

secondary plants than existed to primary plants. If an animal is growing a tumor well he can nearly always be successfully planted again from his own tumors. We have had one instance in which the primary plants were all regressing, while secondary plants were growing steadily. This whole question of immunity in experimentally cultivated tumors is of so great complexity of the tumor graft and the method of transplantation, and there are so many variable factors, such as virulence that at present we can safely say only that immunity is relative; that it may be either natural or acquired, and has thus far been developed experimentally only by the actual growth and regression of a tumor in a selected animal.

The precise nature of the immune factors is not yet understood and the facts are as yet too scanty to permit of very helpful theorizing. We wish, therefore, to put on record the following facts without discussing their bearing on current theories. From some of the experiments carried on in our laboratory which confirm in part these made elsewhere, we are inclined to believe that the blood may contain some expression of the immune condition of an animal, and, if so, the possibility of transferring this immunity to a second individual is open. We have exposed thin slices of freshly removed tumor to a varied environment, such as normal saline solution and defibrinated blood from both susceptible and immune animals, and our impression has been that immune blood is not as favorable a medium for the preservation of these cells as the saline solution or the susceptible blood. Further evidence that the serum from a recovered animal has a deleterious effect on tumor cells is found in the transplantation experiments at the Buffalo Laboratory with cell suspensions in salt solution, normal serum and recovered serum. A smaller percentage of takes was found in the case of the emulsions prepared with the recovered serum, a fact which can scarcely be explained otherwise than by supposing some injurious effect on the tumor cell during its exposure to the serum.

We have had some confirmation of this point and additional evidence that the blood contains immune factors which confer a passive immunity on a susceptible animal. We are indebted to the active cooperation of Dr. George W. Crile for valuable assistance in carrying out these experiments, the detailed results of which will appear later. Dr. Crile has transfused the whole blood from immune animals to animals with actively growing tumors, the latter having been bled prior to the transfusion, and we have seen following this operation a regression of the tumors in the susceptible animal. Such a result has not invariably followed, but if our observations on these experiments are to be summarized we may say that the beneficial results have varied with the condition of the donor and the amount of blood transfused. In one instance the donor was in a very poor physical condition, although his tumors had completely regressed. The dog to whom his blood was given showed no beneficial result whatever, his tumors continuing to grow rapidly. The donor, after the transfusion, was again planted with the tumor with positive results, all four plants taking. As a contrast to such an experiment, we may cite the transfusion from a naturally immune animal in splendid physical condition. In this case a very large amount of blood was exchanged, 600 c.c. having been withdrawn from the tumor animal and 1,500 c.c. having been taken from the donor. Following this transfusion

the tumors regressed rapidly and the animal is now completely recovered.

In the beginning of its course the tumor which we have been studying runs a benign course, with little or no infiltration and with apparently no effect on the health of the animal. Later general metastases appear, the animal becomes cachectic and dies. The cachexia appears late in the course of the growth when there is a comparatively large amount of autolyzed tumor products capable of absorption. The method of origin of this cachexia has been the subject of investigation during the past year. Dr. Weil has found that the extracts from the tumor tissues have a marked hemolytic effect, and that particularly the normal saline extracts from necrotic tumors contain a very active hemolysin. We are inclined to interpret these results as meaning that the necrotic tumors contain substances toxic for normal body cells, this toxicity toward erythrocytes being expressed as a hemolytic effect.

We do not know why this tumor begins to grow, but we can readily understand how it may be transmitted from one host to another, and in neither its origin nor transmission have we any evidence of the action of a micro-organism. There is, therefore, no more reason for applying the term infectious to this process than exists in the case of any other tumor which may be transplanted, and, indeed, following such logic, normal epithelium should be considered infectious. The cause of the tumor will not be found until our knowledge of physiologic growth and development is more complete. So far as these facts can be applied to the human subject, they indicate that there is no reason to consider human tumors infectious to any greater extent than the tumors we have followed in this study of the dogs. It is possible that human tumors can be transplanted in the human species, but we can not argue therefrom that certain houses or water courses are sources of infection. Our efforts to find a direct cure for these tumors has been limited to the study of the mixed toxins advocated by Dr. Coley in the treatment of sarcoma. Dr. Tracy has carried out these experiments in addition to making a thorough study of the toxins of bacillus prodigiosus, the results of which are now in press. The outcome has been very interesting and demonstrates beyond doubt the value of the method. The study has likewise demonstrated the unstable equilibrium which the tumor cell has in comparison with normal cells, since a great variety of toxins affect these growths unfavorably. Apparently the tumor cell has a special function of growth enormously developed, but the means of defense have been sacrificed.

