener, that the retinal arterioles may show both localized and generalized spasm in the toxæmia of pregnancy, suggest that these are probably general throughout the arteriolar system and are closely related to hypertension. The investigations of Wagener and Keith confirm Allbutt's original conception that hypertension is primarily due to spastic constriction of the peripheral arterioles, resulting in a dynamic increase of resistance.

Hypertension

The work of Dumas, Wagener, and Keith has led to the adoption of a subdivision of hypertension into three phases. Sudden blindness due to the occlusion of the retinal arterioles has been observed in all stages of hypertension.

THE FIRST PHASE

In the first or benign phase of hypertension the patients may be in good general health and have very few symptoms. The retinal arterioles are usually narrowed and may show irregularity of lumen as a result of tonic contraction with superadded localized spasm. The patients may complain of transitory blindness affecting one or both eyes, this blindness lasting a few minutes to a few hours. The sight is usually regained, but in many cases such attacks are followed by permanent blindness of the affected eye. These transitory attacks are due to a sudden spasmodic contraction of the retinal arterioles, which may subside quickly, having caused a temporary anaemia of the retina. Prolonged attacks lead to a post-spastic thrombosis of the affected vessel and permanent damage