The Treatment of Malignant Tumors by Bacterial Toxins as Developed by the Late William B. Coley, M.D., Reviewed in the Light of Modern Research

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(Received for publication October 18, 1945)

During the past six years an exhaustive study has been made of the treatment of malignant tumors by injections of bacterial toxins, as developed by the late Dr. Coley and other investigators. The problem has been approached in the following manner: (a) Is there sufficient clinical and experimental evidence to justify the conclusion that the method has therapeutic value? (b) If so, what factors governed success or failure, and why did the method not achieve recognition? (c) If the conclusions to the above questions warrant further study, what can be done to make the toxins consistently effective?

As there is no comprehensive monograph on the subject it was necessary to cover the literature thoroughly, analyzing in detail not only the records of Coley's own cases but those of the other investigators; this entailed extensive correspondence with hospitals, clinics, and physicians here and abroad who have used the toxins. It was considered necessary to study the records of cases in which intercurrent bacterial infections were said to have elicited profound effects upon malignant tumors. The experimental work on malignant tumors in animals has also been reviewed.

The first significant observation to be brought out was that at least fifteen different preparations of Coley toxins have been used since the method was introduced in 1892, of which three were considerably more potent than the rest. It was further observed that the technic of administration has varied considerably as regards site, dosage, frequency, and duration of treatment.

Unfortunately, the only preparation available in the United States since 1921 has been very weak, and the method employed in this later period has been for the most part much less effective than that used in the early years. Therefore, most of the present generation of physicians have not seen the remarkable results originally obtained.

As for clinical studies of the 15 different preparations, a number of physicians in this country and abroad, as well as Coley himself, reported cases successfully treated between 1895 and 1944. Among these were: Johnson (42), Owens (54), Mynter (52), McArthur (49), Stone (72), Battle (5), Fowler (31), Tupper (76), Walton (78), Thomas (73), Winberg (79), Lilenthal (46, 47), Green (34), Spencer (69), Tosier (74), Hertel (39), Greenwood (35), Harmer (36), Lagueux (44), Christian and Palmer (14), Blum and Coley (9), and DaCosta (25).

Others reported failures, and although their experience was based on a limited number of cases the majority seemed to consider it sufficient evidence to condemn the method unequivocally, apparently without considering whether the preparations or the technic employed might not have been at fault. Examples of these are: Emmerich (29), Campanini (13), Sheild (66), Newcomet (53), Babcock and Pfahler (4), and Senn (64).

Except for a few of Coley's longer papers and those of Moulin (51), Fowler (31), DaCosta (25, page 293), and Palmer (55), the method of administration does not appear in detail. However, Harmer described his experience during the period from about 1908 to 1914 (36).

It would appear that few except Coley, here or abroad, considered the possibility that the toxins might be expected to produce a larger number of permanent results in the earlier stages of the disease. An exception was Matagne, who wrote Coley in 1913 that for the previous 18 years he had consistently used the toxins before operation, and that this procedure had given him "a percentage of cures much higher than ordinary surgical treatment alone" (48).

Apparently no one physician or clinic treated a sufficient number of cases of each type of tumor in each stage of the disease to yield adequate material for a comprehensive analysis. It seemed necessary, therefore, to assemble detailed histories of a large number of cases of every type of tumor that has been treated by this method, and to study carefully the technic of administration. Over 600 histories have been abstracted in detail on the same form, covering the period since 1892; approximately 48 per cent were from Coley’s cases. Also included are 65 cases in which
an intercurrent infection, principally erysipelas, caused regression in malignant tumors of various types.

In over 88 per cent of the cases studied the diagnoses were based on reliable microscopical, as well as clinical or roentgenological, findings. The cases were listed according to the type and site of the growth. It was found that the toxins alone or in combination with other methods of treatment had been used in the following groups of tumors in which 5 year survivals were obtained:

It has been possible to abstract 96 histories of carcinoma of various organs in which the toxins were used, as well as 21 cases in which an intercurrent infection occurred. A study of these histories, together with the experimental work of Shwartzman and other recent investigators, indicated the need for a more specialized technic of administration in cases of carcinoma and certain more slowly growing tumors (68); this was not known prior to Coley's death, in 1936. Experiment has demonstrated the presence of factors

<table>
<thead>
<tr>
<th>Type of tumor</th>
<th>Total no. cases</th>
<th>Inoperable</th>
<th>Operable</th>
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<tr>
<td></td>
<td>Total</td>
<td>Five year survivals</td>
<td>Total</td>
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<tr>
<td>Carcinoma</td>
<td>69</td>
<td>45</td>
<td>15</td>
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<tr>
<td>Malignant melanoma</td>
<td>24</td>
<td>19</td>
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<tr>
<td>Bone sarcoma</td>
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<td>98</td>
<td>37</td>
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<td>Soft parts sarcoma</td>
<td>123</td>
<td>91</td>
<td>53</td>
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<tr>
<td>Lymphosarcoma</td>
<td>49</td>
<td>45</td>
<td>24</td>
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<td>Hodgkin's Disease</td>
<td>14</td>
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<tr>
<td>Total</td>
<td>484</td>
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<td>134</td>
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In compiling this table the following cases were excluded: a) 80 that lacked definite histologic confirmation; b) 55 cases of giant cell tumor because of the difficulty in determining which of these were malignant; c) 32 cases in which complete regression occurred but which were followed less than five years.

