of the dose of reserpine administered over the dose range employed (Fig. 1, *A*). Inhibition of the growth of the local tumor was observed consistently in the reserpine-treated mice. Two days after reserpine treatment, the change in the mean tumor diameter was practically nil, even though the tumors of the control (untreated) mice continued to increase in diameter (Fig. 1, *B*). Five days after the administration of reserpine, when all control animals were dead, the change in the mean tumor diameter was an inverse function of the dose of reserpine employed.

Treatment with reserpine frequently appeared to result in complete disappearance of the local tumor at the site of leukemic inoculation. However, transplantation of spleen from several such mice resulted in leukemic growth, indicating that systemic infiltration had not been wholly suppressed. Concomitant with the inhibition of local tumor growth, reserpine also appeared to retard the usual terminal leukocytosis in the peripheral blood.

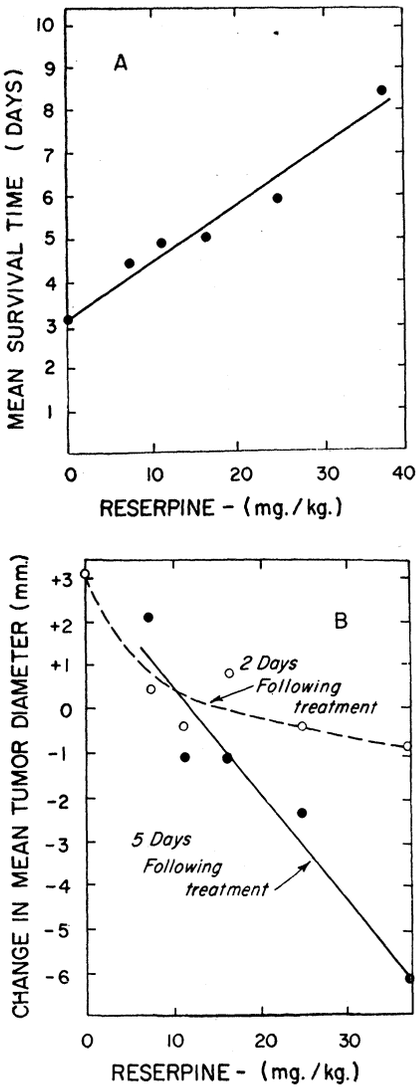

Fig. 1. Dose-response curves for the antileukemic action of reserpine. Reserpine was administered to the mice on the seventh day following inoculation with L1210. The mean survival time shown is the average time, in days, that mice survived after the day of treatment. Each mouse was inoculated with 3.2 million leukemic cells. Groups of ten mice, all with well-developed local tumors (mean tumor sizes ranged from 9.1 to 10.7 mm in diameter) were used for each dose level.

Preliminary experiments indicate that a regime of daily treatment with small doses of reserpine may be superior to treatment with a single large dose of the drug. Other experiments (*6*) have shown that several derivatives of reserpine also possess some antileukemic activity—for example, rescinnamine, deserpedine, and isoreserpine. The vehicle (*7*) used to dissolve the reserpine alkaloids was itself ineffective in inhibiting leukemic growth or in increasing survival time.

The mechanism by which reserpine exerts its antileukemic action is not known. Whether the antileukemic effect is direct or mediated through the host is not clear. Serotonin administration alone did not appreciably influence the course of the leukemia. Overcoming the depression caused by reserpine by administration of *d*-amphetamine did not significantly alter the antileukemic action of reserpine.

At the higher dose levels employed, reserpine-treated animals, both normal and those with leukemia, were severely depressed by the drug and failed to eat or drink for a period of 1 week or longer, and even nonleukemic mice frequently died (of starvation and dehydration?). Drastic loss of weight was invariably observed. Typical data frequently show an average loss of about 30 percent of body weight in 8 days following reserpine administration. Comparable food and water restriction in control animals with advanced leukemia failed to diminish the growth of the local tumors or to increase the survival-time of the mice as compared with untreated controls which received food and water *ad libitum*.

