The Effectiveness of Vaccines Used for the Prevention of Typhoid Fever in the United States Army and Navy

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THE practical results obtained with specific typhoid prophylaxis indicate that, while this procedure does not confer an absolute immunity, it has been of great value in reducing the incidence of typhoid fever among troops, even when living under insanitary conditions. However, because of the lack of a suitable laboratory test of vaccine potency, there has been some uncertainty as to the exact degree of protection afforded by vaccination, and considerable speculation as to the relative merits of different typhoid vaccines.

With the development of newer concepts of the various antigenic changes which may accompany visible dissociative alterations in bacterial cultures, attempts have been made to utilize such information in establishing exact criteria for the selection of cultures most suitable for use in typhoid vaccines. Theories recently evolved from studies of bacterial variation have led certain individuals to question the value of vaccines prepared with sub-strains of the well known Rawlins culture of typhoid bacillus, on the assumption that during their long period of cultivation on artificial media these cultures may have lost certain characteristics necessary to an effective immunizing agent. These theoretical objections to the Rawlins strain have been based largely on cultural and immunological reactions observed in the laboratory, and there has been considerable disagreement as to their validity.

Since a sub-strain of the Rawlins culture has been employed in all typhoid vaccines prepared at the Army Medical School and used by both the Army and Navy, those responsible for the manufacture of these vaccines have naturally been interested in determining whether this culture is still suitable for vaccine production. In searching for the answer to this important problem it must be borne in mind that while theoretical considerations based on antigen-antibody reactions may be suggestive, the only known measure of the protective action of a typhoid vaccine is the incidence of typhoid fever among large groups of vaccinated individuals.

It is the purpose of this paper briefly to review the history of typhoid vaccination; to recount the experience with the U. S. Army sub-strain of the Rawlins culture; to discuss various fac-

tors, including dissociation, which might have influenced the protective action of the vaccines; to correlate these factors with the incidence of typhoid fever; and to determine, if possible, to what degree the vaccines now in use conform to the requirements of a satisfactory prophylactic agent.

EARLY HISTORY OF TYPHOID VACCINATION

The possibility of artificially immunizing human beings by the inoculation of typhoid bacilli was naturally suggested at an early date by the successful use of vaccines for the prevention of certain other diseases, and by the observation that one attack of typhoid fever usually protects against subsequent infection.

During the latter part of the 19th century many investigators attempted to test this possibility by vaccination experiments with laboratory animals, including guinea pigs, rabbits, and mice. Chantemesse and Widal [1] (1888) and subsequent workers showed that such small animals, which could be killed in a short time by the inoculation of large numbers of living typhoid bacilli, might also be protected against the fatal toxic reactions by the preliminary injection of killed typhoid cultures. Because of the natural resistance of these animals to spontaneous typhoid infection, and their failure to develop a disease resembling typhoid fever, such experimental results were not considered as directly applicable to the problem of typhoid prophylaxis in man. However, discovery of the fact that the vaccination of animals produced specific antibodies, including agglutinins, bactericidal bodies, and bacteriolysins, similar to those found in the blood of typhoid convalescents, led to the hope that the presence of these antibodies might furnish a reliable index of immunity. Such observations, and the work of Haffkine on cholera immunization, stimulated interest in the possibility of protecting man against typhoid fever by the use of vaccines.

During 1896 Pfeiffer and Kolle,[2] in Germany, and Wright,[3] in England, inoculated suspensions of killed typhoid bacilli into human beings, observing that this procedure was safe and that it was followed by an increase in antibodies in the blood of such individuals. In 1897, Wright [4] suggested the use of typhoid vaccines in armies and hospitals; and started his pioneer work which constitutes the real beginning of specific typhoid prophylaxis. During the next few years he and Semple [5] prepared vaccines from killed broth cultures of typhoid bacilli, with which they inoculated several thousand British soldiers in India and a number of persons in England. During the Boer War he and Leishman furnished 400,000 doses of vaccine for voluntary use among the English troops; and it is estimated that about 100,000 men were inoculated. While the vaccination records for the Boer War were incomplete, Wright [6] concluded that among those inoculated the incidence was diminished about 50 per cent and the mortality even more. However, for the entire Army with a total strength of 380,605 the typhoid incidence rate was 151.56 per 1,000 and the mortality was 21.08 per 1,000. Such appalling rates created a very unfavorable impression, and there were many reports claiming that the vaccine not only failed to protect but had actually increased the number of cases and deaths. Consequently, toward the end of the War, typhoid inoculation was stopped in the British Army and a commission was appointed to re-investigate the subject.

As a result of their statistical studies this commission,[7] in 1904, made an interim report in which it was recommended that inoculation be resumed as a voluntary measure in the British

Army; and that experimental work be undertaken with a view to increasing the effectiveness of antityphoid vaccination. The investigations, which were continued for several years by Leishman and his associates, produced valuable information, and led Leishman [8] in 1910 to conclude that, while various factors might have played a part, the disappointing results of vaccination during the Boer War were almost entirely due to irregularities in the preparation and standardization of the vaccines. Consequently the older methods were modified in a number of ways including: (a) the selection of a more suitable strain of typhoid bacillus, (b) the use of cultures grown in nutrient broth for 48 hours instead of 10 to 16 days, (c) reduction of the killing point from 60–65° C. to a maximum of 53° C., (d) cooling of the heated suspension before the addition of 0.4 per cent lysol, (e) standardization of the vaccine by Harrison's modification of Wright's counting method, (f) reduction of the dose to ½ and 1 c.c. amounts, of a 1 billion per c.c. suspension, and (g) adoption of an expiration date of 3 months.

ORIGINAL SELECTION OF THE RAWLINS STRAIN OF E. TYPHI

In view of the present question as to the relative immunizing value of different strains of typhoid bacilli, it is of interest to note that this problem was given serious consideration in 1904 by the British commission.[7] In their effort to produce the most satisfactory vaccine possible, for military use—that is, one combining maximum protective action with sufficiently low toxicity—they investigated various strains of *E. typhi*, including old cultures, and others which had recently been isolated from cases of typhoid fever. It was observed that "all strains did not behave alike when grown under identical conditions," and the conclusion was reached that "it was not clear upon what this variability depended as no constant factor such as age, virulence, length of time since isolation, etc., was found." The commission selected as most suitable for use, in the British Army vaccine, the now well known Rawlins strain of typhoid bacillus, which was isolated in 1900 from the spleen of a fatal case of typhoid fever and had already been cultivated on artificial media for a period of 4 years.

In discussing the reasons for adopting the Rawlins strain, Leishman [8] called attention to the current controversy as to the relative merits of cultures of high and low virulence; and stated that an attempt was made to settle this point by animal experiments using two vaccines: "one prepared from our usual non-virulent strain, the other from a strain recently isolated from a fatal case of enteric fever and which was found to possess a high degree of virulence for guinea pigs." The two vaccines were inoculated into rabbits in amounts comparable, on the basis of weight, to the doses used in man; and quantitative estimations were made of the various protective substances which appeared in the blood during the following weeks. While the first experiments showed a slight margin in favor of the vaccine prepared from the recently isolated, more virulent strain, this was not confirmed, as in later experiment both vaccines gave identical results. Consequently Leishman made the following statement:

> We concluded that a virulent strain might perhaps be expected to give rise to a slightly higher degree of immunity but that the difference in this respect was not marked. We have also convinced ourselves by many experiments that the protective substances elaborated in response to inoculations of a non-virulent vaccine are highly active *in vitro* against a freshly isolated and virulent strain of *Bacillus typhosus*. We have made no change then in this respect, but adhere to the use of a strain of typhoid which has been long sub-cultured in the laboratory, and now

possesses but a very slight degree of virulence for guinea pigs. We are influenced in this decision, however, by another factor—namely, the fact that vaccines prepared from a virulent strain of *B. typhosus* give rise in man to more severe reactions both local and general. . . .