Concerning the use of the toxins in carcinoma, Coley stated that until he began using the more potent Buxton preparation in June, 1894, he had seen only temporary improvement in carcinoma. Within the next few months, however, he (17) saw 2 epitheliomas disappear entirely under treatment with the Buxton preparation (Type VI). Unfortunately, other physicians were using less potent products and less effective technic and were meeting with little or no success in treating carcinoma during those first years.

In 1897 Coley began to concentrate his attention on sarcoma because it seemed that the method was more effective here than in carcinoma; hence comparatively few carcinomas appear to have been treated during his lifetime by toxin therapy. But about 1930 he became aware that the results justified a change of attitude, for he stated: “During the first few years, I treated a considerable number of cases of inoperable carcinoma. . . . While in most cases a certain amount of improvement was apparent . . . , in the great majority . . . it was . . . only temporary and I decided to restrict the method chiefly to inoperable sarcoma. Later on, the number of cases of inoperable carcinoma apparently cured by the toxin treatment administered by other surgeons led me to the belief that I had greatly underestimated the value of the toxins in these cases” (24).

It has been possible to abstract 96 histories of carcinoma of various organs in which the toxins were used, as well as 21 cases in which an intercurrent infection occurred. A study of these histories, together with the experimental work of Shwartzman and other recent investigators, indicated the need for a more specialized technic of administration in cases of carcinoma and certain more slowly growing tumors (68); this was not known prior to Coley's death, in 1936. Experiment has demonstrated the presence of factors in some bacterial toxins that are destructive to tumor cells, but it appears that in order to kill the less vulnerable cells it may be necessary to sensitize them by injection into the growth or its immediate periphery, these sensitizing injections being alternated daily or every other day with intravenous injections. The results obtained in a case of carcinoma, generalized in the abdominal cavity and treated by intraperitoneal injections, suggest that the intraperitoneal route should receive further investigation in such cases. From a study of the clinical histories it appears that a longer period of treatment is necessary in the more slowly growing, less sensitive tumors in order to produce complete and permanent regression.

**HISTORICAL OUTLINE**

The treatment of malignant tumors by bacterial toxins is based on the fact that neoplasms of practically all types have been known to regress under acute bacterial infections, principally erysipelas (15). In many cases the inhibitory action was sufficiently powerful to cause complete disappearance of the tumor, and a number remained free from recurrence.

Coley's interest in the phenomenon was aroused by a thrice recurrent inoperable lymphosarcoma of the neck that had recovered under an attack of erysipelas (15). This led him to make a careful study of
the literature, where he found reports of 38 cases of inoperable malignant tumors in which an attack of erysipelas had occurred, either by accident or inoculation. Of these 17 were carcinomas, with 3 permanent regressions, and 17 were sarcomas, of which 7 did not recur. The other 10 sarcoma cases all showed distinct improvement, and in some of them the tumor disappeared completely but later recurred.

Beginning in April, 1891, Coley attempted to produce erysipelas as a therapeutic measure in 10 patients with inoperable malignant tumors. The difficulty in producing an attack and the dangers incident to inoculation with living cultures induced him to seek some method of eliciting the beneficial action of *S. erysipelatis* without the attendant risks. He therefore tried cultures sterilized by heating (at first to 100 °C., later to 58° C.) or filtration, but all were found to be too weak to be effective.

In December, 1892, he learned of the investigations of Roger on *B. prodigiosus* (*Serratia marcescens*) in association with other organisms (60); in this connection are to be noted also the work of Vaillard, Rouget, and Roux (77, 61, 62). As these experiments suggested that *B. prodigiosus* or its toxins increase the virulence of other organisms with which they are associated in their proliferating stage they were incorporated in the formula that became known as Coley’s toxins. Research by Beebe and Tracy (6, 75) and later investigations by Shwartzman (67) have clearly indicated that the toxins of *B. prodigiosus* are more potent in destroying the tumor cell than are various types of streptococci.

The first mixed toxins of these two organisms were prepared for Coley by Alexander Lambert at the College of Physicians and Surgeons, in New York. Cultures were sterilized by passage through a Kitasato filter, and mixed only at the time of use; these are called Type IV in this study. Although a number of tumors never recurred after treatment with this preparation, it appeared to be comparatively weak and distinctly variable.

Early in 1894 Buxton undertook to prepare toxins for Coley, and introduced a modification that consisted of growing the two cultures together. This preparation, also filtered, is called Type V. It appeared to be more potent than Type IV.

In June, 1894, Buxton prepared the first mixed, unfiltered toxins. Cultures were grown together, as in Type V, but were sterilized by heating at 50° to 60° C. The first erysipelas cultures were obtained from a fatal case. After 1894 virulence was maintained by frequent passage through rabbits (12). During 1900 a double sterilization was considered necessary (55). Type VI, made by Buxton from June, 1894, until 1906, appeared to be more potent than any other preparation of the same period; however, its variability appeared to increase and its potency to diminish somewhat during the period from 1900 to 1906.

Some time in 1895 the Lister Institute of Preventive Medicine undertook to prepare the Coley toxins for England. Little is known of the formulas used, but they are believed to have been Buxton’s V and VI. From the scant number of cases in which these early English preparations were used, it appears that they were somewhat less effective than Buxton’s Type VI. A serious handicap of the Lister Institute preparations was that apparently no printed directions or indications for use were enclosed with them, so that unless physicians communicated directly with Coley, or had access to some of his published papers, they had nothing to guide them (63).