It is generally difficult to increase the survival time of mice with advanced leukemia L1210. Only a few drugs, such as amethopterin (*5*) and 6-mercaptopurine (*8*) have been successful in this respect. Even though reserpine has not been as effective an antileukemic agent as amethopterin, the response of mouse leukemia to treatment with reserpine and several of its derivatives has made available for laboratory study a new group of active antileukemic agents.

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3. We wish to express our thanks to Ciba Pharmaceutical Products, Inc., for generous gifts of the reserpine (Serpasil) used in these experiments.

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7. The vehicle was an aqueous solution containing 10 percent polyethylene glycol 300, 2 percent benzyl alcohol, and 0.25 percent citric acid (vehicle "C," Ciba). When appropriate, the reserpine solution in vehicle "C" was diluted with pyrogen-free water.
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26 November 1956

Infection of Chick Embryos by Crithidia from a Phytophagous Hemipteron

The possibility of a nonvertebrate parasite's infecting vertebrates offers a means of gaining some understanding of the evolutionary processes that are involved in the development of parasitism in vertebrate hosts and as such has attracted the attention of investigators for a number of years. Both negative (*1*) and positive results (*2*) have been reported, and considerable controversy has arisen concerning the correctness of the various results. The present paper (*3*) reports the successful transmission of flagellated protozoans of the genus *Crithidia* from the phytophagous bug *Euryophthalmus davisi* (Barber) to the chick embryo.

Crithidial parasites [possibly *C. euryophthalmi,* McCulloch (*4*)] were obtained from the insect, freed of bacteria by the use of antibiotics, and cultured on N.N.N. media. A luxuriant growth ensued. After a few passages on this medium, the flagellates were grown successfully on a medium consisting of nutrient agar (2 parts) and heparinized duck blood (1 part). On either medium, the crithidial morphology was lost and succeeded by that of a leptomonad.

After the parasites had been 5 days on the modified medium, 1 ml of sterile saline was added to the tube, and several drops of the mixture were placed on the exposed chorioallantoic membrane of 9-day chick embryos. Embryos so treated were placed in an incubator in which a temperature of 30°C ± 2° was maintained. This temperature still supported life in the embryo and more closely approximated the temperature in the hemipteran host.

Five days later, material was withdrawn from the inoculated embryos and examined under a phase microscope. Numerous flagellates were present in the

embryo, and they exhibited evidence of considerable multiplication. In some of the embryos there was a stimulation of the monocytic cell series. In certain phagocytic cells, actively moving protozoans were found (Fig. 1). The motion was so violent at times that the plasma membrane was evaginated by the vigorous punching. The nuclei of such cells were not immune to the motion but were frequently indented and temporarily distorted as a result of the violent action. In most instances, the parasites were only one-half the size of the free individuals and so far as could be told were either without flagella or with very short ones. These forms were similar to leishmania or the "intermediate" forms of Trager (*5*). Parasites were also found in the chorioallantoic fluid and the blood.

Material from the chorioallontoic membrane, the chorioallantoic fluid, and the liver was placed on the modified N.N.N. medium. Five days later, a luxuriant growth was observed in all tubes. No forms were observed in liver preparations that were examined under the phase microscope or in stained smears of the same organ, but that they were present was evidenced by the growth on the cultured material.

Transfers of infected chorioallantoic membranes were made to other chick embryos. A heavy growth took place, and again parasites were detected in phagocytic cells. After survival and multiplication of the parasites had been demonstrated, certain of these embryos were placed in incubators held at 37°C. Parasites continued to be present throughout the life of the embryo (10 days). The parasites are now in their fifth transfer from embryo to embryo at 30°C, and they show no signs of adverse effects due to their existence in the new environment.

Examination of untreated embryos has given no evidence of the forms as described in previous paragraphs. The results were negative when attempts were made to culture material from embryos that had been inoculated with saline or material from sterile culture media or pieces of chorioallantoic membranes from uninoculated embryos. Transfers of the flagellates that have been recovered on cultures from infected embryos have been made and have resulted in infection of the inoculated embryos.

