mixtures containing immune ferret or guinea pig sera remained symptom-free and survived in a healthy state for more than 35 days following inoculation.

From the two neutralization tests described above it is apparent that both the egg-adapted virus strain and the parent strain of ferret spleen virus are antigenically (immunologically) related.

Comment. During the course of the work described above, Haig[4,5] reported his studies in which he successfully maintained the *Onderstepoort* strain of virus for more than 90 serial passages on the C-A membranes of chick embryos over a period of 15 months. Through the courtesy of Dr. D. A. Haig and Dr. R. Alexander,[6] Director of the Division of Veterinary Services, The Veterinary Research Institute, Pretoria, Onderstepoort, Union of South Africa, the *Onderstepoort* strain of virus was sent to us in December 1948 and was readily established and maintained in our laboratory on the C-A membranes of chick embryos. Studies concerning the comparative properties of the *Onderstepoort* and *Lederle* strains of chick embryo adapted distemper virus will be published at a later date.

Summary. Studies are reported in which the *Lederle* stock strain of canine distemper virus was adapted and maintained through 74 serial passages on the chorio-allantoic membranes of developing chick embryos. The chick embryo adapted strain retained its capacity to produce lethal infection in ferrets through the 24th egg passage level but apparently lost its ability to do so between the 24th and 28th egg passages. Ferrets inoculated with chick embryo adapted virus of passage levels higher than the 28th transfer failed to show any signs of disease, but were found immune on rechallenge with fully virulent ferret spleen virus. Cross neutralization tests carried out in chick embryos and in ferrets indicate that the chick embryo adapted strain and the parent strain of ferret spleen virus are immunologically related.

The authors wish to express their appreciation to Mr. Frank E. Smith for his able technical assistance during the course of these studies.

[6] Alexander, R., personal communication to H. R. Cox.

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17151. Chymotrypsin in Cancer.

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A trial of chymotrypsin as a therapeutic agent in cancer was initiated at the Laboratory of Experimental Oncology in August 1948. The isolation of this enzyme in the crystalline state was described by Kunitz and Northrop.[1] The basis of interest in chymotrypsin in cancer was not the highly tenuous theoretical considerations that apparently led to its use,[2] but the purported clinical results in one case that has appeared in the literature[3] and additional similar cases that were rumored in this area and elsewhere.[4] These reports claimed prompt relief of pain after 3 or 4 parenteral injections of chymotrypsin, and subsequent decrease or disappearance of lesions stated to have been malignant neoplasms.

Ten patients (Table I) with inoperable neo-

* Aided by Grant 396 from the National Advisory Cancer Council.

[1] Kunitz, M., and Northrop, J. H., *J. Gen. Physiol.*, 1935, **18**, 433.

[2] Gurchot, C., Krebs, E. T., Jr., and Krebs, E. T., *Surg., Gynec. and Obst.*, 1947, **84**, 301.

[3] Krebs, E. T., Krebs, E. T., Jr., and Gurchot, C., *M. Rec.*, 1947, **160**, 479.

[4] Council on Pharmacy and Chemistry, *J.A.M.A.*, 1949, **139**, 93.

TABLE I.
Dose and Time Schedule of Chymotrypsin Therapy.

Case No.	Sex, age	Diagnosis*	Chymotrypsin Dose, mg	Inj. cumulative, No.	Days treated, No.	Total dose, mg
1	M-54	Adenoca. stomach (resected); s.c.met.	60 20	7 18	26	640
2	M-27	Anaplastic ca. lung with met.	20	43	43	860
3	M-31	Adenoca. stomach l.n., s.c. met.	20	60	60	1200
4	M-48	Epidermoid ca. lung; l.n. met.	20 60	27 48	48	1800
5	M-72	Epidermoid ca. alveolus; l.n. met.	20 60	49 60	60	1640
6	M-54	Epidermoid ca. esophagus (resected) l.n. met.	20 60	43 60	61	1880
7	F-65	Adenoca. breast, l.n., s.c. met.	60 20	12 39	44	1260
8	M-60	Adenoca. rectum, liver, l.n. met.	40 20	6 40	40	1040
9	M-38	Melanoma, l.n. met.	20 60	12 53	57	2700
10	M-45	Melanoma, s.c. met.	40	35	38	1400

* Abbreviations: ca.—Carcinoma. met.—Metastases. l.n.—Lymph node. s.c.—Subcutaneous.

plastic disease received repeated administrations of 20 to 60 mg of crystalline chymotrypsin for 26 to 61 days.† The crystalline material was dissolved in approximately 2 cc of sterile isotonic saline and injected intramuscularly. The first patient initially received 7 doses of 60 mg every other day, but the remaining patients received daily injections with an occasional interruption of one to 3 days.