Seven years after Coley introduced toxin therapy two important studies were made of this method. The first was that of Moullin (51), who stated in outlining the evolution of the method: “It has been known for many years, according to Fehleisen since the seventeenth century, that not only malignant growths, but chronic ulcers of the skin, lupus nodules, syphilitic sores and other affections, occasionally disappear with great rapidity after an attack of erysipelas. The number of cases that have been recorded with sufficient accuracy and detail is not a very large one it is true. They are undoubtedly of exceptional occurrence. Many of them are of somewhat ancient date, as might be expected from the fact that erysipelas is much less common now than it used to be, and that attacks are less severe than in pre-antiseptic days, but after making full allowance for all these defects, there still remains a sufficient number of well-authenticated instances to dispel at once the idea that the disappearance can in any way be due to mere coincidence.

“. . . Such cases as these—isolated though they were—could not fail to attract attention, and even before the microbic origin of erysipelas was known, several attempts were made at inducing an attack by means of inoculation. When Fehleisen discovered the streptococcus, and it was shown that pure cultures could be obtained, it was naturally not long before a systematic attempt was made. Fehleisen himself was the first.

“. . . Coley and others quickly followed suit, but two things very soon became apparent. First, that it was exceedingly difficult in many instances to induce an attack. . . . Secondly, that it was still more difficult to limit the effect of an attack when it did occur. There are 25 cases recorded in which an attempt was made to induce an attack of erysipelas by inoculation. In nearly all, pure cultures were injected or rubbed in after the skin had been scarified; in one the patient was placed in a bed which had a notoriously bad
history. Six never had a genuine attack. . . . Four of the remaining nineteen died as a result of the attack.

"Fourteen of these cases were definitely sarcomata. One of them under the care of Dr. Coley, a sarcoma of the neck that had already recurred twice, was cured; that is to say four and a half years afterwards the patient was well in every respect. Another, a lymphosarcoma of the neck which had resisted arsenic, under the care of Kleeblatt, disappeared entirely. . . . In two others, under the care of Coley, after repeated inoculations with living cultures, each of which was followed by temporary improvement, a cure resulted from the use of the mixed toxins. . . . Two others, an enormous sarcoma of the neck . . . and of the tonsil . . . almost disappeared, but as soon as the effects of the erysipelas had passed off, began to grow again. Two of Fehleisen's diminished considerably for a time. Four were scarcely affected, and in the remaining two erysipelas never occurred at all.

"It is worth noting that in every single case in which erysipelas occurred the tumors showed some change, although in four of the twelve it was very slight." 1

Moullin describes the effects produced by the toxins on malignant tumors as follows: "When there is an ulcer or fungating sore, inflammation and sloughing appear to be the rule. In one or two instances the whole tumor has been thrown off in this way. On the other hand, with an unbroken surface, sloughing is the exception. . . . The difference seems to depend upon whether pyogenic organisms are present and can gain access through some accidental abrasion. If they are present the tumor sloughs, if they are not it undergoes a process which for rapidity and thoroughness can only be compared to acute yellow atrophy of the liver" (51, page 21).

As to the microscopic changes produced, Moullin stated: "... The only definite description that I can find is in the accounts of three cases, one of sarcoma, two of carcinoma, in which the tumors were . . . disappearing during an attack of erysipelas when it proved fatal. . . .

"One of these was a round-celled sarcoma of the neck, recorded by Busch. . . . The sarcoma cells had undergone fatty degeneration . . . around its outskirts, portions of the growth that were still unaffected allowed its character and the nature of the changes it was undergoing at the time of death to be ascertained (11, page 245).

"Very much the same appearances, making allow-

1 The experiences cited above appear to indicate that permanent results occur rather rarely following the brief toxic effect of an attack of erysipelas or some other acute bacterial infection. Apparently a more sustained toxic action, or a larger quantity of toxins than is usually generated during a single attack of erysipelas, is necessary to destroy the tumor completely and prevent recurrence.

It is interesting to analyze the type of investigation that helped to throw doubt on the effectiveness of bacterial toxins. To quote Moullin: "Friedrich, for example, after trying the toxins (of erysipelas alone) on nineteen cases, came to the conclusion that they had no specific effect of any kind, and that the changes the tumors sometimes undergo are 'merely the expression of the cumulative action of a number of injurious influences working together.' Of the nineteen cases he records, however, four only were sarcomata and two lymphosarcoma. The rest were carcinoma. . . . Moreover, though the streptococci were taken from many different cases, it is by no means certain that they were sufficiently virulent. . . ."

Moullin's conclusions as to the value of toxin therapy are: (a) That a considerable number of hopelessly inoperable sarcomata, many of which were recurrent, have completely regressed under this treatment, and that there is no other of which this can be said. (b) That the disappearance of sarcoma is not due to inflammation, but to an intensely rapid form of fatty degeneration comparable only to that which affects the hepatic cells in acute yellow atrophy of the liver. Inflammation and sloughing, when they do occur, are septic complications. (c) That degeneration or absorption may occur whether the toxins are injected directly into the tumor or at a distance. (d) That the toxins are of no value unless derived from a virulent case of erysipelas, or are made virulent by passing the streptococcus through rabbits. (e) That B. prodigius immensely increases the reaction. (f) That the effect is most striking in rapidly growing sarcomata; more slowly growing ones appear to have much more resistance. (g) That patients often gain in weight and strength while under treatment. (h) That the toxins may not be so effective against a recurrence. (i) That the severity of the reaction is variable, apparently depending upon the rapidity with which the toxins are absorbed. (j) That disappearance of the growths is not the result of high temperature alone, since fever from other causes is not followed by this result (51).