THE TREATMENT OF EXPERIMENTAL TUMORS WITH BACTERIAL TOXINS.*

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The use of injections of the combined sterilized cultures of *Streptococcus* and the *Bacillus prodigiosus* in the treatment of inoperable sarcoma has acquired considerable prominence since its introduction by W. B. Coley of New York some fifteen years ago.

It is well known that intercurrent attacks of ery-

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* From the Huntington Fund for Cancer Research of the General Memorial Hospital. The laboratory work forming the basis of the report was done at the Loomis Laboratory, Cornell University, Medical College, New York.

sipelas have been observed in a number of cases to exert a restraining and even curative influence on the course of malignant tumors, and investigators have endeavored by inoculation with streptococcus cultures to bring about artificially equally beneficial results. Roger of Paris, in experimenting on rabbits, believed that by an admixture of the *B. prodigiosus* with his streptococcus he could enhance the virulence of the latter cultures, and Coley¹ applied this idea to the treatment of sarcoma, beginning in 1892 a systematic clinical study of the therapeutic effect of such mixed toxins. The striking results attained in an increasing number of cases by this method of treatment have directed attention to the possible significance of the *B. prodigiosus* in the preparation, and have seemed to us to warrant an investigation of the subject from this point of view. Since, also, we have been able at the Loomis Laboratory to maintain by transplantation a strain of lymphosarcomas in dogs, we have had at hand material offering a valuable opportunity for experimental work of this sort.

It seemed of importance to study with some care the

bacillus has been well established and needs no comment here. The streptococcus used was obtained from a fatal case of septicemia, and no attempt was made to keep up its virulence by passing it through a series of animals. At first the organism was grown for three weeks in ordinary peptone broth, glycerin to the strength of 20 per cent. was then added, and a small piece of thymol as a preservative, and the suspension then heated to 75 C. (167 F.) for one hour. Later, in order to obtain a preparation which would be more powerful, bulk for bulk, than the above, the broth cultures were centrifugalized and the bacteria washed several times with sterile physiologic salt solution, in a very little of which they were finally suspended. To this now concentrated suspension, glycerin and thymol were added as before, and the mixture sterilized at 75 C. (167 F.).

The *Staphylococcus aureus* was from the laboratory stock, and was grown on agar for from 48 to 72 hours, was then rubbed off in salt solution and prepared as described above. The *B. coli communis* was also grown on agar and prepared as was the staphylococcus.

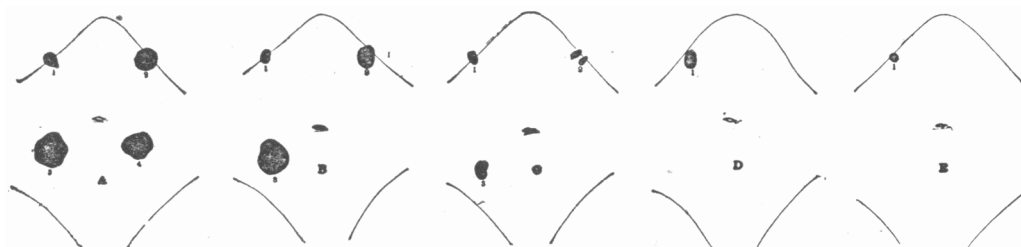


Fig. 1.—Dog 268. (Experiment 2.) Treated with suspension of *B. prodigiosus*. Measurements: A, May 13; B, May 22; C, June 1; D, June 15; E, July 15. Last trace of tumor gone July 22.