It is extremely probable that not all strains of non-virulent typhoid bacilli are equally suitable for use in vaccines; we have reason to believe that that which we employ is exceptionally so. Wassermann considers that the virulence of the organism is not of so much importance as its affinity for the specific amboceptors and in this our non-virulent strains excel.

In view of the recent interest in dissociation it is of importance to note that certain physical growth characteristics were also considered in selecting the Rawlins strain. To quote Leishman [9]:

It was originally intended to employ for this purpose a strain "G," isolated from the spleen at Netley 5 years ago, which had been largely employed by Dr. Wright and myself in the preparation of vaccine, and is still employed by Dr. Wright for this purpose. In our preliminary work, however, it was found to possess the disadvantage of being a strain which could only be emulsified from an agar culture with great difficulty and at the sacrifice of more time than we were likely to be able to afford. Further experiment resulted in the selection of another strain "R," of similar origin and of about the same age, which had also been extensively employed at Netley in the preparation of vaccine. This strain was one which furnished a very even and satisfactory emulsion from an agar culture and was thus more suitable for some parts of the delicate experimental work which lay before us. Both these strains being of low virulence, and of proved suitability for inoculation, preference was accordingly given to that which promised to give more regular results in our test experiments, and the strain "R" was, therefore employed, both in the preparation of the vaccine and in the daily quantitative tests of the protective substances developed in the blood of the inoculated.

Thus it appears that when adopted for use in the British Army vaccine in 1904, the Rawlins strain, which was then 4 years old, was selected because of its relatively low toxicity for guinea pigs and man, its satisfactory stimulation of certain antibodies and because it had at least one characteristic of what has since been designated as a smooth type of *E. typhi;* that is, it produced a uniform diffuse growth in broth, and suspensions from agar cultures were not agglutinated by physiological salt solution.

This strain was used by the British Army from 1904 until 1933; and a sub-strain obtained in 1908 has been used since that date in all typhoid vaccines manufactured for the United States Army and Navy. Still other sub-strains have been furnished to public health and commercial laboratories throughout the world; and it seems safe to say that the descendants of the so-called Rawlins culture of typhoid bacillus have been used more extensively than any other for vaccine production. Thus by virtue of extensive use in prophylactic vaccines, if for no other reason, this strain has acquired an importance which demands for it serious consideration.

THE U. S. ARMY SUB-STRAIN OF THE RAWLINS CULTURE

Typhoid vaccination was introduced into the U. S. Army in 1908 by General F. F. Russell who, after a thorough study of the procedures used in the English and German Armies, adopted a modification of the two methods and selected for use in the American vaccine a sub-strain of the British Rawlins culture. In a discussion of antityphoid vaccination in 1913, Russell [10] commented on this strain as follows:

The English vaccine is, therefore, a killed broth culture made from a single strain of the bacillus, which was originally selected because it emulsified well from agar slants. Preliminary trials of this organism showed that it agglutinated well in immune serum, and produced in good quantities all measurable kinds of antibodies in animals and man. This strain is still in use in England and in our military service, and although we have searched from time to time for a strain with greater antigenic properties, none has yet been found.

As this same Rawlins sub-strain has been used continuously in our Army, it has been responsible for whatever protection we have derived from typhoid vaccination. However, from time to time theoretical objections have been raised concerning its relative effectiveness.

THEORETICAL OBJECTIONS TO THE RAWLINS STRAIN

In 1917 Weiss [11] questioned the advisability of using the Rawlins strain in vaccines, on the assumption that it belonged to a relatively small subgroup which differed from the majority of typhoid cultures. He reported that, of 31 typhoid strains which he investigated, 6 failed to produce acid in xylose broth, and that 4 of these appeared to be atypical antigenically in that they did not absorb agglutinins for the xylose-fermenting strains. Observing that a Rawlins strain was one of those that failed to ferment xylose, Weiss made the following statement:

> We wish also to emphasize that on a basis of these results we believe that in typhoid prophylaxis the single "Rawlings" vaccine may not give complete protection. Recent cases of typhoid fever among soldiers who have received the prophylactic treatment have come to our attention and lend weight to our belief. We would recommend that a polyvalent vaccine, or one made from an organism which is more representative of the whole group should be used.

However, Teague and McWilliams,[12] by plating and selecting different colonies of a single culture of *B. typhosus*, showed that differences, with regard to the absorption of agglutinins, could be obtained at times with these subcultures, similar to those obtained by Weiss when using his xylose-fermenting and non-fermenting strains. Therefore, it was decided that the evidence offered by Weiss did not warrant the addition of other strains to the vaccine prepared at the Army Medical School. During 1918, strains of typhoid bacilli that apparently failed to ferment xylose were encountered in cultures obtained from certain American soldiers in France; and the question of xylose fermentation was investigated under the direction of General F. F. Russell, by Teague and Morishima.[13] These workers studied 116 strains of typhoid bacilli including the U. S. Army, Rawlins culture, and discovered that all of them fermented xylose, although about 8 per cent were slow fermenters—acting through the production of "daughter colonies." Similar observations were made concerning the fermentation of arabinose, and other sugars; and it was concluded that there was no evidence to justify Weiss's suggested division of typhoid bacilli into separate groups.

More recently there has been some question as to the value of typhoid vaccines in general, because of observations which indicate that the inoculation of vaccines into man fails to produce the so-called somatic or "O" type antibodies in such concentrations as are observed after recovery from typhoid fever. While flagellar and somatic antigens were differentiated in 1903 by Smith and Reagh,[14] their significance was not recognized until later when Weil and Felix [15] and their followers investigated the "H" or flagellar and the "O," or somatic antigens, of Proteus X19, and later extended these observations to organisms of the typhoid-paratyphoid group. In 1924 Burnet [16] showed that both the "flocular or H" and the "granular or O" types of agglutinins were developed about equally in the serum of cases of typhoid fever, while the agglutinins in the serum of vaccinated persons were mainly of the H type. Felix,[17] who associated the O type agglutinins with immune body, failed to demonstrate such anti-bodies in sera from vaccinated men. Consequently he and his followers believe that our present typhoid vaccines are not likely to give adequate

protection. Experiments performed by Arkwright,[18] in 1927, appeared to support this view of the importance of the O antigen, as he observed that guinea pigs were protected as well by vaccines containing only the O antigens as by others which contained both O and H antigens. However, Springut [19] and Ibraham, and Schutze [20] in vaccinating mice against *Bact. aertrycke* infection, obtained results which indicate that although the H antigen alone was of little immunizing value, the combination of H and O antigens was more effective than the O alone.

While the importance of the O type antigens is now generally recognized, there remains considerable doubt concerning the significance of O antibodies as indicators of protective immunity; and it appears that theories as to the supposed ineffectiveness of typhoid vaccines based on such reactions may be erroneous. This is suggested by such results as those obtained by Greenwood and Topley [21] in experiments with mice vaccinated with various antigens of *Bact. aertrycke*. These investigators observed that, among those animals which were protected against infection by vaccines containing O antigens, only a minority developed O type agglutinins in detectable amounts. Whitehead [22] in 1927 failed to demonstrate granular agglutinins in the sera of 6 out of 7 persons inoculated with the English Army vaccine, but he also obtained negative results with the sera of 12 typhoid convalescents. However, inoculation of the vaccine into rabbits was followed by production of granular agglutinins in large amounts. The fact that vaccination usually produces a higher O agglutinin content in the serum of rabbits than in man has been noted by many observers, including the authors, Cox,[23] and others. In spite of the speculation as to the possible significance of O antibodies, we have no proof that their titer indicates the degree of protection afforded by typhoid vaccines and we concur in the following comment made by Topley and Wilson [21] in 1931:

> It would seem that circulating antibodies in very low titer or a heightened reactivity of the tissues to the O antigen afford an adequate degree of protection.