At about the same time Coley, too, described the macroscopic changes following treatment. "The toxins produce marked decrease in vascularity. . . . The
tumor soon becomes much more mobile and . . . . decrease in size is often noticed within two or three days after the treatment is begun.2 Cessation of pain is in many cases caused by the treatment, and anodynes, though indispensable before, are no longer needed.” This beneficial effect upon pain had already been noted by Finney (17, page 161) and later was observed by a considerable number of other physicians. Among these was Lagueux, who, in reporting cases of successfully treated carcinoma, said that the pain entirely disappeared almost immediately after the first injection and that it was not necessary to lose time by giving small doses; the more quickly fever was induced the more often was success achieved. He added that while he did not wish to seem more enthusiastic than Coley himself, he believed that at least 60 per cent of inoperable sarcomas and carcinomas could be successfully treated (44, page 470).

The second comprehensive study was that of Fowler (31). After reviewing the literature rather thoroughly, including the effects of intercurrent infections on malignant disease as recorded by Fehleisen (30), Busch (11), Billroth (8), Biedert (7), Plenio (56), Bruns (10), Coley (15), Misholt (50), Stein (71), Kleeblatt (43), and Wyeth (80); as well as the attempts to produce erysipelas in cases of malignancy recorded by Répin (57), Holst (40), Janicke (41), Lassar (45), and Spronck (70), he described the evolution of Coley’s final product, the mixed toxins of erysipelas and B. prodigiosus.

Fowler called attention to the varying effects that followed injection at different sites in Répin’s experience. Répin used the subcutaneous route at first, but because of the irritation and edema changed to intravenous injection, which, he found, caused no local disturbance and at the same time elicited a more prompt, decided, and uniform reaction. Fowler stated that the intensity of the general reaction varied with the dosage and method of administration; when the injection was made subcutaneously a larger quantity was required to produce the desired reaction, whereas a few drops were sufficient when the intravenous route was used. He added: “The vascularity of these tumors explains the ease with which a reaction can be produced by Coley’s method of interstitial injections, the latter being quite analogous, if not identical with, the intravenous method.” In Fowler’s experience a chill, the first noticeable symptom, occurred from 15 minutes to 2 hours after injection and rarely lasted longer than 10 minutes. In further describing the effects of the injections Fowler said:

“The symptom of elevation of temperature likewise depended on the dose. This followed the chill after three to six hours, if the latter occurred, and in some instances reached 40° C. The fever lasted from eight to ten hours. It was accompanied by the usual correspondingly increased pulse rate and frequency of respiration.

“Cephalalgia sometimes occurred, as well as nausea and slight vertigo. Herpes labialis, sweating of the palms of the hands, and fugacious eruptions were observed in some cases in which the fever was well marked. Estimates of the amount of urea showed this at times to be somewhat lessened, albumin was not found at any period.

“Examinations of the blood made ten minutes after the intravenous injections showed slight diminution in the number of leucocytes present. The subcutaneous injections were followed by no change in this respect. Leucocytosis was occasionally observed, this being more noticeable after the intravenous injections.

“Emaciation supervened rapidly with the use of large doses . . . . This, according to Répin, need excite no alarm, and should not constitute sufficient justification for discontinuing the treatment.”

It should be noted that a study of several hundred cases with various types of tumors indicates that emaciation occurs only where the neoplasm is extensive and large quantities of necrotic tumor are being absorbed under toxin therapy. These degenerative products appear to be hemolytic, and where tumors break down rapidly their absorption may cause toxemia or cachexia, which disappears when free drainage is established or the tissue has been absorbed.

As regards Coley’s technic of administration prior to 1898, Fowler stated: “All of Coley’s patients have been treated by interstitial injection into the tumor itself. This circumstance rather enhances the value of the evidence . . . . While it is true that decided effects can be produced by irritating substances . . . . such . . . . as turpentine . . . . these are not of curative value; furthermore, they have no elective action when injected at a distance . . . . Once the specific action of the toxins is admitted, it is highly probably that this should be exerted more energetically in situ than when injected at a distance.”

Not until after Coley’s death, in 1936, did other investigators demonstrate that to be most effective the toxins must reach the tumor via the blood stream as rapidly as possible, before they are neutralized by the tissues (67). This would indicate why intratumoral and intravenous injections produced more rapid destruction of the growths. Unfortunately, however, Coley gradually ceased using intratumoral injections in the period between about 1912 and 1915. He did not begin using intravenous injections until about 1926 to 1928. This route, tried at the suggestion of B. L. Coley, proved considerably more effective than intra-
muscular injections and more easily tolerated by the patient.

