

# Toxin Therapy of Experimental Cancer

## The Influence of Protozoan Infections upon Transplanted Cancer

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(Received for publication July 29, 1945)

Experiments were undertaken to determine the influence of various infections and toxins upon transplanted cancer. After a careful search *Schizotrypanum cruzi* was selected because of its ability to produce a chronic form of schizotrypanosomiasis, chiefly by reason of its organotropism. Our experiments consisted in infecting mice with trypanosomes and inoculating them with the Ehrlich carcinoma. A count of parasites in the peripheral blood and a record of the tumor growth were made every 4 to 5 days. At first a decrease in the development of the infection as well as in that of the tumor occurred in comparison with controls. This was followed by increase in the infection, which finally led to the death of the mouse. The tumor at the same time regressed or disappeared.

The tumors receded in 30 of 45 test animals under the influence of trypanosome infection, while in 15 the growths were inhibited. The grafts grew in all the 45 control mice. In most cases mice simultaneously inoculated with cancer and trypanosomes lived longer than those inoculated with cancer alone, and even longer than the animals inoculated with trypanosomes alone. This experiment, repeated several times with similar results, suggested that trypanosome infection exerts an antagonistic action on mouse cancer.

Another group of mice was infected with relapsing fever (*Sp. duttoni*). The purpose was to determine whether the effect of the *Schizotrypanum cruzi* infection was specific, or whether it was but another form of nonspecific fever therapy. Relapsing fever did not affect the development of grafted tumors.

The third experiment consisted in inoculating 45 guinea pigs with hypernephroma and then infecting them with *Trypanosoma equiperdum*. This form of trypanosomiasis flourishes in guinea pigs, and assumes a chronic form with low mortality. Here trypanosome infection did not reduce the percentage of tumors. The death rate of infected and control guinea pigs with grafted tumors was the same. A slight delay in the appearance and development of these growths in their early stages was noticeable in the infected animals, but retrogression or disappearance of a tumor has never occurred. Spirochetes of

relapsing fever and *Trypanosoma equiperdum* differ, therefore, from *Schizotrypanum cruzi*.

Under the influence of *Schizotrypanum* infection, the mouse tumor appeared to melt away. Sections revealed large areas that stained poorly and contained scattered groups of typical cancer cells. Sections from regressing tumors showed accumulations of parasites in the lumina of blood vessels. Many cancer cells contained in their cytoplasm 1 to 3 leishmania-like forms of *Schizotrypanum*, and degenerated or totally disintegrated nuclei.

Extracts of *Schizotrypanum cruzi* had no effect upon cancer cells.

It is possible that the parasite, in disturbing normal metabolism and exhausting the organism, deprives the tumor of substances necessary for its development. Cytologic observations on the tumors of infected mice suggest that trypanosomes reproduce in the cancer cell and eventually cause its degeneration. Tumor cells appear more vulnerable to parasites than normal cells, since in control sections from various normal organs of mice few or no parasites have been found. This suggests that specific toxins may be secreted by *Schizotrypanum cruzi* to which cancer cells may be particularly sensitive.

### THE ACTION OF *Schizotrypanum* ENDOTOXIN ON TUMORS

Endotoxins were prepared in the following manner: Heart's blood taken at the height of the disease in a mouse infected with *Schizotrypanum cruzi* was mixed with a 2 per cent sodium citrate solution and centrifuged. The trypanosomes remained in the supernatant fluid, which was stored overnight in an icebox. It was then placed in a bath at from 40° C. to 50° C. for 20 minutes, which killed the trypanosomes. The prepared trypanosome containing plasma thus was injected into mice on 8 consecutive days, 24 hours after tumor inoculation, in doses of 0.25 cc., 0.35 cc., and 0.50 cc. On storage the serum lost its potency.

These experiments were made in order to determine whether the endotoxin specifically affected cancer cells, without producing any effect upon other organs and

tissues of the mouse. Seventy cancer-bearing mice were divided into 3 groups. The first group, of 43, was treated with trypanosome plasma. Ten mice, comprising the second group, were injected with plasma from healthy mice, prepared in the same manner. Seventeen cancer-bearing mice were untreated. The size of the tumors was recorded as follows: (a) scarcely palpable; (b) diameter up to 0.5 cm.; (c) 0.5 to 1 cm. in diameter; (d) 1 to 1.5 cm. in diameter; (e) very large.

On the 30th day the tumors in animals treated with prepared plasma measured on the average 1.01 cm., whereas those of control mice had a mean size of 3.23 cm. In all control animals the tumors developed well, whereas 19 of 43 mice injected with trypanosome plasma showed no tumors and 24 showed a prolonged latent period and slow growth. The 10 mice injected with normal mouse plasma and the 17 untreated controls developed the usual tumors.

Microscopic sections were made from tumors treated with the *Schizotrypanum cruzi* endotoxin. On the third day of treatment there appeared slight lymphocytic infiltration around the growth and small, scattered areas of necrotic cancer cells. On the fifth day these changes were more pronounced; numerous cancer cells were in various stages of necrosis, but among them healthy cells were present. A regressing tumor on the seventh day of treatment showed massive necrosis with replacement by spindle-shaped fibroblasts, while the intercellular spaces were filled with connective tissue fibers and lymphocytes. Ten days after the beginning of treatment an occasional cancer cell was noted, surrounded by granulation tissue.

Further experiments consisted in studying the therapeutic action of *Schizotrypanum* endotoxin obtained from trypanosomes cultivated *in vitro*. The culture was prepared in the same way as the *Schizotrypanum* plasma.