Another problem of considerable current interest is the question as to the relative effectiveness of vaccines prepared with the so-called " smooth " and " rough " types of typhoid bacilli. The investigations on bacterial dissociation, by Baerthlein [24] 1918, Arkwright [25] 1920–1927, and deKruif [26] 1921, gave evidence that certain of the many obvious cultural changes, which are to be observed among the descendants of a single pure strain of bacteria, may be associated with alterations in other important characteristics including physiological reactions, chemical and antigenic structure, virulence, etc. Arkwright [27] from his studies of the colon-typhoid-dysentery group, called attention to the fact that the so-called normal or " smooth " types of these organisms possess certain common characteristics, including: (a) the formation of typically round, domed, smooth, shining, translucent, non-granular colonies on solid media; (b) the development of a uniformly turbid growth in broth; and (c) the production of agar growths which form uniform suspensions in 0.85 per cent solution of sodium chloride. He observed that such smooth cultures might give rise to several different variants, of which the most important was the form designated as " rough." This so-called rough variant type is characterized by: (a) colonies which are dry, flattened, irregular in outline and surface, and coarsely granular; (b) by granular, precipitated growths in broth; and (c) by the fact that organisms from agar cultures spontaneously agglutinate in 0.85 per cent salt solution. Such S

to R dissociation is of considerable practical importance because, when complete, it is often accompanied by other changes including a decreased virulence, and by marked antigenic alteration in which the specific somatic O antigen of the smooth type is lost and is apparently replaced by a rough somatic antigen which may be common to several related species. As it has been shown that the smooth to rough, and the motile to non-motile types of variation may occur independently, or simultaneously, it is now recognized that motile organisms of the colon-typhoid group may conform to either of the following forms (1) smooth motile, containing both H and O antigens; (2) smooth non-motile containing O antigen but no H; (3) rough motile retaining H antigen but with the O replaced by R or Ø antigen; and (4) rough non-motile containing only the R or Ø antigen. The work of Zinsser and Parker [28] (1923), Furth and Landsteiner [29] (1928), White [30] (1928), etc., suggests that the S—R change in the typhoid-paratyphoid group may be associated with chemical alterations similar to those observed during the dissociation of pneumococci (Heidelberger and Avery[31] 1923) (Reimann [32] 1925). In other words, it seems possible (Zinsser [33]) that the complete somatic antigen of the smooth type consists of two parts, a nucleo-protein common to all forms, and a soluble specific substance which alone produces no antibody but gives its specificity to the antigen as a whole; while the rough variants lose the SSS, retaining the nonspecific nucleo-protein antigen.

In vaccination experiments using guinea pigs and *Bact. paratyphosus* A, Arkwright [18] observed that "smooth" vaccines protected against a subsequent test injection of living smooth organisms while "rough" vaccines failed. Similar, but less striking, results were obtained with the typhoid bacillus. Likewise, in experiments with mice and *Bact. aertrycke*, various workers, including Springut,[19] Ibraham and Schutze,[20] and Greenwood and Topley,[21] have also shown that vaccines prepared with rough cultures were much less effective than those made from cultures of the smooth type. In 1930 Grinnell [34] observed that vaccination of rabbits, and of man, with a rough sub-strain of *E. typhi* Rawlins produced little or no increase in the specific bactericidal power of the blood as compared with similar vaccinations with smooth typhoid bacilli. However, noting that the rough vaccine produced agglutinins for the smooth strains, he concluded that the agglutination reaction is not a satisfactory test for immunity, and that, in so far as bactericidal action is an index of resistance, rough strain vaccines are of relatively slight prophylactic value. Grinnell [35] (1932) made a study of 12 Rawlins cultures obtained from different laboratories engaged in making antityphoid vaccine, comparing them with freshly isolated smooth strains with respect to morphology, motility, colony form, nature of the growth in broth, growth in 10 per cent normal horse serum broth, and stability of suspension in normal saline solution. He concluded that 1 of these Rawlins strains corresponded in cultural and serological characteristics to the classical rough variant, and that the remaining 11 gave cultural reactions intermediate between the rough and smooth types; also that "in the stability of their suspensions in normal salt solution and in their agglutinability in the serum of rabbits immunized with non-motile smooth antigens—criteria on which Arkwright lays great stress—they corresponded to the smooth phase." However, he states that "as judged by the more significant tests of the bactericidal action of normal blood and by their virulence for mice, they should

be considered rough." Grinnell reported several experiments in which he observed: (a) that organisms from 18 hour broth Rawlins cultures were killed by quantities of fresh defibrinated guinea pig blood which had no bactericidal effect on the smooth cultures; (b) that when 0.1 c.c. amounts of 18 hour broth cultures were inoculated into mice, 95 per cent of those which received the smooth cultures died, while death occurred in only 3.3 per cent of those injected with the Rawlins culture; and (c) that a large proportion of mice, vaccinated with smooth strains, survived a subsequent intraperitoneal dose of a smooth culture, whereas most of those, vaccinated with a Rawlins strain, failed to survive this toxic dose.

Grinnell concludes from these experiments that

> . . . since the Rawlins strain differs from the smooth phase of *Bact. typhosum* in cultural characteristics and in virulence, and is much less efficient than smooth strains as a protective antigen; . . . it would seem but logical to substitute virulent smooth cultures for the very old Rawlins strain, if we are to expect the maximum protection from antityphoid vaccination.

This conclusion appears to us to be predicated entirely upon the assumptions (a) that the protective value of a typhoid vaccine can be measured by demonstrable antigen-antibody reactions; (b) that the infectivity for man of a typhoid culture is proportional to its toxic action when introduced into the peritoneum of a mouse; (c) that the degree of protection afforded a mouse against the toxicity of a strain is an index of the protection afforded man against natural invasion and infection with the same strain; and (d) that the Rawlins strain is a fixed entity irreversibly dissociated to a degree rendering it unfit for vaccine production, and that none of its sub-strains have been, or can be, maintained in a satisfactory dissociative state. On the other hand, Maltaner [36] found that, measured by protection of rabbits against intravenous inoculations of living cultures, rough typhoid vaccines were distinctly superior to the smooth, even against smooth organisms. These results led him to the following conclusion:

> Certainly the suggestion that smooth organisms be substituted for those of intermediate types, which have been most commonly employed, is not at present justified.

Such contradictory observations emphasize the fact that we still have no satisfactory laboratory criterion for determining the practical value of a typhoid vaccine, and leave unanswered the question of the relative merits of smooth and rough vaccines. Therefore, we propose to review the history of the U. S. Army Rawlins sub-strain and to look for evidence of dissociation both in the culture and in the practical experience with the vaccine. If this sub-strain has dissociated during the 25 years of its use, and if dissociation has significantly affected the protective action of the vaccine, then such alteration should be clearly reflected in the practical results obtained with the vaccine.