Fowler's appreciation of the importance of producing definite reactions should be emphasized. He wrote: "While it is true that the more decided the reaction as shown by the temperature, the better the outlook for a favorable influence upon the disease, it is not desirable that this should exceed 103⁰ to 104⁰ F. This is surprisingly well borne." As to the frequency of injection he said: "In the absence of excessive reaction or great debility, the injections may be given daily, with the expectation of obtaining two or three well-marked reactions during the week. With the occurrence of marked diminution in the growth, frequency may be diminished." Fowler believed that the injections should be kept up 3 or 4 months, with occasional intervals of rest lasting 3 or 4 days.

Fowler stated that, unlike most therapeutic agents, the toxins varied greatly in their effects upon different patients; so much so that it became necessary to establish the dose in each case by commencing with the minimum amount that would produce an effect in those most susceptible. Thus safety was assured, although a greater amount of time was required.

In making the present study the data on the toxins used prior to Fowler's observation were thoroughly reviewed, and it became evident that the variable response may have been largely due to the use of at least 8 different preparations. Fowler was apparently aware of the danger that immunity to the toxins might develop, for he wrote: "There can be no question that the general effects of the toxins become rapidly less pronounced in the course of treatment as immunization becomes established." He added that whereas a comparatively small dose was sufficient to produce a decided rise in temperature early in the treatment, this had to be increased many times to obtain the same effect as treatment progressed. He further stated: "Inasmuch as the local reaction upon the tumor follows a course parallel with the intensity of the general reaction, it necessarily follows that as immunization becomes established the effect upon the growth becomes less and less until at last it becomes nil, and the neoplasm, released from the restraint exercised upon it by the toxins, resumes its former progressive march . . . ." (31).

In discussing the rationale of the action of bacterial toxins on neoplasms, Fowler discussed the theory that the occurrence of high temperatures exerted an influence on the vitality of the tumor cells: "It is difficult to understand why conditions unfavorable to cell life created by the fever of erysipelas should differ in this respect from those arising from the fever, the result of other diseases." It was not until over 30 years later that the exhaustive research of Shwartzman and several other investigators indicated that not all bacteria generate toxins capable of eliciting necrosis and regression in malignant tumors. However, some of these investigators, including Andervont (1) and Aptiz (3), indicated that the destructive process may be enhanced by thermal hyperemia, apparently as a result of increased tissue permeability.

First Commercial Preparation

In October, 1899, Parke, Davis and Company undertook to produce the Coley toxins commercially, using Buxton's Type VI formula. This preparation, Parke, Davis Type IX, was made from late in 1899 until 1907 and letters found in Coley's files clearly indicate its comparative weakness and variability. One reason for its uncertain strength was that the streptococcus and prodigiosus cultures grew with variable luxuriance and no method had been devised to standardize the concentration of the suspensions. Cases in which both the Buxton Type VI and the Parke, Davis Type IX toxins were used further confirm the observations as to the comparative weakness of the latter. However, the technic of administration used by most physicians in this period was a favorable one; that is, injections were generally made into or near the tumor, and a certain number of successful results were obtained when the less potent Parke, Davis preparation was administered aggressively.

Huntington Research Fund Preparations (Tracy's X and XI)

Beebe and Tracy (6) appear to have done the first experimental work in this country on the effect of bacterial products on malignant animal tumors. They employed sterilized suspensions of four types of bacteria: B. prodigiosus, Streptococcus pyogenes, Staphylococcus pyogenes aureus, and B. coli communis (Escherichia coli). The streptococcus was from a fatal case of septicemia. The organisms were grown for 3 weeks in ordinary peptone broth; glycerine to the strength of 20 per cent was then added and a small piece of thymol as a preservative, and the suspension was heated to 75⁰ C. for 1 hour. Later, in order to obtain a more potent preparation, the cultures were centrifugized and the bacteria washed several times with sterile physiological saline, in a very little of which they were finally suspended. To this concentrated suspension glycerine and thymol were added as before, and the mixture was sterilized at 75⁰ C.

The toxic properties of B. prodigiosus had received little attention since Roger's work (60), so a preliminary study was undertaken by Tracy. The organism was shown to contain highly toxic substances lethal to animals in very small amounts.
oculation of a nonlethal dose of the suspension produced coagulation necrosis, while autolysis of the bacilli at 38°C for 2 weeks set free agents that passed easily through a Berkefeld filter and produced toxic effects identical with those elicited by the suspension. These toxic substances could be separated into two distinct fractions, the alcohol-insoluble fraction being highly toxic, whereas the alcohol-soluble fraction was chiefly hemolytic.

As preparations of these two fractions failed to produce a local lesion comparable to that caused by suspensions of the whole organisms the authors felt that little effect upon tumors was to be expected, and experiment verified this conclusion.

They standardized their bacterial suspensions by determining the nitrogen content, expressing dosage in mgm. of nitrogen. In order to bring the preparation of the mixed toxins into line with this method of measurement, a definite quantity of prodigiosus suspension of determined nitrogen content was added to the broth culture of streptococcus so that each cc. of the mixed product contained 2.5 mgm. of prodigiosus nitrogen.

These preparations were all tried on dogs bearing large transplanted lymphosarcomas, with the following results: (a) Concentrated suspensions of streptococcus were necessary to produce effects. (b) Killed cultures of B. prodigiosus were definitely destructive to the tumor, as were the toxins of B. coli. (c) The toxins of Staphylococcus pyogenes aureus had no inhibiting influence, even though injected directly into the growths. Necrosis set in at once in tumors that had been directly injected and within 24 hours considerable discharge of necrotic tissue took place, so that by the second day only the smallest remnant of tumor was still palpable. On the other hand, no sign of regression occurred in a dog treated by intramuscular injections remote from the tumor until the end of the second week of treatment. The need for surgical drainage where fragments of necrotic tumor tissue are not absorbed was established.