The patients selected for trial were those with easily palpable or visible lesions or metastases which could be observed and measured objectively for evaluation. Two patients (Cases 1 and 5) had lost considerable weight, but the rest were in fair or good state of nutrition. Two patients (Cases 5 and 7) had received roentgen therapy to the lesions some months previously, and the remaind had had no therapy other than surgery f the primary lesion. Appropriate biopsies we performed before chymotrypsin treatment all cases, and in 4 patients additional biop material was obtained during the course injections.

In none was there the slightest objective e dence of effect, upon either the neoplastic pr cess or the clinical condition of the patie No alleviation or decrease of pain, or a subjective improvement were noted. All patients have died after the usual progress their disease. Necropsies were obtained 7. No histologic evidence of effect was se in the biopsy or necropsy sections of t tumors.

All patients receiving chymotrypsin co plained of pain and swelling at the site injection. Sudden severe reactions, consisti

† Supplied by Kuster Laboratories, Inc., 571 Seventh Street, San Francisco 3, Calif.

of marked diaphoresis, weakness, loss of consciousness, rapid thready pulse, faint heart sounds, and peripheral vascular collapse were encountered in 3 patients, (Cases 8, 9 and 10) within 5 minutes following the 40th, 53rd and 29th doses, respectively, of the enzyme. Prompt use of artificial respiration, epinephrine intramuscularly, oxygen inhalations and intravenous saline probably prevented fatal termination of these anaphylactoid reactions in patients apparently sensitized by previous injections of the protein enzyme. Another patient (Case 6) had a similar but less severe reaction with spontaneous recovery after the 22nd dose.

Further trial of chymotrypsin was discontinued, not only because it had no effect whatsoever in 10 patients with advanced malignant neoplasms, but because repeated administrations of the protein enzyme are of definite serious hazard.

Summary. Parenteral injections of chymotrypsin had no effect on the course of 10 cases of neoplastic disease in man.

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17152. Ineffectiveness of Chymotrypsin Therapy in Malignancy.*

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An elaborate theory has been proposed by Krebs and co-workers[1,2] which assumes that the cancer cell and the trophoblast cell are fundamentally alike, and that both are destroyed selectively by the pancreatic enzyme chymotrypsin. On the basis of this reasoning, but with few supporting facts, chymotrypsin has been suggested as the palliative of choice in neoplastic disease. This report summarizes the negative results of chymotrypsin therapy in experimental and clinical cancer.

Experimental cancer. The toxic dose of crystalline chymotrypsin† in mice subjected to repeated daily intraperitoneal injection was established at 40 to 80 mg per kilo of body weight. The dose used in treatment was 2.0 mg per kilo, which is 5 times the recommended dose for humans. Forty mice with subcutaneously transplanted sarcoma 180 and 40 without tumor were employed in the experiment. Half of each group received chymotrypsin intraperitoneally 7 days a week for 14 days, the remainder serving as controls. Weight gains were uniform in all 4 groups of animals. Treatment was begun when tumors were measurable, on the fifth day after transplantation, and tumor size was recorded on alternate days thereafter. The average increase in tumor area at the conclusion of the experiment was 3.8 times in the treated animals and 3.7 times in the controls. No change in chymotrypsin inhibitor concentration of the blood as determined by the method of West and Hilliard[3] resulted from the treatment in either normal or tumor-bearing animals.

Clinical Cancer. All patients selected for chymotrypsin treatment had received the maximum benefit from accepted forms of therapy but still had active disease. Their clinical course was followed by an impartial group of observers, Drs. G. M. Leiby, J. A. Weinberg, F. Isaac, and F. W. Wilkins of this hospital. Weekly laboratory determinations included,

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1 Gurchot, C., Krebs, E. T., Jr., and Krebs, E. T., *Surg., Gynec. and Obst.*, 1947, **84**, 301.

2 Krebs, E. T., Krebs, E. T., Jr., and Gurchot, C., *M. Rec.*, 1947, **160**, 479.

† Crystalline Chymotrypsin was supplied by Spicer-Gerhart Co., Pasadena, Calif., and Worthington Biochemical Laboratory, Freehold, N. J.

3 West, P. M., and Hilliard, Jessamine, in press.