DISSOCIATIVE CHARACTERISTICS OF THE U. S. ARMY RAWLINS SUB-STRAIN

As indicated above, the Rawlins strain was isolated 33 years ago from the spleen of a fatal case of typhoid fever. After 4 years of cultivation on artificial media it was selected by Leishman for use in vaccines prepared for the British Army, because of its relatively low toxicity for guinea pigs, its satisfactory antibody production, and because of the fact that it formed a uniform suspension in salt solution. After the strain had been cultivated in England for a period of 8 years it was selected by General Russell for vaccine production in the U. S. Army.

Since its introduction at our Army Medical School, this Rawlins sub-strain has been maintained under conditions

which have probably kept its dissociative characteristics rather stable and uniform. The stock vaccine culture has been kept on agar slants, in sealed tubes, at refrigerator temperature; and at intervals of 20 to 30 days the cultures have been checked for purity and transferred to new media. For the production of vaccine, transplants are made from stock cultures to nutrient agar in Petri plates, and from these cultures about 12 typical colonies are selected for transfer to large, nutrient-agar slants, and to Russell's double-sugar media. After incubation, the cultures on double-sugar are tested with stock agglutinating serum containing both H and O agglutinins; and broth is added to the agar slant cultures. After several hours, the latter broth suspension of the "seed" culture is used for the inoculation of nutrient agar in Kolle flasks, which are then incubated at 37° C. for 24 hours. The organisms are then removed, suspended in buffered physiological salt solution, killed by heating in a bath at 56° C., and, after counting, 0.25 per cent cresol is added. The bacterial content is estimated with the Helber counting chamber, after which the vaccine is diluted to the desired concentration with 0.85 NaCl solution. The control tests carried out with each lot of vaccine include: (a) microscopic examination of unstained and Gram stained preparations, (b) various checks to rule out contamination and insure sterility, (c) the inoculation of mice, guinea pigs, and rabbits to detect abnormal toxicity, (d) study of the growth on agar to insure obtaining only "typical" colonies, (e) checks to avoid the use of organisms which produce a granular type of growth in broth or clump in physiological salt solution, (f) tests for agglutination with typhoid antiserum, and (g) tests for the production of a satisfactory agglutinin titer in rabbits inoculated with the vaccine. With a few modifications noted below, this is essentially the technic followed since 1908.

The question will now be considered as to the classification of the U. S. Army Rawlins sub-strain in terms of Arkwright's criteria for smooth and rough dissociative types.

Colony Morphology—While the early records of the vaccine laboratory contain no detailed description of the morphology of the colonies, it is obvious that an attempt has always been made to select colonies of a uniform appearance since, from the beginning, it is recorded that only "typical" forms were used for the "seed" vaccine cultures. In Russell's [37] original records it is noted, at intervals, that "the most even looking" or "most uniform colonies" were picked; and at other times, it is stated that uneven growths appeared which had to be scraped off the agar, and that they were discarded. However, since most of the earlier texts on bacteriology described the typical typhoid colony as being of the "grape-leaf" or "veined" type on gelatin, it seems quite probable that, from the beginning, the U. S. Army Rawlins colonies have been somewhat irregular in outline. In this connection it is of interest to note that Arkwright [27] states that the veined or medusoid colonies, of the typhoid and paratyphoid groups, may have the same serological properties as those of the normal smooth colony.

During the past 5 years the colonies have been studied rather intensively; and an attempt has been made to select those more nearly conforming to the so-called smooth type. As a rule these colonies are not completely circular, showing some irregularity of outline, but otherwise they have the morphological characteristics of the smooth type. It should be added that while the criteria used routinely in the selection of colonies for use in the vaccine

have not been radically changed, it has been shown experimentally that it is possible to produce perfectly typical, smooth colonies with the U. S. Army Rawlins by various methods.

Suspensibility in Physiological Salt Solution—On rare occasions during the past 25 years, individual cultures of this strain have shown a tendency to agglutinate spontaneously in salt solution; but all such cultures have been discarded. Thus, throughout this period, there has been an unconscious but consistent selection of organisms possessing the smooth characteristic of uniform suspensibility in normal salt solution.

In recent experiments at the Army Medical School, it has been shown that both the Rawlins and a recently isolated, smooth, typhoid strain reacted similarly when suspended in NaCl solutions of 0.2, 0.4, 0.8, 1.6, 3.2 and 6.4 per cent. After incubation at 37° C. and at 50–55° C. for 19 hours, both strains remained in suspension; while a rough control strain (Dorset) showed clumping in salt solutions of 0.8 to 6.4 per cent.

Growth in Broth—For the reason given above, the character of the growth in broth has also been of the smooth type. While variants can be obtained which produce a granular growth in fluid media, such variants have not been used in the vaccine. When grown in nutrient broth media, containing 0.5, 1, 2, 3, 4, and 5 per cent sodium chloride respectively, there was no noticeable difference in the turbidity or sedimentation produced by the Rawlins and the recently isolated smooth strains; while a rough strain showed agglutination in all tubes within 24 hours.

Antigenic Structure—Since the beginning it has been an arbitrary requirement that the culture used for each lot of vaccine shall agglutinate, to a satisfactory titer, with a stock diagnostic antiserum; and that, when inoculated into rabbits, the cultures shall produce agglutinins to a satisfactory titer. In more recent years it has been observed that the agglutinins produced in rabbits by U. S. Army Rawlins vaccines include both the H and O antigens. Livesay,[?8] in unpublished experiments, has observed that with rough and smooth antisera the H and O antigens of this strain gave agglutination reactions similar to those obtained with smooth strains, including one recently isolated from a case of typhoid fever. As noted by Craig,[39] in 1929, individuals inoculated with the U. S. Army Rawlins vaccines may show in their sera other antibodies including precipitins and complement-fixing bodies.

Virulence—The original Rawlins strain was selected because of its relatively low virulence (toxicity?) for guinea pigs. Nichols [40] observed in 1915 that the U. S. Army sub-strain showed a relatively low virulence for guinea pigs and rabbits, but that it was distinctly toxic. We have no recent data concerning its virulence for man. However, Bulloch [41] mentions two severe cases of typhoid which occurred "at the Royal Army Medical College in the vaccine department in workers who were employed in preparing vaccines with the old culture (Rawlins)."

It appears that the Rawlins substrain, as maintained at the Army Medical School, produces colonies which are slightly irregular in outline but do not conform to the morphological picture of a rough organism; and that in its other cultural, physical and antigenic reactions it behaves as a typically smooth organism. Furthermore, the records of the vaccine department indicate that there has probably been little change, in the dissociative state of this sub-strain, during the 25 years of its use.

At this point it may be well to warn against the present tendency to attrib-

ute fixed dissociative characteristics to any particular strain of organisms, without considering the environmental conditions to which it is exposed. While it is true that certain typhoid strains appear to have a greater tendency to variation than others, it is obvious that even such stable organisms as those descended from the original Rawlins culture are susceptible to such influences, and may be obtained and kept in either the motile or non-motile, smooth, intermediate or rough condition as desired. Among the many Rawlins sub-strains, which have been cultivated in different laboratories, it is possible to find some which are typically smooth in all their characteristics, many that are intermediate, and others that are entirely rough. In fact, sub-strains of the different types may be developed in any laboratory by a variety of methods. Larkum,[42] for example, recommends for the production of smooth cultures, frequent intraperitoneal passage through guinea pigs. Others have obtained similar results by passage through other animals. Cox [23] has advised the use of Huntoon's hormone agar for maintaining cultures in a smooth state. In unpublished experiments performed at the Army Medical School, Simmons [43] and Livesay [38] have shown that typical, smooth-type colonies can be obtained by various selective culture methods.

