While the problem arose in the physiological laboratory where other research on heavy water was being carried on, the identification and cultural isolation of the diatoms were the work of E. E. Cupp, of the Phytoplankton section here; she also made the population counts. G. F. McEwen, of the section of Physical and Dynamic Oceanography, performed the mathematical analyses and wrote the discussion of their significance.

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The Effect of Filtrates of *Cl. histolyticum* upon the Growth of Animal Tumors.

HAROLD P. RUSCH* AND WILLIAM S. PRESTON. (Introduced by Walter J. Meek.)

From the Departments of Physiology and Medical Bacteriology, University of Wisconsin.

Connell[1] has recently reported a small series of cancer patients who, he believed, were benefited by the use of proteolytic enzymes, which were obtained by the growth of *Cl. histolyticum* upon tumor tissue. Torrey and Kahn[2] had previously produced an enzyme by the growth of *Cl. histolyticum* on a 3 to 4% peptone meat infusion broth and following the injection of this material into the Flexner-Jobling rat carcinoma, reported a 50 to 75% cure. Parenteral injections were of no value. Des Ligneris,[3] in quoting some earlier work of his along similar lines, reports results of an entirely negative character. Gye[4] was unable to confirm Connell's results, using mice as test animals.

The experiments here reported were done for the purpose of testing the effect of certain bacterial filtrates prepared according to the method of Connell upon the rate of growth of 2 animal tumors, whose characteristics are well known and thoroughly established. According to Connell's statement, such solutions should contain proteolytic enzymes.

Preparation of the bacterial filtrates. Three types of tissues were used separately as sources of media for the production of the fil-

*Jonathan Bowman Cancer Research Fellow.

1 Connell, Hendry, *Canadian Med. J.*, 1935, **33**, 364.

2 Torrey and Kahn, *J. Cancer Research*, 1927, **11**, 334.

3 Des Ligneris, *Brit. Med. J.*, Dec. 28, 1935, p. 1280.

4 Gye, *Brit. Med. J.*, *Oct.* 19, 1935, p. 760.

trates: the viable portions of the Flexner-Jobling rat carcinoma, mouse sarcoma No. 180, and rat muscle, the latter serving as a control. These tissues were removed with aseptic precautions, and about 15 gm. of each tissue placed in separate Erlenmeyer flasks to which normal saline was added in the proportion of about 10 cc. to one gram of the tissue. The media were then inoculated with a pure culture of *Cl. histolyticum* and incubated at 37.5°C. under anaerobic conditions for from 4 to 6 days until the contained tissue had become disintegrated. The liquid contents of the culture were then filtered first through filter paper and then through Mandler candles, following which each was placed in a sterile container and kept in the refrigerator until used.

The 3 different solutions obtained by this procedure will be referred to as "rat tumor filtrate," "mouse tumor filtrate," and "rat muscle filtrate." These solutions were given by one of 3 routes: intramuscularly (thigh muscles), intraperitoneally, or directly into the tumor in 0.5 cc. doses daily in rats and 0.2 cc. amounts every other day in mice, continuing until the animals died. In preliminary work it was found that larger, more frequent doses in mice would result in a loss of weight and early death due to toxicity. The rat tumor filtrate was given to rats and the mouse tumor filtrate to mice, except for 6 animals of each species, where cross injections were performed to test for specificity of the solutions. The rat muscle filtrate injected into the thigh muscles of the rats also served to detect any proteolytic action on normal tissue. In the rats injected directly into the tumor, only animals were used which had previously been inoculated with tumor material in 2 sites. Injections were made into one tumor with the filtrate and with normal saline into the opposite one.

Injections were begun only after the inoculated tumor cells were apparently established and by the size of the growing mass it could be more readily determined which tumors would be likely to grow and not regress spontaneously. This was about a week after the inoculation in the mice and about 3 weeks in the rats. All tumors were measured at weekly intervals. An inbred strain of mice, "C-57" (black) of Little, susceptible to sarcoma No. 180, and a closely inbred strain of stock rats having about an 85% susceptibility to the Flexner-Jobling carcinoma were the animals used.

In vitro tests were done to test for proteolytic enzymes and for any specificity which they might have. Pieces of rat tumor, mouse tumor, and rat muscle were removed with aseptic precautions and placed in test tubes to which 5 cc. of one of the 3 filtrates was added. Five tubes were used for each test. These were then

TABLE I.

Animal used	No. animals in exp.	Source of bacterial filtrate	Site of injection	Amt. injected	Average length of life (days)
Rat	10	Flexner-Jobling rat carcinoma	Into tumor	0.5 cc. daily	28
"	19	" " " "	Intramuscularly	"	44.8
"	15	Normal saline solution	"	"	50
"	6	Flexner-Jobling rat carcinoma	Intraperitoneally	"	40
"	6	Normal saline solution	"	"	45
"	5	Rat muscle	Intramuscularly	"	38
"	6	Mouse sarcoma No. 180	"	"	41
Mouse	15	" " " "	Into tumor	0.2 cc. every other day	9
"	15	Normal saline solution	" "	"	7
"	19	Mouse sarcoma No. 180	Intramuscularly	"	11
"	18	Normal saline solution	"	"	10.1
"	18	Mouse sarcoma No. 180	Intraperitoneally	"	5.3
"	15	Normal saline solution	"	"	12
"	6	Flexner-Jobling rat carcinoma	Intramuscularly	"	9

placed in an incubator for one week and observed for tissue disintegration at the end of this time.

No inhibition in the rate of tumor growth was noted in any of the treated animals as compared with the controls except when the mouse tumor filtrate was given intraperitoneally to mice. Following this procedure, the tumor growth was halted, the mice lost weight, appeared toxic, and died in a few days. That the solution was toxic for normal animals was demonstrated by the fact that when somewhat larger doses, 0.4 cc., were given intraperitoneally to healthy mice, the animals died in from 2 to 6 days. No difference in the number and frequency of metastases were noted in the treated and control animals.

The variation in length of life noted in the different experiments, except in the group mentioned in the preceding paragraph, is not considered of any significance, because of the small number of animals used in each series.

The early breakdown and necrosis which occurs in a tumor following the injection of many types of solutions directly into it appeared to be much more severe in the tumors in which the solutions were given as compared with those in which normal saline was used. However, the tumors continued growing at parts not reached by the solution, and this finding could be determined only, in degree, by comparative observation.

Sections of thigh muscles which had been injected with the solutions used were removed at autopsy and examined for histological changes. While evidence of inflammation was present in almost all, and infection in several, no evidence of a proteolysis could be observed by this method.

In the *in vitro* tests for proteolysis, none of the tissues suffered a disintegration such as that noted in those in which *Cl. histolyticum* is growing. Only softening of the tissues in varying degrees was noted. This was probably due to autolysis, since it was not more pronounced than that seen in the saline controls. According to this method of testing, we were unable to demonstrate any enzymatic activity in the filtrate.

Conclusions. The solutions prepared according to the method of Connell and tested upon mouse sarcoma No. 180 and the Flexner-Jobling rat carcinoma were without any effect in prolonging the life of the animals, in preventing or decreasing the number of metastases or in inhibiting the rate of tumor growth.

NOTE: Since this paper was written, Pommerenke (*J. Am. Med. Assn.*, 1936, **106**, 1654) has reported results of a negative character in the use of similar bacterial filtrates tested upon the Brown-Pearce rabbit epithelioma.