

THE EFFECT OF TRYPSIN ON CANCER AND ON THE  
GERM CELLS IN MICE.\*

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In January, 1906, there appeared in the *British Medical Journal*<sup>1</sup> an article under the caption, "The Effect of Trypsin on the Living Cells of Jensen's Mouse Tumor." It was from the pen of Mr. Beard, Lecturer on Embryology at Edinburgh University. In this article is the statement that the action of trypsin upon the cancer cell "is to pull down the cancer albumen" — "to cause cancer to degenerate." In a previous article<sup>2</sup> Beard states that for the treatment of cancer, in trypsin, "the physician has had forged for him a light and not dangerous weapon, only second, if not equal, in potency to the surgeon's knife." These and similar statements have attracted considerable attention, and the question has arisen, "Will trypsin cure cancer?"

Numerous investigators have put the question to the test of experiment in the treatment of human patients. The reports as to the efficacy of this new therapeutic agent are conflicting, or at least are not in agreement, and in one case there was actual conflict of opinion as to whether the patient was cured or not.<sup>3, 4, 5</sup>

The question would seem to be easy of access by experiment on animals, and Beard himself applied this test. His experiments are not conclusive, however. He employed three mice with tumors. Two were treated with injections of trypsin, the third was kept as a control. In about ten days one of the animals under treatment died from some unknown cause. In about three weeks the control died, with a tumor as big as the "terminal phalanx of a man's thumb." The second mouse under treatment, the tumor of which was then the size of a lentil, was killed, and the tumor examined under the microscope. Of this Beard says: "The tumor was in advanced degeneration, shrinking away to

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nothingness, and quite harmless. It appeared probable to us that, at the time we killed it, its 'cure' from cancer was not far distant, and the microscopical examination confirmed this opinion. Even without further treatment, the tumor would in all probability have been absorbed shortly, or its remains cast out." This is the experimental evidence for the hope that trypsin will cure cancer.

At the suggestion, and at first under the direction of Dr. Wm. P. Graves of Boston, and with the cordial assistance of Dr. E. E. Tyzzer, a further experimental study was undertaken.

The scope of the experiments may be briefly outlined as follows:

To determine the effect of trypsin on the growth of tumors in various stages of advancement.

(1.) Very large tumors.

(2.) Moderately large tumors, but apparently definitely advanced beyond the stage where spontaneous regression might take place.

(3.) Early tumors, at a time when spontaneous regression might occur.

(4.) In a fourth experiment, the effect of relatively large amounts of trypsin in normal animals was noted.

(5.) Then the effect on conception and pregnancy of the injection of non-fatal doses of trypsin.

In addition, histological changes were studied in three groups of cases:

(6.) Tumors into which or near which trypsin had been injected.

(7.) Sexual glands of normal adult animals, into the general circulation of which trypsin had been introduced.

(8.) Sexual glands of the male guinea-pig into which trypsin had been directly injected. For the material of this last experiment I am indebted to Dr. Graves. In all the other experiments mice were used, for which I am indebted to Dr. Tyzzer. The tumor was an adenocarcinoma—the Ehrlich tumor. The trypsin employed was a stock preparation put up in sealed glass ampoules, containing about twenty

minims in each. By the manufacturers it was stated that it is not possible to make a quantitative determination of the amount of the trypsin; the determination of the strength is made by means of the digestive power of a given quantity of the solution. As, therefore, accuracy is not possible, the stock preparation was arbitrarily called one hundred per cent trypsin for the sake of convenience. This, however, contains about sixty per cent glycerine. The quantity used varies in different experiments and is there stated. In human patients doses larger than one ampoule, or twenty minims, have been used with safety. Regarding seventy kilograms as the average weight of a patient, and twenty minims as an average dose, it is evident at once what enormous amounts of trypsin were used; approximately one-fiftieth of the dose for a human patient was employed for a mouse. The injections were made at intervals of two to three days.

(1.) In the first experiment three mice were taken; the tumors were very large, evidently in a late stage; the general condition of the mice was good. Injections of fifty to one hundred per cent solutions, two to four minims, were made directly into the tumors, with slight decrease in size, which might easily be explained by the sloughing of the tumor. The mice died in from six to nineteen days after the beginning of the treatment with practically no appreciable local improvement. The tumors were measured before each injection.