A most instructive example, of the ease with which a Rawlins culture may be altered, is furnished by the recent experience of Perry, Findlay, and Bensted [44] with the English Army vaccine strain. Cultures of this strain, examined by Whitehead [22] in 1927, were of an intermediate-smooth type, with characteristics quite similar to those of the U. S. Army sub-strain. However, Perry, Findlay, and Bensted reported in April, 1933, that a culture of the English vaccine strain which they studied was extremely rough, having changed both culturally and antigenically. Using Grinnell's technic for estimating virulence by mouse inoculation, it was found that this rough variant had a minimal lethal dose estimated at 500 millions, when compared with more recently isolated smooth cultures, including an Indian strain, "Allahabad," which was lethal in doses of 40 millions. They observed that this rough Rawlins culture " failed consistently to protect mice against a subsequent lethal dose of a virulent strain of *Bact. typhosum*, whereas a series of mice previously inoculated with a vaccine manufactured from a recently isolated virulent strain were effectively protected against a similar test dose." Incidently it was noted that, even though the smooth strains had been recently isolated, careful colony selection was necessary, due to the fact that the usual rough variants were constantly encountered; also that, while vaccine prepared from the Allahabad smooth strain were most effective, the vaccine made from its rough variant gave almost as satisfactory results. The authors concluded that, in view of Grinnell's work, and of their own investigation, they were " of the opinion that the Rawlings strain should be discarded and the strain Allahabad substituted for it in the Army typhoid-paratyphoid vaccine."

However, it is of interest to note that within a few months' time the same authors [45] published a second report in which they observed that, while most workers acquainted with the Rawlins typhoid bacillus are in agreement as to the predominance of its rough character, they had recently received two cultures of the strain, both of which produced definite smooth colonies, which were antigenically pure and showed little or no tendency to throw off rough variants; also, that the virulence of these smooth Rawlins sub-strains was in sharp contrast to that of their rough Rawlins culture. Moreover, they re-

ported that they had since rejuvenated their rough Rawlins culture by simply passing it through mice in doses just sufficient to kill in 24 to 48 hours. Cultures from the heartblood of such animals yielded profuse growths of pure smooth colonies, and these organisms consistently killed mice in a dose of 40 millions. Their comments on this smooth Rawlins culture follow:

> It is now apparent from the mouse experiments that have been detailed that rejuvenation of this senile strain can be effected by certain *in vivo* methods. By such methods the original Rawlings strain has been so transformed that its virulence is possibly of a higher degree now than when it was originally employed as a typhoid vaccine. At the present time it is not possible to state how permanent this change may be; no alteration in this respect, however, has been noted during the last 2 months. *It has also been ascertained that this smooth Rawlings strain is as effective in protecting mice as any of the more recently isolated strains with which we have worked* (Italics ours). Possibly, further investigation may prove its superiority in this respect, and these comparative estimations are in progress. *It is, however, questionable whether so highly virulent an organism is suitable for inoculation into man* (Italics ours). In addition to the criterion of its protective properties, antecedent experience should prove that its administration is not followed by severe after-effects. The strain of typhoid bacillus (Allahabad) at present included in the Army vaccine was subjected to extensive trials, both from the protective and reaction aspects, before its selection was approved. Until such time as more experience is obtained of the after-effects of the inoculation of this smooth Rawlings strain into man it is not proposed to include it in the typhoid-paratyphoid vaccine.

This experience with the English Rawlins vaccine strain indicates quite clearly that, by a single passage, even a very rough culture can be transformed into a smooth type which is virulent for mice, and effective in Grinnell's mouse protection test. It also indicates that if " smoothness " and " roughness " are to be held as important criteria for the selection of vaccine strains of the typhoid bacillus, our practical problem is not so much concerned with the search for recently isolated cultures as with the question as to what dissociative state is desirable, and what methods are most suitable for the maintenance of our present culture in the most effective condition.

Since the question as to what constitutes the most desirable dissociative state has not been satisfactorily answered by laboratory tests, the following analysis of the practical results obtained with typhoid vaccination in the U. S. Army and Navy should be of considerable interest as a practical approach to the possible solution of the problem.

The ideal method of determining the relative effectiveness of a typhoid vaccine would be to administer virulent typhoid bacilli by mouth to large groups of vaccinated individuals; but such experiments are not practicable. Consequently the best information available is afforded by statistical studies dealing with the incidence of typhoid fever among vaccinated persons.

Before proceeding with the statistical analysis it should be noted that on several occasions vaccinated individuals have accidentally swallowed living typhoid bacilli without developing typhoid fever.

LABORATORY EXPOSURE TO INFECTION

In a case observed by Grant [46] in 1921, an assistant in the 8th Corps Area Laboratory sucked into his mouth 0.5 c.c. of a heavy suspension of living typhoid culture, strain K110. His mouth was washed out with 50 per cent alcohol and he was given 0.5 c.c. of Army vaccine. Four days later he developed headache and malaise, but there was no fever, or other symptom, except slight headache on the 8th day. Typhoid bacilli were isolated from the feces on the 12th day after the accident, and again on the 3 succeeding days,

but none were found in the blood. This man had received the U. S. Army vaccine 14 months previously.

In 1922 Sergeant George F. Luippold, while working in the vaccine department at the Army Medical School, swallowed about 25 c.c. of a saline suspension of the U. S. Army Rawlins culture containing about 3,000 million organisms per c.c. After rinsing his mouth with a cresol solution and with alcohol, he was given a subcutaneous injection of vaccine and kept in bed for 1 week. Cultures of stool, urine, and blood taken during this week were negative. On the 4th day he developed a temperature of 101 which disappeared after 2 days, but aside from a sore throat there were no other symptoms. Prior to this accident, the Sergeant had received 3 complete courses of Rawlins vaccines, the last in 1920.

THE U. S. ARMY VACCINE AND TYPHOID FEVER

We know of few convincing data bearing directly upon the effectiveness of antityphoid vaccination. Many of the available data are from the British experience in the Boer War, which was with a vaccine now known to have been faultily prepared. When universal compulsory vaccination was introduced in the United States Army and Navy, obviously there were no controls. Therefore, the only approach to the question of the effectiveness of our vaccine lies in an inquiry into the effectiveness of the control of typhoid and paratyphoid fevers before and after the advent of the vaccine.

FIGURE I

Logistic Curve Fitted to Mortality from Typhoid Fever, Registration Area of 1900 (Less District of Columbia), 1900–1932
The shaded band defines the limits of 4 P.E. of the ordinates of the curve.

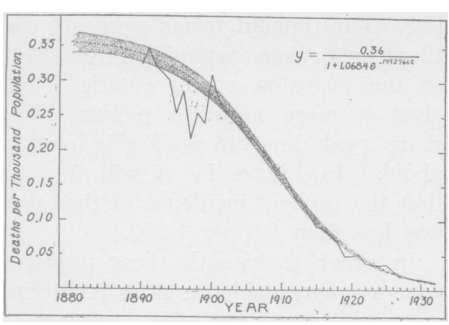
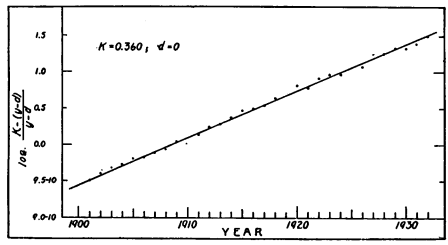


FIGURE II

Plot of Log. $\frac{K-(y-d)}{y-d}$ for Curve in Figure I
If the curve fit perfectly, all dots would fall upon the line.