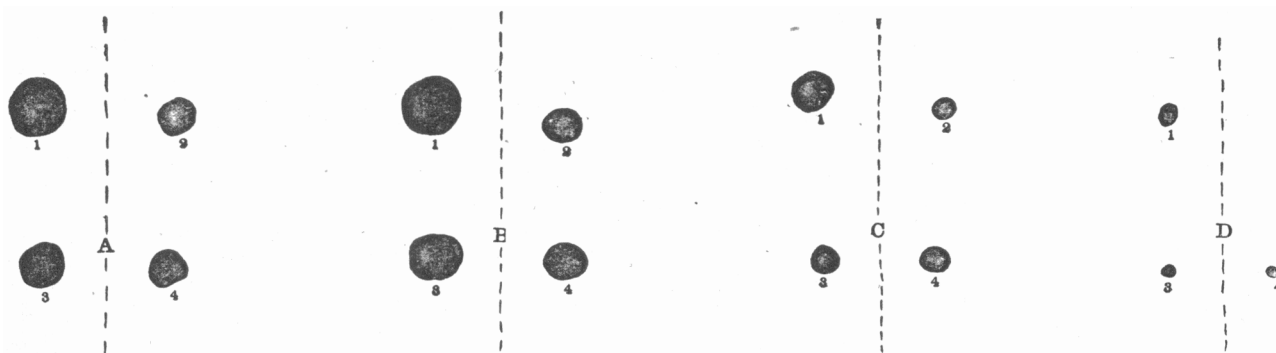


Fig. 2.—Dog 416. (Experiment 3.) Treated with suspension of *B. prodigiosus*. Measurements: A, June 22; B, June 28; C, July 15; D, July 30. Inoculation at no time into tumors. June 25 inoculation into left thigh; June 28 inoculation into right thigh; July 3 inoculation subcutaneously on abdomen; July 5 inoculation subcutaneously on abdomen.

effect of inoculations, not only with the *B. prodigiosus*, but with other bacterial toxins as well, on the actively growing dog tumors, with the hope of determining the rationale of this method of treatment, and if possible placing it on a more scientific basis.

The observations to be reported briefly at this time have covered a period of one year, and, while much further study of the subject is desirable, the facts already established are of considerable interest.

The bacteria thus far used in the work are but four, the *B. prodigiosus*, the *Streptococcus pyogenes*, the *Staphylococcus pyogenes aureus* and the *B. coli communis*. As these organisms exert their poisonous influence by means of so-called endotoxins the preparations used consisted, for the most part, of sterilized suspensions of the whole germ cells.

The toxicity of the streptococcus and of the colon

The toxic qualities of the *B. prodigiosus* have not received much attention from investigators, and a preliminary study of this organism was undertaken and has recently been reported by one of us.² Some of the conclusions reached in that investigation may with advantage be restated briefly here. It was shown that "the bacterial cell of the *B. prodigiosus* contains highly toxic bodies capable in very small doses of causing death in animals;" that the "subcutaneous inoculation of a non-fatal dose of prodigiosus suspension produces a local lesion consisting of a central area of coagulation necrosis, surrounded by a zone of active round-cell infiltration;" that "autolysis at a temperature of 38 C. (100.4 F.) for two weeks set free from the germ cell soluble toxic substances which passed freely through a Berkefeld filter and which were capable of producing in animals toxic effects identical with those which had been

1. Amer. Jour. Med. Sci., March, 1906.

2. Tracy (M.): Jour. of Med. Research, May, 1907.

observed to follow inoculations with the whole germ substance; that a solution of these toxic substances could be divided chemically into two distinct fractions, and with each fraction could be correlated certain poisonous qualities, an alcohol-insoluble fraction being highly toxic, while an alcohol-soluble fraction was chiefly hemolytic in action."

In the light of this study it seemed advisable to use not only a sterilized suspension of the whole prodigiousus germ cells, prepared in a way similar to that given for the colon bacillus, but also preparations of the two soluble fractions described above. Inasmuch, however, as subcutaneous inoculations with these soluble fractions had failed in the preliminary experiments to produce any local lesion comparable to that caused by the whole prodigiousus germ cell, little effect on the tumor was to be expected from the use of these solutions, unless the action should prove to be a systemic one.

In order that the action of these preparations might be accurately compared, it was necessary to devise some method of measurement which could be used for the soluble fractions of the prodigiousus toxin as well as for

is impossible to say what part of the bacterial body is non-poisonous. Experimentation has shown, however, that such estimations of toxicity form a satisfactory working basis for dosage.

The suspensions of the *B. coli communis* and of the staphylococcus, both of which were grown on agar, could be measured in the same way. With the streptococcus, however, which grows more readily in broth, the proteid of the medium must enter into consideration; although in the concentrated streptococcus suspensions referred to above the repeated centrifugalization of the cultures with washing in physiologic salt solution removed this difficulty.

In order to bring the preparations of mixed streptococcus and prodigiousus toxins into line with this method of measurement, a definite quantity of prodigiousus suspension, of determined nitrogen content, was added to the broth culture of streptococcus, so that each c.cm. of the mixed product contained 2.5 mg. of prodigiousus nitrogen.³

The tumors treated were, as we have said, lymphosarcomas grown in dogs as a result of transplantation