On the second day after tumor inoculation test animals received 0.3 cc. of endotoxin injected subcutaneously for 8 successive days. The mean index of tumor growth for experimental mice was 1.73 and for controls 4.52; tumors from 1 series weighed an average of 0.5 gm. for 12 treated animals and 3.39 gm. for 12 controls. In 10 of 23 treated mice complete recovery occurred.

#### THE ACTION OF *Schizotrypanum* ENDOTOXIN *in Vitro*

A mouse tumor was reduced to pin-head fragments, and each one was placed in a drop of plasma containing *Schizotrypanum* endotoxin at room temperature (14° to 15° C.) for 6 hours and then implanted subcutaneously into 18 mice in the usual manner. Small fragments of the same tumor were immersed in normal

mouse plasma at the same temperature and for the same time, and then grafted into 20 mice. Of the mice inoculated with grafts exposed to endotoxin, only 3 showed scarcely perceptible tumors on the 30th day, whereas those grafted with tumor kept in normal plasma developed tumors on the eighth to tenth day. On the 15th day tumors had grown in 10 of the 20; on the 30th day 6 mice died of their tumors, while the survivors developed large growths. The experiment clearly demonstrated a direct inhibiting effect of endotoxin on the tumor cell. Small tumor fragments kept in normal plasma for 24 hours after preliminary exposure to endotoxin plasma did not grow.

The effect of the reticuloendothelial system was studied by a preliminary administration of endotoxin serum and subsequent tumor inoculation. The reticuloendothelial system was blocked by *ferrum saccharum* in 15 mice, and by trypan blue in 15 additional ones. Twenty-two mice were splenectomized and then inoculated with tumor. Injections of *Schizotrypanum* endotoxin inhibited the growth of mouse cancer by its direct effect upon cancer cells and by stimulating the reticuloendothelial system. When the latter was blocked the therapeutic effect was absent.

#### THE ACTION OF *Schizotrypanum* ENDOTOXIN ON HUMAN TUMORS

Treatment was carried out by Dr. Bonhard in 3 patients with incurable cancer of the pharynx, chosen because changes could be easily observed.

Endotoxin was prepared in the following way: 4 cc. of heart's blood was taken at the height of infection from a guinea pig inoculated with *Schizotrypanum cruzi*, 1.5 cc. of a 2 per cent citrate solution was added, and the mixture was slowly centrifuged for 30 minutes. The plasma was drawn off and placed in an icebox (1° to 2°) for 20 hours, then inactivated at 58° C. for 30 minutes, and 1 cc. of a 1:1,000 rivanol solution was added to 10 cc. of plasma after it had cooled. Finally, the plasma was tested for contamination and stored in 1 cc. ampules. It lost its potency in about 10 days.

Injections were made into the tumor with a special syringe fitted with a platinum needle; the first dose was 0.5 cc., the second 1 cc., and the final one 2.0 cc. The treatment was repeated every other day.

One case history is given below.

K., male, aged 42, complained of severe dysphagia. Bilateral cervical adenopathy present. The pharynx showed a large bleeding tumor and biopsy verified the diagnosis of a malignant growth. The Oncologic Institute did not consider the patient a good risk for x-ray therapy. After 2 months' treatment with 16 endotoxin injections the nodes diminished in size, pain ceased, the tumor shrank, bleeding had stopped, and

the arytenoid cartilages showed mobility. The patient, who had gained 2 kgm. in weight, was then sent again for x-ray treatment. Three months later the pharynx showed considerable improvement. The general condition was good and the patient remained under further observation for about 2 years. In this one case treatment with the plasma reduced pain, prepared the case for x-ray treatment, alleviated inflammation, and stopped hemorrhage.

Our tentative trials suggest this form of therapy on more extensive material.

#### THE ACTION OF BACTERIAL TOXINS

The effect of other bacterial toxins upon experimental cancer was then studied. Diphtheria and tetanus toxins, dysentery anatoxin, and *B. oedematiens* and *B. tumefaciens* toxins were tested.

The first two toxins gave positive results. The dysentery anatoxin and the *B. oedematiens* and *B. tumefaciens* toxins did not influence the development of tumors. In addition we carried out a series of experiments with preparations of nonpathogenic bacteria of the coli type; all failed to affect the tumor growth.

After a series of preliminary experiments we adopted the following procedure. Tumors were grafted in 1,000 animals and at the same time subcutaneous injections of toxin in various dilutions were given for several days. The size of the growth was recorded and at the end of the experiment all the tumors were excised, weighed, and their weights compared with those of tumors from control animals. Tetanus toxin

caused regression in 19 of 48 treated mice, while 6 showed definite inhibition of tumor growth. The average weight of receding tumors was one-fifth that of controls. The effect of the toxin treatment was enhanced by ultraviolet irradiation of the experimental mice.

Diphtheria toxin gave better results. The amount and concentration of the injected diphtheria toxin (M.L.D. 0.002) is important in treatment, and was established after a number of tests. In definite doses this toxin not only inhibited tumor growth but also caused recession in a considerable number of animals. This effect appeared to a lesser degree in 10 day old tumors; their average weight in experimental animals was 1.63 gm. and in control mice 3.63 gm. Complete recovery occurred in 39 of 65 mice, while in 19 animals tumor growth was inhibited. The mean tumor weight was one-tenth that of control, untreated ones. The combined use of 1:400 toxin and ultraviolet radiation gave better results.

#### SUMMARY

1. Cancer cells may be particularly sensitive to certain protozoan endotoxins and bacterial toxins, while normal cells of a given animal species are immune.
2. Some bacterial toxins and protozoan endotoxins in adequate dosages inhibit the development of certain experimental tumors and cause complete regression of others.
3. Toxin therapy may become one of the methods for treating malignant tumors.