Mortality from typhoid fever has been declining in the civil population of the United States since about 1900. The Registration Area of 1900 comprised the states of Connecticut, Indiana, Maine, Massachusetts, Michigan, New Hampshire, New Jersey, New York, Rhode Island, Vermont, and the District of Columbia. It would be difficult to select another comparable area in the United States where public health agencies were better organized and supported; and the experience of this area will be accepted by most observers as representing an experience decidedly above average in effectiveness in the control of typhoid fever by sanitation.

The mortality from typhoid fever in the Registration Area of 1900 (less the District of Columbia), from 1890 to 1932, is shown in Figure I. A logistic curve, with 4 times the probable errors of its ordinates, has been fitted to the observations from 1900 on. The fit is remarkably good and this curve can be accepted as indicating the trend of typhoid mortality in this area between these dates. The trough during the

90's need not concern us; it may be due to indifferent reporting (since a large part of the area was not then in the Registration Area), or it may be due to unexplained events in the natural history of typhoid fever. The important facts are: (a) that typhoid fever was at a certain level of incidence in 1900, and (b) that this level has declined since that time in a surprisingly orderly fashion. The fit of this curve is better shown, perhaps, by Figure II.

The equation of the logistic curve is $y - d = \frac{K}{1 + Ce^{rt}}$. This says, in simple language, that the upper and lower limits of the variable y are K and d, respectively; the rate of variation of y is expressed by r as the exponent of the Napierian e, and t (time) expresses the duration of the influence of r.

r, then, is the inherent rate of change of y. Consequently, in this case, r is the index of the influence of *all the factors* tending to reduce the mortality from typhoid fever. Since this curve fits very well throughout the range 1900–1932, and the value of r remains constant (0.1492966), the sum of the influence of all the factors tending to reduce typhoid mortality in this population has not changed significantly since 1900. It is conceivable, but scarcely probable, that different factors, exerting exactly the same amount of influence, were substituted from time to time. The most logical inference is that the same factors were in play in 1930 as in 1900, and that these were improvement of water supplies, sanitary sewage disposal, and possibly improvement in the personal and occupational hygiene of food handlers.

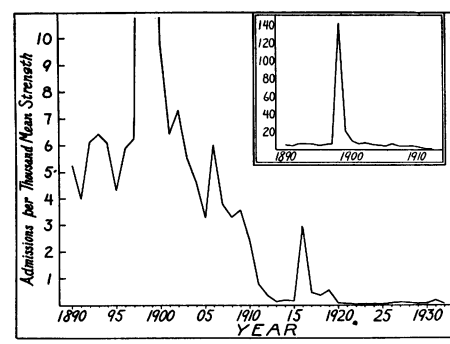
Now there is some indication that at least one other factor was introduced around 1920. The best fitting curve for the observations from 1900 to 1921 appears to be one asymptotic to 0.015 (deaths per 1,000), or $d = 0.015$. The curve shown in Figure I is asymptotic to zero. This difference is, so far, too small to be regarded seriously. It *may* represent the effect of the increasing use of vaccines among the civil population.

In any event, whatever r may represent, mortality from typhoid fever in the Registration Area of 1900 has declined in the past 30 years in a regular and orderly fashion that is satisfactorily expressed by a logistic curve, and this curve may be accepted as the measure of typhoid fever in a representative civil population provided with health agencies above the average in accomplishment.

The incidence of typhoid and paratyphoid fevers in the U. S. Army from 1890 to 1932 is shown in Figure III.

FIGURE III

Admission Rates, Typhoid and Paratyphoid Fevers, All American Troops, U. S. Army, 1890–1932

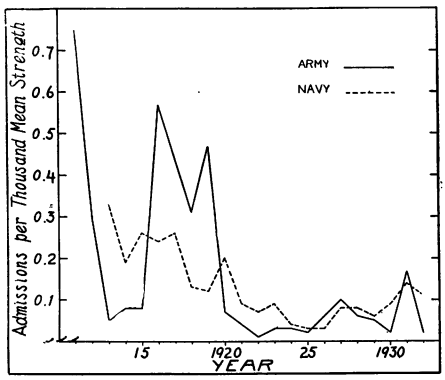


The smaller population, the variations in exposure due to war, mobilizations, geographic distributions of troops, etc., and the possible effect of immunity acquired by attacks of the disease—these all contribute to the irregularity of the line. Paratyphoid fevers were not distinguished from typhoid until 1911; so the inclusion of all enteric fevers gives a more accurate picture. The sharp peak in 1916 is largely paratyphoid. In Figure IV it will be seen that the typhoid incidence of that year was less than 0.6 per 1,000.

In order to smooth these peaks, 3 year average incidence rates, centering

FIGURE IV

Admission Rates, Typhoid Fever Only, U. S. Army and Navy, After Introduction of Typhoid Vaccination



upon each year, are shown in Figure VI. Merely to place the curve where comparison is easiest, and not to suggest case fatality rates, the Registration Area curve x 18 is plotted in this graph. It is evident that the curve expresses fairly well the trend of typhoid in the Army from 1891 to 1908 (the Spanish American War excepted) and in the Navy from 1898 to 1910. Since 3 year average admission rates are used, the effect of the introduction of vaccine is spread over some 5 years, but, even so, the decline in both the Army and Navy is very sharp.

It is to be expected that the advances in sanitation in the military services kept pace with civil improvements. It has been argued that military sanitation has advanced much more rapidly than civil sanitation and that this largely explains the sharp decrease in incidence of the enteric fevers in the Army and Navy in 1911–1912. Aside from the fact that no one knows of any radical innovations in military sanitation adopted at that time, this argument would be much more convincing were the sources of typhoid fever among soldiers to be found only within the sentry lines. As a matter of fact, within recent years at least, the only demonstrable sources of infection have been outside military control, so that the status of typhoid in the civil population is a fair index of the exposure of the soldier to infection.

During war, and in large concentration camps, this index is unquestionably faulty, but the greater part of our experience with typhoid vaccine has been in small garrisons in time of peace. Those who attribute the sudden drop in incidence in 1911 for the Army and 1912 for the Navy wholly or largely to improvements in sanitation, exhibit a naïve ignorance of the history of typhoid fever in the military services. The experience of the Spanish American War resulted in immediate stressing of the importance of sanitation. Chlorination of water supplies was commenced shortly thereafter, but this practice was extended gradually and relatively slowly. Why the improvements in sanitation should exhibit such a cumulative effect and select the years immediately following the introduction of vaccine to become so prominent has never been explained by critics of vaccination.

Finally, if the vaccine has exerted any influence upon the incidence of typhoid fever in the military service, radical changes in the vaccine itself, or the method of administration, must be reflected in the admission rates for this disease.

It is unnecessary to defend the assertion that the law of diminishing returns operates in the control of typhoid fever. This is to say that, as the incidence of the disease approaches the vanishing point, greater efforts are required to

FIGURE V

Admission Rates, Paratyphoid Fevers Only, U. S. Army and Navy, After Introduction of Typhoid Vaccination

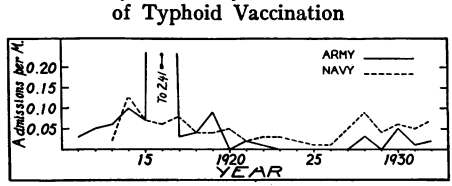
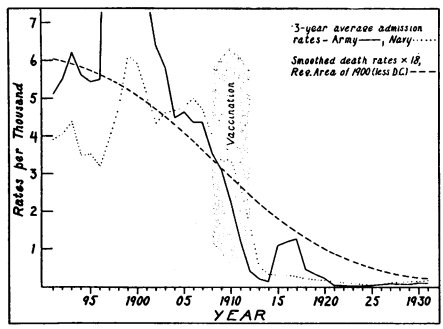


FIGURE VI

Trends of Typhoid and Paratyphoid Fevers, U. S. Army and Navy and Registration Area of 1900 (Less District of Columbia), 1891–1931



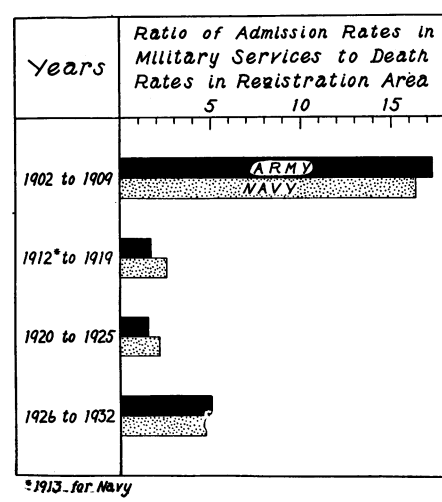
effect further reduction. It is a fairly safe prediction that it will require more time and effort to eradicate the final death per 100,000 per annum than it has required to reduce these deaths from 30 in the Registration Area of 1900 to a single one, if such an effort can ever be justified economically.