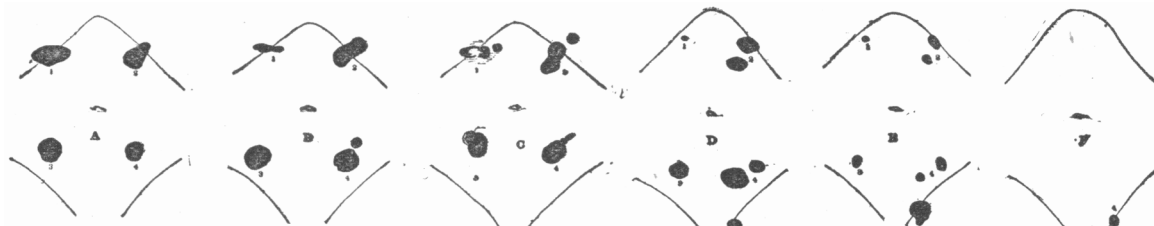


Fig. 3.—Dog 193. (Experiment 4.) Treated with suspension (sterilized broth culture) of streptococcus. First inoculation into tumor 2, April 23; inoculation into tumor 1, April 26; reinoculation April 27 and 30; inoculation of tumor 1, May 1, inoculation into tumor 3, May 10; reinoculation into tumor 1, May 17, 18 and 20; reinoculation May 22, 27, 28 and 31 divided between tumors 1 and 3; reinoculation of tumor 3, June 12, 14 and 17; reinoculation of tumor 4, June 19 and 21; no further inoculation. Measurements: A, April 23; B, May 10; C, May 22; D, June 11; E, June 28; F, July 30.



Fig. 4.—Dog 123. (Experiment 5.) Treated with toxins of streptococcus and *B. prodigiousus*. Inoculation of tumor 1, February 11; reinoculated February 18, 21, 25 and 28; no inoculation into tumor 2 at any time. Measurements: A, February 11; B, February 25; C, February 28; D, March 12.

the suspensions of the whole germs. One difficulty heretofore encountered in the use of the mixed toxins has been the varying power of the preparations and lack of any standard for measurement thereof. In the paper already referred to it was shown that this difficulty lay in the unequal growth of the prodigiousus broth cultures, a matter which it was exceedingly difficult if not impossible to control. It was found, however, that by cultivating the bacillus in large flasks on agar a luxuriant growth was obtained, and the thick pellicle of bacteria could be removed practically free from contamination with the medium, and containing, therefore, no proteid but that of bacterial origin. An even suspension of such bacterial growth was made, using sterile physiologic salt solution, and the nitrogen content of this suspension determined by the Kjeldahl process. The dosage could then be expressed in milligrams of nitrogen.

It is, of course, obvious that such a method does not give the measurement of the actual toxic proteid, for it

from a spontaneous tumor removed by operation at the veterinary hospital of Dr. T. G. Sherwood.

As soon as palpable tumors could be recognized, following such transplantations, the animals were kept under careful observation, and the growths measured at intervals, graphic records being kept of the condition. When the tumors had reached a considerable size, inoculation with the described bacterial suspensions was begun.

Up to the present time three animals have been

3. The formula of the mixed toxins of streptococcus and bacillus prodigiousus as now prepared for the use of Dr. Coley and others is as follows: To each 100 c.c. of three-weeks-old broth culture of streptococcus is added 30 c.c. of a suspension of *B. prodigiousus* containing 375 mg. of prodigiousus nitrogen. This suspension of prodigiousus is obtained by growth of the organism on agar, removal of the mass of bacteria after ten days, and rubbing it into a smooth suspension with physiologic salt solution. The nitrogen content of 1 c.c. of the suspension is determined and if too concentrated it is brought to the desired dilution by the addition of more salt solution. To this 130 c.c. of mixed bacterial suspension is then added 20 c.c. of glycerin, and after bottling, a small piece of thymol to each bottle. The whole is then sterilized at 75 C. (167 F.) for two hours.

treated with suspension of the whole prodigiousus germ cells, one with the alcohol-insoluble toxic fraction of prodigiousus filtrate, one with the alcohol-soluble hemolytic fraction of the same, one with the streptococcus suspension; three with suspensions of streptococcus and prodigiousus mixed, the so-called "mixed toxins," one with the staphylococcus suspension, and one with suspension of the colon bacillus. Briefly the clinical history of these animals is as follows:

CLINICAL HISTORIES.