In conclusion Tracy and Beebe stated: "The results . . . . certainly demonstrated the destructive action . . . . on tumor cells of this type by bacterial toxins. Such action, while chiefly local, is at the same time something more than this, for it is repeatedly observed that tumors at a distance . . . . undergo regression simultaneously with those inoculated, in while in one instance the entire treatment was by inoculation remote from the tumor."

As a result of these studies, Tracy evolved the first stable preparation of Coley's mixed toxins, and it proved to be considerably more potent than any other prepared from 1892 until 1942 (19, 20). A streptococcus culture from a fatal case of septicemia was used. The two organisms were grown separately and heated to 75°C for an hour in a water bath. The prodigiosus was then reduced to a dry powder and the amount employed, determined by Kjeldahl's method, was 5 mgm. per cc. of the mixed toxins. After mixing and bottling the preparation was again sterilized in the water bath at 75°C for 2 hours, and thymol and glycerine were added as preservatives. Coley found this product, Tracy's Type X, much more stable as well as more powerful in its action, and said that the results in inoperable sarcoma had shown a distinct improvement over those obtained with the older preparations (20). Type X was made from early in 1906 until the latter part of 1907, when the amount of prodigiosus was reduced to 2.5 mgm. per cc. of the mixed toxins (21, 22). This preparation, Type XI, was made by Tracy from the latter part of 1907 until June, 1920. From then until late in 1921 the same formula was prepared by Dr. Morton Kahn, with only the modification that he used the single cell method to insure purity of the cultures.

It appears that Coley recognized the importance of technic of administration at the time Tracy's preparations were introduced, for he said in 1909: "Much depends upon a judicious determination of the dosage for a given case. As a rule I give as much as the patient can safely stand. I always begin with ½ minim, diluted in sufficient boiled water to insure accuracy, injected either in the buttocks or the pectoral region. After the individual's susceptibility has been ascertained, one can inject into the tumor itself if it is not in an inaccessible region. The initial dose into the tumor should always be less, not more than one-fourth that used elsewhere. [He advised alternating between the tumor and the muscle.] Daily injections should be given, increasing by one-fourth minim until the desired reaction, a temperature of 102°-104° is obtained. This should be modified to suit patients in a weakened condition. Having secured the desired reaction, the dose should no longer be increased until it fails to give a reaction . . . . the highest dose given in many of the cured cases has been seven or eight minims." He usually preferred to give the injections locally, for he had found the effect more rapid and more definite than when given subcutaneously and remote from the tumor. "The dose depends largely upon the vascularity of the tumor and upon the condition of the patient." (22).

The highest number of successful results in various types of tumors was obtained in the period from 1906 to about 1912, when Tracy's potent and stable preparations were first being used, and when the effective technic described above was being advised.

It is evident, however, that Coley realized there were certain weak and unreliable preparations being dis-
tributed as “Coley’s Fluid” prior to 1907, for after describing in detail the formulas used by Tracy he stated: “It is important . . . to know how [the toxins] have been prepared, for the results vary greatly with their composition and manner of preparation. Many . . . now on the market have been so weak as to produce hardly any reaction and have been found . . . of little value. Parke, Davis and Company, we know, have made great efforts to keep their product up to the standards of the [Huntington] Research Laboratory; of other preparations I have no personal knowledge” (20).3

Late in 1907 or early in 1908 Parke, Davis and Company adopted the Tracy Type XI formula (designated as Parke, Davis XII). Unfortunately, the formula Tracy sent to Parke, Davis apparently did not specify at which stage the nitrogen determinations should be made or the need for using chromogenic cultures of B. prodigiosus. For these and other undetermined reasons the Parke, Davis preparations continued to be weaker than those of Tracy. This statement is based on the experience of a considerable number of physicians here and abroad, as well as with some of Coley’s cases. While some of these men realized that something was wrong, a far larger number undoubtedly did not, condemning instead the fundamental principles of the treatment.

Finally, in April, 1915, a particularly clear-cut example of the comparative weakness of the Parke, Davis product was reported to Coley by Tuholske (23). At Coley’s suggestion, Parke, Davis, in collaboration with Tracy, attempted to make their product more effective. The resulting preparation was designated as Parke, Davis Type XIII. Since 1915 the following slight modifications have been made in this formula: (a) Some time between 1915 and 1922 the attempt to maintain virulence by passage through rabbits was abandoned. (b) In January, 1922, a new streptococcus culture was obtained from a case of erysipelas.

It should be noted that Tracy also prepared filtered toxins in the period during which the unfiltered Type XI was available (1906 to 1921), as did Parke, Davis. These appeared to be considerably less potent than the unfiltered toxins, although the formulas are believed to have differed only as regards the method of sterilization. It was observed in a number of cases in which both the filtered and the unfiltered toxins were used that the patients did not seem to develop immunity to the filtrate as they did to the unfiltered preparations. However, from the evidence available, it appears that the filtrates used by Coley and other physicians were not so effective.

The Lister Institute adopted Tracy’s Type XI formula some time in 1907 or 1908, and has continued to make this preparation until the present time. However, the cases available for study are not sufficient to justify full comparison of its potency with that of Tracy’s Type XI, or the Parke, Davis products available in the same period.