It is to be expected then, that, all other things being equal, the effect of vaccination will be less and less evident as typhoid fever declines in incidence from other measures of control. Figure VII shows the ratio of admissions for typhoid fever in the Army and Navy to deaths from typhoid fever in the Registration Area of 1900 (less D. C.) for 4 different periods. Paratyphoid fevers were not distinguished from typhoid in the military services until after vaccination was introduced. But, since the mortality from paratyphoids is much less than from typhoid, it is necessary to subtract them from the admissions in order not to exaggerate the effect of the vaccine. Omitting the Army's epidemic year of 1916, 83 per cent of all admissions for enteric fevers from 1911 to 1932, in both services were for typhoid fever; and the proportion is surprisingly constant. In order that any error might be on the side of conservatism, for the admissions in the Army and Navy from 1902 to 1909, 20 per cent were deducted as having probably been paratyphoid fevers.

For this period just prior to the introduction of the vaccine, based upon such an estimate, there were approximately 17 admissions for typhoid fever in the Army and Navy for every death from typhoid fever in the Registration Area of 1900 (per 1,000 exposed to risk in each case). Had the control of typhoid fever in the military services and the civil population proceeded apace, this ratio would remain constant; but in the 8-year period immediately following the introduction of the vaccine, this ratio dropped to 1.67 admissions from the Army and 2.55 admissions from the Navy to each death in the Registration Area. This means that, following the introduction of the vaccine in the military services, typhoid control became 9 times as effective as it had been just before; and this improvement took place largely in less than 2 years. Also, it will be remembered that this comparison takes into

FIGURE VII

Number of Admissions per 1,000 for Typhoid Fever Only in the U. S. Army and Navy to Each Death per 1,000 in the Registration Area of 1900 (Less District of Columbia)



account the rapid decline of typhoid fever in the Registration Area during these periods.

In the next period, 1920–1925, there is again a slight advantage for the military services, the ratios for the Army and Navy declining to 1.54 and 2.12, respectively—this despite the fact that the incidence of the disease was rapidly approaching zero in the services and control measures were therefore, less productive of quantitative results.

In the final period of the experience, 1926 to 1932, typhoid fever declined some 56 per cent in the Registration Area. In the Army, the incidence increased from 0.063 to 0.090 per 1,000, while it remained stationary in the Navy (0.087 to 0.084). This resulted in an increase of the ratios of admissions in the military services to deaths in the Registration Area to 5.00 and 4.67 for the Army and Navy, respectively.

In brief, Figure VII shows that typhoid control was decidedly more effective in the military services than in the Registration Area of 1900 for the period 1912–1919; that it was even slightly more satisfactory in the 6 years following, but that something happened to typhoid control in the military services in the 1926–1932 period.

It will be shown later that a radical change was made in the vaccine just prior to this last period. We believe this reversal of the trend of the control of typhoid fever in the military services, following the alteration of the vaccine, to be the most convincing evidence of the rôle played by the vaccine in the control of the disease. Admitting that military sanitation has progressed since 1911, admitting for the sake of argument only that this has been solely responsible for the great reduction in typhoid fever, who will now advance the argument that military sanitation has gone backward since 1924? There have been no wars nor even mobilizations in this period. The vaccine was changed; and this change has been reflected in the typhoid experience of both the Army and Navy.

MODIFICATIONS OF THE VACCINE

From the time of its introduction until July, 1917, the vaccine consisted of a saline suspension of the Rawlins strain, grown upon beef-extract agar, and killed at 53°–54° C. for 1 hour. The vaccine was standardized at 1 billion organisms per c.c. by the Harrison method in which washed red blood cells were used for comparison. Later experience has shown that the actual bacterial count is only about one-half that of the count estimated by this method, so that during this period the vaccine consisted of approximately 500 million typhoid organisms per c.c.

In July, 1917, 750 million paratyphoid-A organisms, and a like amount of paratyphoid-B, were added to the vaccine, by the same Harrison method of counting which resulted in a vaccine that, by actual count, approximated 500 million typhoid and 750 million paratyphoid organisms.

In July, 1919, the direct method of counting in a Helber chamber was adopted which resulted in doubling the quantities of each of the components of the vaccine; and in April, 1920, the nephelometer method of counting was introduced, using standards established by counts in a Helber chamber.

These nephelometer standards became more and more opaque with age, resulting in higher and higher counts in the vaccine, a fact that was not recognized until some time in 1923. Meanwhile, the reactions had become so severe that in February, 1922, the paratyphoid constituents were reduced to 500 millions each—by the nephelometer estimate, which was still inaccurate, and actually there remained almost 1,500 million paratyphoid organisms per c.c.

The concentration of the vaccine continued to increase with the age of the

standards until, when checked with the Helber chamber late in 1923, the actual count was found to be approximately 1,750 million typhoid and a like amount of paratyphoid organisms per c.c.—a total count of around 3,500 million organisms per c.c., although the prescribed strength was only 2,000 million. The severe reactions again forced a reduction in the strength of the vaccine and, in March, 1924, the vaccine for the Army was re-standardized by direct count with the Helber chamber at 500 million typhoid, 250 million para-A and 250 million para-B organisms per c.c. At this time the Navy discontinued the paratyphoid fraction and their vaccine was standardized at 1,000 million typhoid organisms per c.c. Two other changes were made at about the same time; veal infusion agar was substituted for the beef-extract media and the killing point was increased to 56° C. for 1 hour.

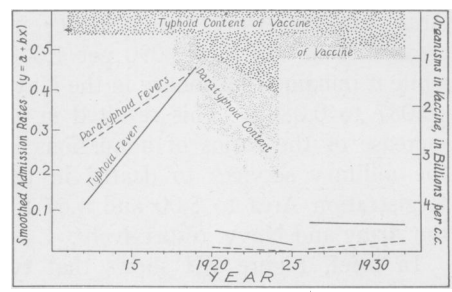
In October, 1927, the Army vaccine was again modified by discontinuing the para-B fraction and increasing the typhoid fraction to 750 million per c.c.

Based upon the physical characteristics and method of production, the total experience with the Army typhoid vaccine falls into three more or less homogeneous periods: (1) From 1911 to July, 1919. During this period, with the exception of less than 2 years during the World War when the paratyphoid fractions were first added, the vaccine consisted of about 500 million typhoid organisms per c.c. (2) From July, 1919, to March, 1924. In this period the dose was greatly increased by design and continued to increase for the reason set forth above. (3) From March, 1924, to the present. In this period the dose was reduced, the method of preparation was slightly changed, and the proportion of the constitutents of the vaccine for the Army was again altered in 1927.