Experiment 1.—Dog 1 at the beginning of treatment presented five abdominal tumors, the largest measuring $3\frac{1}{2}$ by 2 inches. The first inoculation of a prodigiousus suspension was made May 9, 1906, 6 c.c. containing 6.0 mg. of prodigiousus proteid,* being injected into the large tumor, and 1 c.c. into each of the smaller growths. A severe chill and vomiting followed about an hour later, and in 3 hours the temperature had risen to 41.2 C. (106.4 F.). In 24 hours the tumors were softened, especially the large one, which was much swollen also. The dog was very sick, and its temperature did not go below 39.7 C. (103.6 F.). On the second day the large tumor showed distinct fluctuation, and on tapping with trocar and canula about 100 c.c. of semifluid necrotic tissue were re-

This preliminary experiment was suggestive in several points: In the first place it indicated that the *B. prodigiousus* itself exerts a powerful toxic action of some sort on the tumor cells. Secondly, the prompt reaction which followed the inoculations with the boiled suspension demonstrated that even this high temperature failed to injure those substances in the germ cell which were responsible for the local and systemic effect. And, finally, it was made clear that the treatment must not be pushed to the extent of reducing the general condition of the animal if good results were to be obtained.

Experiment 2.—Dog 268 (Fig. 1) presented four tumors, three of a diameter of $1\frac{1}{2}$ to 2 inches, and one smaller. The first inoculation was made May 13, into one of the large tumors (Fig. 1, tumor 4). A well-sterilized thick suspension of prodigiousus was used, the dose of 0.1 c.c. containing 3.6 mg. of proteid material. The animal's temperature rose to 41 C. (105.6 F.). In 24 hours there was swelling and fluctuation of the inoculated tumor, and tapping with the trocar permitted the removal of considerable thick necrotic material. By the second day all inflammation had subsided and only the smallest possible bit of tumor was palpable. Two of the remaining tumors were softened and decreased in size. A second

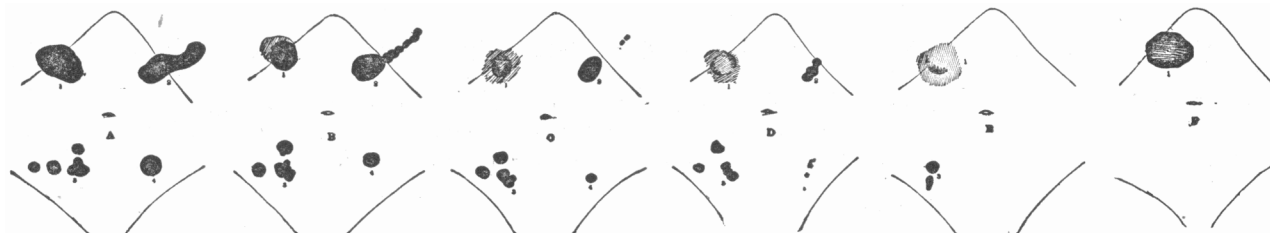


Fig. 5.—Dog. 180. (Experiment 6.) Treated with mixed toxins of streptococcus and *B. prodigiousus*. Inoculation of tumor 1, March 18; reinoculated March 21, 26, 27, 30, April 2, 4, 5, 8, 12, 20, 23, 24, May 10, 17, 18, 27, 28 and June 12. Shaded area in B represents softened tumor; in C shaded area represents inflammatory swelling masking outline of tumor. Tumor 4 in D was removed for histologic study. Measurements: A, March 18; B, March 30; C, April 7; D, April 17; E, April 26; F, June 15.

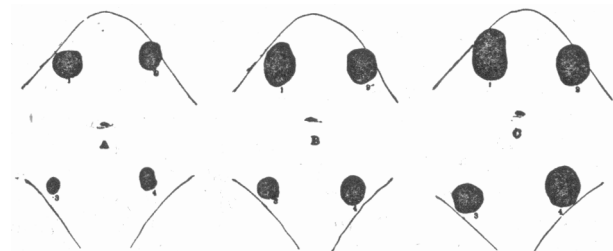


Fig. 6.—Dog 244. (Experiment 8.) Treated with suspension of *Staphylococcus pyogenes aureus*. Inoculation into tumor 1, June 25, 27, 28, July 3, 5, 8, 15, 17, 22. Tumors continued to grow. Measurements: A, June 22; B, July 6; C, July 30.

moved. The smaller tumors were softened, but did not break down. For several days reaccumulations of fluid in the large tumor cavity were removed, and the dog's temperature remained high, varying between 38.6 C. (101.5 F.) and 39.4 C. (103 F.). A culture taken from the tumor discharge gave an active growth of prodigiousus, demonstrating the insufficient sterilization of the material used for inoculation, and indicating the possible existence of a prodigiousus septicemia to account for the temperature curve.

Further injections were made with boiled suspensions, at intervals of five or six days for about a month. A severe reaction, chill and high fever, followed each inoculation, and all the tumors broke down and discharged freely. The animal continued in bad condition, however, lost weight in spite of the fact that he ate a large amount of food, suffered severely with the mange, which we were unable to control, and was finally killed in July. At the time of death there still remained growing margins around the discharging tumor areas. At autopsy intra-abdominal metastatic growths were found.