The success of this experiment is, in my opinion, a result of three factors: (i) the extreme adaptability of the crithidia, (ii) the use of the chick embryo as the experimental host, and (iii) the lowering of the temperature of the experimental host. Thus, it may be concluded that, under certain optimal conditions and using techniques that had not been perfected at the time earlier experimenters worked, it is possible for crithidia from a phytophagous bug to infect a vertebrate host. It is hoped that future investigations of this phenomenon will shed some light on the little understood subject of evolution of parasites.

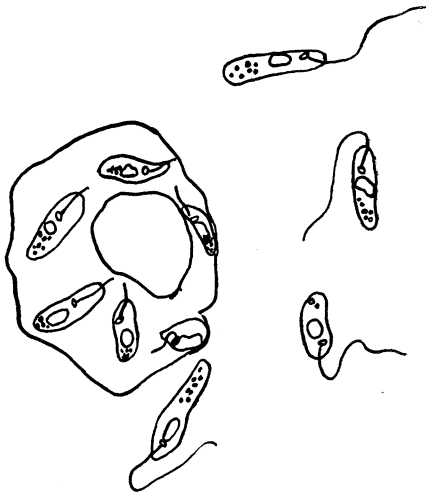

Fig. 1. Camera lucida drawing of a smear of chorioallantoic membrane showing flagellates both free and within a phagocytic cell. Dry film, fixed with methyl alcohol, stained with Giemsa's stain (× 1000).

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31 October 1956

Homotransplantation of Human Cell Lines

The development of human neoplastic cell lines that can be grown serially in tissue cultures (*1, 2*) and in heterologous hosts (*3*) has made necessary the investigation of the capacity of such cells to grow in a homologous (human) recipient. Such studies are of fundamental importance to our knowledge of tissue transplantation and host defense mechanisms. In addition, there is the possible danger of initiating neoplastic disease by accidental inoculation during laboratory investigation or by injection with such cells or cell products if they should be used for production of virus vaccine. This article is a preliminary report of a continuing study of (i) the persistence and growth of neoplastic human cell lines after homologous transplantation and (ii) host reactions to such implants.

All recipients were volunteers who were aware of the general purposes of the study and the nature of the implanted materials and who were agreeable to subsequent biopsies (*4*).

Tissue cultures were grown on glass in a mixture composed of 20 percent human serum, 5 percent beef embryo extract, and 75 percent bovine amniotic fluid. Within 4 hours of implantation the cultures were trypsinized, centrifuged lightly, and resuspended in Gey's saline to yield between 3 and 5 million cells per 0.5 ml. This dose was inoculated subcutaneously on the flexor surface of a forearm by tuberculin syringe and a No. 20 needle. When the inoculum was obtained from irradiated and cortisone-treated rats or chick-embryo chorioallantoic membrane (CAM), it consisted of four to six tumor fragments 1 to 2 mm in diameter, and it was implanted through a No. 13 trocar.

All implantations were marked by India-ink tattoos. Usually a single preparation was inoculated at one or two sites, but a few recipients received two to four cell types simultaneously, and one received a total of seven preparations on two occasions. Complete excisional biopsies (*5*) were usually performed as soon as a definitely palpable nodule appeared. In some recent studies, excision was delayed to study duration of growth and the process of regression.

Initial studies were restricted to volunteer patients with advanced incurable cancer and a very short life expectancy. Many had infectious and metabolic complications and chachexia. None had received treatment with steroid hormones, ACTH, marrow-depressing agents such as nitrogen mustard, or x-rays during the three months preceding the studies, and none received any of these treatments during the course of the studies.

Slight local induration and erythema frequently followed inoculations but subsided completely by the third day. Human embryonic fibroblasts with normal cytology were inoculated in three patients. No growth was detected, but neoplastic cells inoculated simultaneously into the same patients did grow. No other normal cells were available for study. Four epithelial cell lines of normal origin (*1*) were inoculated in seven patients, usually produced nodules, and one of these recurred in one patient. These cells cannot be considered normal because they had developed neoplastic characteristics during tissue culture passage, and the nodules were histologically diagnosed as cancer (*6*).