The most radical changes in the vaccine have been in the concentration of the antigen although it is recognized that these may have affected the protective value less than the change of media and the raising of the killing point. It will be seen, however, that a great decrease in the incidence of typhoid and paratyphoid fever followed the increase in dosage of vaccine prepared exactly as before.

FIGURE VIII

Trends of Typhoid and Paratyphoid Fevers, U. S. Army, With Relation to the Composition of the Vaccine



In Figure VIII is plotted: (a) the approximate strength of the vaccine from 1911 to 1932, and (b) the trend of typhoid and paratyphoid fevers in the corresponding periods. A lag of approximately 1 year has been allowed after each major change in the vaccine in order to afford the new vaccine an opportunity to show its effect. The trend is shown, in each case, by a straight line ($y = a + bx$) fitted to the observed incidence rates.

The influences of the border epidemic of 1916 and the World War are exerted toward the end of the first period so that the trend was decidedly upward from the low rates of 1912 to 1915. The strength of the vaccine was low during almost all of this period, and the demands upon the vaccine in 1916 and the World War were much greater than during the garrison days of 1912 and 1913. Extrapolation of a straight line is, of course, decidedly unwise; and it must not be inferred that a continuation

of the World War would have resulted in an increasing amount of typhoid fever. The evidence is to the contrary, it appearing probable that the incidence of enteric fevers approached the peak in 1919. It is evident, however, that the vaccine of that day was inadequate for the maintenance of as low a rate under field conditions as under garrison conditions, although, based upon past experience, the typhoid rate during the World War was phenomenally low, and generally considered satisfactory.

During the second period (1920–1925), characterized by garrison environment and a very strong vaccine, paratyphoid fevers disappeared from the Army, and typhoid approached the vanishing point. The trends of both were decidedly downward with the increasing strength of the vaccine.

The environmental conditions of the Army during the third period were not significantly different from those during the second. In this period, paratyphoid began to appear (see Figure X) and the level of the incidence of typhoid was higher. The trend, measured in this manner, is slightly downward, but this must be accepted with considerable scepticism since the very favorable years of 1930 and 1932 may not be quite representative. The Navy trend is upward during this period.

The Navy experience, shown in Figure IX, is very similar. The Navy, possibly by reason of the similarity of hygienic conditions in war and peace, has escaped the epidemics of the Army. Actually, the personnel of the Navy probably are less exposed to the enteric infections in war than they are in peace. The trends of both typhoid and paratyphoid fevers in the first period are downward. In the second period, the rates of decrease are faster than in the first period, while there is a rise in both typhoid and paratyphoid fevers in the third period.

FIGURE IX

Trends of Typhoid and Paratyphoid Fevers, U. S. Navy, With Relation to the Composition of the Vaccine

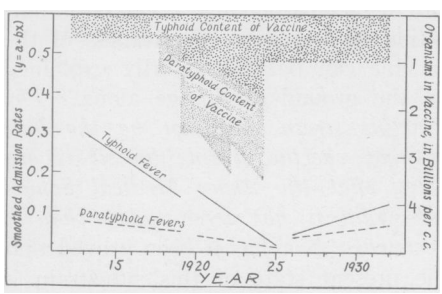
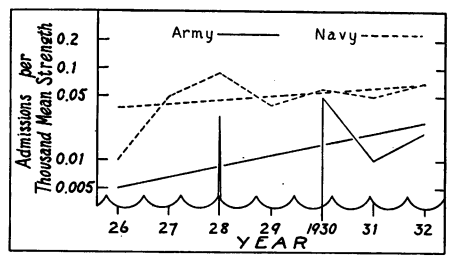


FIGURE X

Trends of Paratyphoid Fevers, U. S. Army and Navy, 1926–1932. During this period the Navy vaccine contained no paratyphoid organisms while the Army vaccine retained a paratyphoid fraction.



These widely separated experiences are in complete agreement and indicate (1) that the low dosage of vaccine from 1911 to 1919 was sufficient to effect a marked reduction in the incidence of typhoid fever, especially under garrison conditions; (2) that the very large doses of vaccine from 1919 to 1924 greatly reduced the incidence of enteric fevers, and this reduction progressed as the vaccine dosage increased through ageing of the standards; and (3) that the reduction of the dosage of the vaccine in 1925 was followed by an increase in the incidence of enteric fevers, but the level has remained appreciably lower than during the 1911–1919 period when the dosage of vaccine was least.

There is another significant reversal of trend shown in Figure VII. So long as the same vaccine was furnished both

the Army and Navy, there was more typhoid fever in the Navy than in the Army. Since the dose of the typhoid fraction was reduced in 1925 in the Army to 500 million and in the Navy to 1,000 million, there has been less typhoid in the Navy than in the Army. This is also illustrated very well in Figure IV.

THE EFFECTIVENESS OF THE PARATYPHOID FRACTION

Nichols [40] once said that "clinical experience is unanimous that there is no immunity to the paratyphoid infections after typhoid immunization." Clinical experience is, however, notoriously unreliable.

Before the addition of the paratyphoid fraction to the vaccine, there was one epidemic of paratyphoid fever in the Army (1916). This was, however, during a period in which the dosage of the typhoid vaccine was, as is now known, entirely inadequate. Paratyphoid fevers declined in incidence in the Navy before the paratyphoid fraction was added to the vaccine, and, *since the paratyphoid fraction was dropped from the Navy vaccine, paratyphoid fevers have increased less rapidly than typhoid fever in that service.* They have also increased less rapidly than has paratyphoid fever in the Army (see Figure X), which retained a paratyphoid fraction. However, we commend these figures to you with reservations, since there are too few years in which there was any paratyphoid in the Army to make the trend entirely significant.

It is our opinion that the experience suggests that a potent typhoid vaccine may control paratyphoid fever, and that there is no evidence that the reverse is true to the same degree. We feel that, if any reduction of dosage must be made because of toxicity, this reduction should be entirely in the paratyphoid fraction. Further experience with increased dosage of a monovalent vaccine may lead us to change our opinion.

SUMMARY

The experience with the Army Medical School vaccine has been divided into the periods corresponding to major changes in the production of the vaccine.

During the first two of these periods only the dosage of the vaccine was changed. In the third period the dosage was decreased and, also, the medium used for growing the organism and the temperature at which the vaccine was killed were changed. Any effect of change of media and killing-point cannot be separated from the effect of the reduction of dosage but, from the experience of the first two periods, it would appear that the reduced dosage alone accounts, in whole or in large part, for the increased incidence of enteric fevers in the last period.

Studies of the dissociative characteristics of the U. S. Army sub-strain of the Rawlins culture show that this organism, as used for the production of vaccine, forms an irregularly shaped colony but, otherwise, possesses all of the characteristics of a typical so-called smooth type of organism. Furthermore, the available records indicate that, if there has been any dissociative alteration in this strain since it was brought to the Army Medical School in 1908, it has not been toward roughness. As a matter of fact, there is no laboratory or statistical evidence of any dissociative change. The only indication of decreased effectiveness of the vaccine can be satisfactorily explained on the ground of dosage alone. *The Rawlins strain may not be the best typhoid vaccine strain; but it is our belief that the Army Medical School sub-strain is no worse today than it ever was.* Nor has it been proved that the present state of this sub-strain is

not the optimum for the prevention of typhoid fever in man; or that, when the optimum dissociative state has been determined, the Rawlins strain cannot be so maintained.

In view of these facts, we believe that the Army vaccine can be improved by increasing its bacterial content within the limit of safety.

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