4. Where any quantity of bacterial proteid is referred to the figure is obtained from the nitrogen content by multiplication with the usual factor, 6.25.

inoculation was given into one of the remaining tumors (Fig. 1, tumor 3), and this was repeated on alternate days for four more doses. The last dose was of 0.3 c.c., as some tolerance had been acquired and the smaller dose failed to produce any reaction. By May 27 all trace of the second tumor inoculated had disappeared and the two remaining were noticeably smaller. No further treatment was given, and the regression continued, until by July 22 no trace of any tumor remained.

Experiment 3.—Dog 416 (Fig. 2) was treated with prodigiousus suspensions injected at a distance from the four tumors, which were on the back. At no time was an inoculation made into or near any of the growths. Four treatments only were given, two of the injections being intramuscular, into the thighs, and two subcutaneous on the abdomen.

Following the first intramuscular injection the animal's temperature rose to 41.6 C. (107 F.). There was necrosis at the site of each injection, but rapid healing after the discharge of thick semifluid material. At the end of the second week of treatment the tumors first showed signs of regression, and a month later, with no further treatment, had disappeared completely.

The history of the two animals treated with the soluble toxins of prodigiousus may be given in a word. It was entirely negative. Repeated inoculations with the toxic fraction, in spite of a good general reaction as evidenced by a temperature of 40 C. (104 F.), failed to produce locally more than a slight inflammation which subsided without effect on the tumors. The hemolytic fraction in large doses failed to produce either general or local result. In both of these animals the tumors continued to grow steadily.

Experiment 4.—Dog 193 (Fig. 3) received inoculations with suspensions of the streptococcus. The animal presented four abdominal tumors, and during six weeks' treatment the two

tumors inoculated (Fig. 3, tumors 1 and 2) softened and regressed to a certain extent with comparatively little sloughing. The uninoculated tumors (Fig. 3, tumors 3 and 4) at first continued to grow, but later remained stationary for some weeks, or at most showed the slightest possible tendency to regression. June 11, six weeks after the beginning of treatment, what appeared to be a metastatic involvement of an inguinal lymphnode was noticed in the left groin. At this time the use of denser germ suspensions was begun and a slow but steady regression of all tumors except the metastatic growth set in. No treatment was given after June 22, and by July 15 only the groin tumor remained, and this had begun to decrease in size. The dose in this case was rapidly increased to 1 c.c. of the weaker, or 0.5 c.c. of the stronger suspension, at a single inoculation, but on no day did the temperature rise above 39.7 C. (103.6 F.), and rarely was there such active destruction of tumor tissue as occurred in the dogs cited above.

Experiment 5.—Dog 123 (Fig. 4) was inoculated with the mixed toxins, and is an example of a rapid and complete recovery of all tumors under treatment. It presented two abdominal growths and received during a period of two weeks, from February 11 to February 28, five inoculations with the mixed cultures of streptococcus and prodigiosus. Twice the injected tumor (Fig. 4, tumor 1) was tapped with a trocar and considerable necrotic tissue drained out. After the third injection the second tumor which was at no time inoculated, began to decrease in size, and by March 6 had entirely disappeared before the inflammatory reaction resulting from the inoculations into tumor 1 had entirely subsided. There has been no recurrence up to the present time. May 1 the animal was replanted with similar tumor tissue, and as no growth re-

made into one of the tumors (Fig. 6, tumor 1), but in spite of a slight localized necrosis, the growth inoculated as well as the others continued to grow steadily, and on August 3, five weeks after treatment was begun, showed no sign of any real regression.

Experiment 9.—Dog 260 (Fig. 7), the last animal treated, has received inoculations of *B. coli communis*. Six doses of bacterial suspension, 0.1 c.c. and later 0.2 c.c. at each dose have been injected into one of the four tumors (Fig. 7, tumor 4). The highest temperature reaction, and that on one occasion only, was to 41.1 C. (106 F.). Following the first inoculation, considerable necrotic tissue was removed by tapping, and from that time the regression of the inoculated tumor was slow but steady. Regression of the other tumors followed, and at the end of five weeks all had disappeared completely.

The accompanying charts (Figs 1 to 7, inclusive) give a graphic representation of the behavior of these tumors under treatment. It is noteworthy that the general health of the animals was at times much affected by the inoculations, and loss of body weight made it necessary to intermit the treatment until the physical condition was restored. If, on the other hand, the dosage was carefully regulated, the dogs frequently gained under treatment in spite of the presence of the softening and sloughing tumor.