(2.) In the second experiment, two groups of mice were taken. The first consisted of old mice with tumors well advanced—slowly growing—no sign of sloughing. In one mouse there was noticeable improvement: in about four weeks the tumor had disappeared. There remained a questionable trace in the form of a very small crust at a point where there had occurred slight sloughing. The treatment was stopped, but ten days later the tumor was again palpable, and caused the death of the animal, in spite of treatment, in about one month. The other mice in this experiment,

including the group of younger mice in which the tumors grew more rapidly, died after varying intervals, from the growth of the tumor, while under treatment. Two to ten minims of from two to ten per cent solution were injected.

(3.) In the third experiment ten young mice of a susceptible strain were inoculated with cancer tissue. Before the treatment with trypsin was begun, two of these died, of which one showed no sign of tumor; the other showed a small growth. Of the remaining mice, one showed no tumor at any time. Treatment was begun in seven on the fifteenth day after inoculation, at a time when the tumors might spontaneously disappear. They varied from the size of a split pea to one centimeter in diameter. The treatment consisted of three injections a week for nine weeks into the subcutaneous tissue, at a distance from the tumor. For two injections trypsin was used; for the third, amylopsin. (This use of amylopsin also is recommended by Beard.) In three mice the tumor disappeared in about twelve days. Treatment was stopped for a week, then resumed. There was no reappearance of the tumor. In four the tumor progressed much as in the mice not under treatment, ultimately causing death. This gives a percentage of cure or spontaneous recovery, according to the point of view, of forty-three, a little higher than the percentage usually given for spontaneous recovery. But since the different strains of mice vary and the number is so small, this slight difference of from ten to fifteen per cent may not be attributed to the effect of the trypsin. Four minims of ten per cent solution of trypsin or amylopsin were used at each injection.

(4.) In the fourth experiment, large amounts of trypsin were injected into three normal mice. All became toxic after a few days of treatment, and two died; the third recovered after stopping the treatment. The tissue from these animals was not studied under the microscope.

(5.) In the fifth experiment there was one mouse belonging to the group in Experiment III. in which a tumor disappeared. While under treatment this animal became pregnant and gave birth to normal young. At autopsy

there was no trace of the tumor, which had been present at an early stage. The case is interesting on account of the claims made by Beard as to the relation of cancer to germ cells, and the specificity of the action of trypsin on both kinds of cells. Functionally, at least, there was no interference with the action of the germ cells in the ovary.

(6.) The study of histological changes covers three experiments. From one to four injections were made into or near small tumors, which were removed later at operation or autopsy, and fixed in Zenker's fluid. If trypsin has a deleterious effect on the tumor cells, we should expect to find it extending directly from the point of injection, or by the blood stream. In the tumors examined, which showed the usual central necrosis of even early tumors, the cells around the blood vessels were in the best condition, and there could be made out no especial injury of cells lying on the periphery of the tumor nearest the point of injection.

(7.) In the seventh experiment six normal young adult mice were taken — three males and three females. They were treated with trypsin solution for three weeks, three injections a week. They were then killed and the tissues fixed in Zenker's fluid. Under the microscope the sexual glands cannot be distinguished from those of normal animals.

(8.) In the eighth experiment, trypsin was injected directly into the testicle of guinea-pigs, and the tissue removed at autopsy forty-eight hours later. As the trypsin is prepared with glycerine, about sixty per cent, glycerine alone was injected as a control. Microscopical examination showed focal necrosis of the injected area, the epithelial cells being more completely destroyed than the connective tissue. The destruction was slightly more marked with the trypsin than with the glycerine alone. There could be made out no difference in the character of the effect of the trypsin and glycerine, and the glycerine alone.

These uniformly negative results suggest the question as to the presence of any active enzyme in the solution employed. Test-tube experiments were conducted with the amylopsin as well as with trypsin, employing fibrin from horse's blood.

A rather active proteolytic enzyme was present in each, the two solutions being apparently of approximately the same digestive power for fibrin.

In these experiments the number of animals studied is small, but the results did not seem to justify further investigation along just this line.

#### CONCLUSIONS.

The conclusions may be stated briefly. In these experiments trypsin had no appreciable effect in causing the cancer to disappear or in inhibiting the growth and function of the germ cells.

#### REFERENCES.

1. Brit. Med. Jour., 1906, Vol. 1, 140.
2. Brit. Med. Jour., 1905, 281, Feb. 4.
3. Med. Rec., 1906, Dec. 8, 893, W. J. Morton.
4. Med. Rec., 1907, Jan. 12, 69, E. W. Peet.
5. N.Y. Med. Jour., 1907, Mar. 2, 385, W. S. Bainbridge.