No experimental work seems to have been done in this field between 1907 and about 1930. Interest in the bacterial toxins was revived in 1931 by Gratia and Linz (33), who, having attempted to produce local tissue reactivity in a liposarcoma of the guinea pig, found the filtrates capable of eliciting the phenomenon, and also hemorrhagic necrosis, in the tumors without any preparatory injections.

Shwartzman and Michailovsky described the same phenomenon in a rather extensive series of mice bearing transplanted sarcomas (68). Duran-Reynals pointed out that besides their intrinsic value two considerations make these findings particularly interesting: (a) That mice are insusceptible to the ordinary Shwartzman phenomenon (local tissue reactivity), and it would seem that special conditions in the tumor render its vessels susceptible to the toxins. (b) That this state of reactivity in the tumor in either susceptible or nonsusceptible animals is a permanent one so that no sensitizing injection is required, as is the case with the ordinary Shwartzman phenomenon in rabbits and guinea pigs (26, 27). Another significant observation of Duran-Reynals was that animals in which tumors had regressed after injections of bacterial toxins were resistant to reinoculation. He reported on the vascular reactions to a B. coli toxin of low potency of a series of growths ranging from benign embryomas and granulomas to highly malignant transplantable carcinomas and sarcomas of mice and rats. He concluded that only those growths showing at the same time malignancy and rapidity of growth present the phenomenon described by Gratia and Linz. Duran-Reynals stated: “It is obvious that the phenomenon is conditioned by two sets of factors: (a) The intrinsic factor, depending on the sensitivity of the tumor itself. (b) The extrinsic one, depending on the activity, quantity and route of inoculation of the toxin.” Additional studies showed that very susceptible tumors required smaller doses of toxin to produce the same effect, and the same result was obtained with filtrates of low toxicity as with the more active ones (26). Duran-Reynals also noted that the route of inoculation did not seem a matter of much importance in treating these rapidly growing tumors. In conclusion he stated: “The facts brought to light in transplanted as well as in spontaneous tumors establish the principle that the newly formed vessels of malignant growths . . . are extremely sensitive to . . . bacterial toxins, and . . . this fact . . . creates . . . the tumor vulner-
The exact nature of the effect of bacterial filtrates on transplanted tumors has not been elucidated. It is now known that it resembles closely, if it is not identical with, local tissue reactivity (Shwartzman's phenomenon) (67). The most striking macroscopic and microscopic finding is hemorrhage in the tumor about 4 hours after injection of the toxins. Apitz noted edema of the tumor cells, which he believed to be independent of the hemorrhage, for while both were produced by large doses, only edema was seen with smaller ones (3). Apitz observed no effect unless a lethal or sublethal dose was used—further evidence of the need for aggressive treatment in human patients.

Andervont found that the macroscopic reaction, a bluish discoloration within the tumor, that increased in intensity for several hours, was clearly visible 2 to 4 hours after injections (1). When complete destruction of the tumor occurred the discoloration continued until the growth became black, and within 24 hours all that remained was a hard, dry mass of dead tissue. This scab usually persisted for a week or two and then dropped off, exposing a mass of granulation tissue.

Microscopic studies of tumors removed at 2 hour intervals revealed an accumulation of white blood cells in the capillaries about 2 hours after injection. This was followed by congestion and eventual diffuse hemorrhage. Some of the tumor cells were swollen prior to hemorrhage, indicating that the toxins may have a direct effect. Andervont and Shimkin (1) believe that transplanted tumors regress after these injections only when gross hemorrhage is produced in the tumor, and that regression is directly proportional to the amount of hemorrhage; thus if only the center is affected growth of the nonhemorrhagic periphery is uninterrupted.

The need of early treatment is suggested by the work of Duran-Reynals, who stated: “... mice having larger spontaneous tumors and receiving larger doses of toxins had a greater intensity of local reaction, a higher early mortality and a lower per cent of partial or total inhibition than ... the group with ... small tumors receiving smaller doses of the toxins” (27, 28).

Gardner, Bailey, and Hyde found that maximum hemorrhagic reactions after intravenous injection are determined by an optimal dosage, which causes a prolonged and intense systemic reaction and depends upon the sensitivity of the host. Reaction in the tumors was discernible 30 minutes after injection. Twenty-four hours later, sections showed definite hemorrhagic discoloration in those areas outlined by necrosis (32).

Perhaps the most persistent and exhaustive experimental studies in this field have been made by Shear, who has isolated an active fraction, apparently a polysaccharide, from the toxins of B. prodigiosus (Serratia marcescens) that appears to be 1,300 times more potent than the Parke, Davis Type XIII preparation (38, 65).

The Lankenau Hospital Research Institute recently began a study of the effects of Shear's polysaccharide upon human neoplasms, a significant feature of which is biopsy, not only before but during treatment.

DISCUSSION

The various preparations of Coley's toxins, and laboratory research in this field during the first 50 years after their introduction, has been reviewed in considerable detail because there has never been a comprehensive analysis of this method. The data indicate rather clearly the importance of maintaining closer cooperation between research and commercial laboratories and the physicians who administer toxin therapy.