The results of this preliminary study certainly demonstrate the destructive action exerted 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 dis-

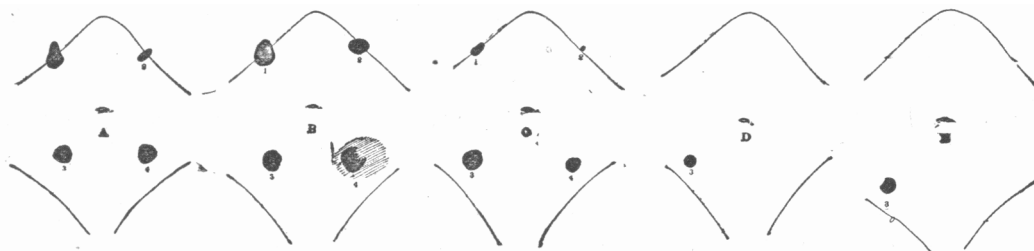


Fig. 7.—Dog. 260. (Experiment 9.) Treated with suspension of *B. coli communis*. Inoculation into tumor 4, May 14, 16, 17, 18, and 20. Inoculations were at no time made into any tumor but tumor 4. Measurements: A, May 10; B, May 16; C, May 27; D, June 1; E, June 15.

sulted, was once more planted six weeks later. There was no evidence of growth August 1, when the dog was bled to death in a transfusion experiment.

Experiment 6.—Dog 180 (Fig. 5) presents a similar history. The tumors at first yielded rapidly to the treatment with the mixed toxins, and by May 1, six weeks after inoculations were begun, there remained only a small part of tumor 1 (Fig. 5), into which the toxins had been injected. This remnant of indurated swelling resisted treatment for six weeks longer, but then gradually disappeared. A small regressing nodule (Fig. 5, D, tumor 4) was removed for histologic study, and was found to consist entirely of homogeneous necrotic tissue which failed to take the hematoxylin stain. The dog was in excellent condition throughout the treatment.

Experiment 7.—In dog 140 the tumors also yielded, though more slowly, to the treatment with the mixed toxins. After three months there remained of three actively growing tumors only a part of that one which had received the inoculations. For two months further injections seemed to produce no effect whatever. Doses were still given, however, at varying intervals, and finally a breaking up of the resistant fragment took place. Through sinuses which had persisted for some time, it was possible to press out fragments of tumor tissue as large as a small bean, and all that remained of the tumor was soon discharged in that way.

Experiment 8.—Dog. 244 (Fig. 6) was treated with suspensions of *Staphylococcus pyogenes aureus*. A rise of temperature to 40 C. (105 F.), or 40.6 C. (105 F.), followed nearly all of the nine inoculations given. The injections were

tance from the site of injection undergo regression simultaneously with those inoculated, while in one instance the entire treatment was by inoculations at a distance from the tumors.

On the other hand, when the soluble toxins of prodigiosus were used, and thereby systemic effect only obtained, the toxins in soluble condition being apparently too rapidly removed from the site of inoculation to bring about any local reaction, no effect whatever was produced on the tumors.

One can only theorize concerning the mechanism of the reaction, systemic and local, when it does occur. It is conceivable that the tumor cells have acquired their power of uncontrolled multiplication at the expense of other properties, including that of self-defense. They may, therefore, be more susceptible to the destructive action of these chemical poisons than are the normal body cells.

Furthermore, the absorption of such dead tumor cells may give rise to some sort of antibody in the body fluids, thus raising the resistance of the animal against tumor cells not yet destroyed by the toxins.

Regarding the action of the mixed toxins, Coley states: "The fact that all of my own successes, as well as those of other surgeons, have been obtained with the combined toxins, not a single permanently successful

case having been observed from the use of the erysipelas toxin alone, goes far toward establishing the importance of the *B. prodigiosus*." The experiments here outlined are certainly confirmatory of this opinion. It seems unquestionable that this bacillus, an organism which has been shown to possess highly toxic properties, exerts in itself a decidedly destructive influence on the cells of this particular tumor at least, and that its rôle in the effect produced by the mixed toxins is an active and independent one, and by no means merely that of enhancing the virulence of the streptococcus. It seems possible, however, that an equal bulk of any equally toxic organism would exert a similar destructive action.

We appreciate that the data presented at this time are insufficient to base any definite conclusions on, and we offer them only for what they are worth, believing, however, that, taken in connection with Coley's results in the human subject, they may be accepted as of some value.

THE EXPERIMENTAL PRODUCTION OF EPITHELIAL PROLIFERATION.*

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ST. LOUIS.

The artificial reproduction of conditions simulating those found in malignant growths has been attempted for a long time, but until very recently without any very definite results.

Since Virchow elaborated the irritation theory of origin of tumors many experiments have been made along that line. Brosch¹ painted the skin with a solution of paraffin and xylol and obtained a slight thickening and lengthening of the epithelial papillæ. Ribbert,² by means of repeated scratchings of the epithelium of the lower lip of a rabbit, produced a papillomatous outgrowth.

As is well known, there may be marked epithelial proliferation following in the course of chronic inflammation. Bernard Fischer³ undertook to bring about this condition without causing any lesion in the overlying epidermis. Many substances were injected without result until agar was used. This caused some change in the connective tissue with the formation of giant cells. Olive oil was then used, and in the course of a few weeks, after repeated injections, there was a considerable increase in the thickness of the epithelium and in the length of the papillæ. The proliferation was, however, not atypical, and in no way resembled a carcinomatous change.

Fischer then added Scharlach R. to the olive oil, and this solution was then injected into a rabbit's ear. As a result of this there followed a most interesting condition. By repeating the injections two or three times on alternate days he found that in the course of a week or more the superficial epithelium began to thicken and become roughened and scaly. By the end of three weeks the following microscopic picture was observed: The squamous epithelium showed a marked increase in thickness, with a cornification of the outer stratum. Projecting into the deeper tissues were long and compound finger-like papillæ. These extensions were quite irregular in shape,

and lying apparently free in the subcutaneous tissue were numerous cell nests. In many of these the central cells had undergone a very marked keratin degeneration with the formation of what appeared to be typical epithelial pearls.

Throughout the injected area were seen many clear spaces that in frozen section were found to be filled with the Scharlach oil. In some of these there were present cells around the periphery enclosing the foreign substance. There was also present a well-marked increase in the amount of connective tissue. The Scharlach oil penetrated throughout the lymph spaces, acted as a stimulant and gave rise to a condition simulating a non-infectious inflammation. Microscopically speaking, the picture so closely resembled that of a squamous epithelioma that it could not be differentiated from such.

From the results obtained, Fischer elaborated a theory in explanation of them. The proliferation could not be due to the mechanical irritation as such was ruled out by long-continued experiments in that line, all of which proved negative. For a period of months he painted the ear of a rabbit with the Scharlach oil without any results. In consequence of which he concluded that the proliferation of the epithelium following the injections is not the result of irritation, but is due to a chemotactic influence exerted by the Scharlach R. and olive oil. Similar results also followed the use of Sudan III and indophenol.

The oil penetrates the interstices of the tissue and the epithelium is then attracted to those places. It gradually surrounds the oil, forming a small cyst. As the oil is absorbed the epithelium still further proliferates until the foreign substance is entirely replaced. The epithelium then begins to degenerate and becomes converted into masses of keratin.

The opinion finally expressed by Fischer is that this injected material contains within it some substance that has the power of attracting epithelial cells. To this substance he gives the name "attraxin." To a certain extent it might be said to be related to Ehrlich's "Gewuchstoffe" or "Specific X-body," that according to his belief is necessary for the growth of malignant tumors.

As has been shown by Loeb in his experiments of parthenogenesis, it would seem that an alteration in the physico-chemical relations of the tissues could give rise to a proliferation of certain cells. Although Scharlach R. and the other substances used by Fischer are insoluble in water, yet they are to some extent soluble in the body juices, and therefore do not necessarily act entirely as insoluble bodies. It is possible that by their solution the power of the attraxin is exerted with the consequent proliferation of the epithelium.

That there may be specific attraxins would seem to be indicated by the fact that the injection of the Scharlach oil was unable to produce the epithelial changes anywhere else than in the skin, with possibly one exception. In some instances in both rabbits and dogs after injecting the above oil there were found small epithelial nests in the lungs. These were composed apparently of alveolar epithelium.

As a result of Fischer's article I undertook further experiments along the same line. Various animals were used, Belgian hares, guinea-pigs and white rats. The results obtained with the hares were the most satisfactory.

Nov. 7, 1906, I injected the external surface of the right ear of a large Belgian hare with about 4 minims

* Read in the Section on Pathology and Physiology of the American Medical Association, at the Fifty-eighth Annual Session, held at Atlantic City, June, 1907.

¹ From the laboratory of the St. Louis Skin and Cancer Hospital.

1. Virchow Archiv., 1900, clvii, 32.

2. Geschwulstlehre, 1904, 352.

3. Münch. med. Wochschr., 1906, lili, 2042.