A brief review of some of the other factors that hindered the acceptance and further development of toxin therapy may be of value. One of the first was the explanation that disappearance of a tumor under the treatment was probably due to spontaneous regression, and thus merely coincidental. These critics do not appear to have considered that the majority of so-called spontaneous regressions recorded in the literature occurred during or following an acute bacterial infection of some sort, including erysipelas, pneumonia, pyemia, typhoid, and others. Furthermore, the frequency with which neoplasms failed to recur after the administration of Coley's toxins precludes serious consideration of this criticism. Another objection most often heard was that the successful cases had not been unequivocally found malignant rather than benign tumors. Fortunately, Coley recognized the need for searching diagnostic tests in his cases, and the clinical and roentgenological data were supplemented by microscopic examinations by such leading pathologists as Ewing, Wolbach, Welch, Whitney, and Stewart, or the pathological departments of the larger hospitals. The majority of the bone cases were reviewed by the Bone Sarcoma Registry Committee.

A somewhat later criticism, namely, that if toxin therapy had the value claimed by Coley it should have become universally adopted was answered by Coley as follows: "I will call attention to one fact, apparent to anyone familiar with the history of medical discoveries; that the relative value of such discoveries bears not the slightest relation to the rapidity of acceptance by the medical profession" (24). We would add that even where scientific discoveries are accepted fairly soon, enormous difficulties have to be overcome before any new method becomes generally adopted.
This is particularly true in the field of cancer therapy, which for more than two thousand years has been ridden with quacks and so-called cures. In the case of toxin therapy the natural reluctance of physicians to embrace any radical new therapeutic measure was strengthened by the special problems already described: variability of the preparations, and ignorance of the optimum technic of administration. In addition, it is believed that the discovery of x-rays and radium so soon after toxin therapy was introduced may have played a part, for these commanded the attention of the profession everywhere, stimulating research and attracting munificent endowments. Thus radiology achieved popularity at a period in which toxin therapy had not yet been adequately developed.

Still another handicap was the lack of readily accessible literature. Coley's earlier papers and those of Fowler (31), Moullin (51), Harmer (37), Beebe and Tracy (6, 75), as well as the reports of successful cases treated by other surgeons here and abroad, are probably to be found only in the larger medical libraries, and considerable time and effort are required to locate and read them. Studied separately, they give only a fragmentary and rather confusing picture of what was accomplished.

Another factor that has received little or no consideration is the possible effects that other forms of treatment, given before or during toxin therapy, might have had upon the latter. In making the present analysis this was carefully studied. It was found that any agent that alters or destroys the vascular or lymphatic channels through which the toxins must reach the neoplasm or inhibits the regeneration of normal tissues, such as heavy radiation or repeated incomplete surgical procedures, appears to limit the effectiveness of subsequent toxin therapy. Since recent cytological studies indicate that the neoplastic cell is most responsive to toxin therapy during division, it would appear that anything that inhibits the rate of mitosis during toxin therapy may also slow up or minimize the destruction of tumor tissue by this treatment. These factors suggest that where the use of both toxins and radiation is contemplated, toxin therapy should invariably be completed before radiation is instituted. It has been further noted that any process that decreases tissue permeability may have a deleterious effect on toxin therapy. This point deserves further study with a view to using invasive strains of bacteria, or including a "spreading factor" such a hyaluronidase, as a means of possibly enhancing the effect of bacterial products (28).

To establish the treatment of malignant tumors by bacterial products on a more scientific basis, the following program is suggested:

1. Publish clinical data, to provide a comprehensive history and an analysis of the treatment. This would serve as a guide to the method, prevent some of the mistakes and misconceptions of the past from being repeated, and stimulate further research.

2. Encourage bacteriological research to make more potent and dependable preparations available. Many organisms are known to possess high titers of tumor-destructive toxins, and many more will need to be investigated. The ultimate goal is to isolate the active principles and produce stable and dependable preparations.

3. Institute clinical research on all types of tumors where the diagnosis is unequivocally established, using purified and concentrated preparations. Such methods as surface application of toxins to fungating tumors; the injection of minute doses into different parts of a tumor; continuous intravenous drip; dermoclysis; the establishing of a blood level, as in the use of the sulfonamides; are possible lines of experimentation.

4. Make physiological studies to determine the possible effects of toxin therapy upon the organism. The existing evidence indicates that certain beneficial effects may occur, including decided relief of pain, increase in appetite, better sleep, stimulation of wound healing, and regeneration of bone and other tissues destroyed by the neoplasm. These appear to vary according to the stage of the disease, the toxin used, the dosage, and the route of inoculation. The physiologic approach might include a study of the effects of toxin therapy on conditions other than malignancy, i.e., arthritis, paresis, various eye affections, etc., conditions in which the toxins have been used empirically by various physicians other than Coley with apparent benefit.

5. Establish a central clearing house where detailed records of histories may be registered and progress analyzed. (For additional data address H. C. Nauts, 1290 Madison Avenue, New York 28, N. Y.)

SUMMARY

This study provides sufficient evidence, both clinical and experimental, to justify the conclusion that toxin therapy has clinical value, and that further extensive research is warranted in order to produce better preparations and further refinements in the technic of administration. Reasons are given to explain why the method has not achieved wider recognition in the past.

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Note: This is only a part of the total bibliography used in the course of making this analysis.
The Treatment of Malignant Tumors by Bacterial Toxins as Developed by the Late William B. Coley, M.D., Reviewed in the Light of Modern Research

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