blood count, sedimentation rate, bleeding and coagulation time, serum protein, alkaline phosphatase, cephalin flocculation, thymol turbidity, NPN, and serum chymotrypsin and rennin inhibitors.[3] When feasible, patients were followed roentgenographically as well.

The diagnosis in each instance had been proved by biopsy. There were 15 cases in the series: bronchogenic carcinoma, 3; carcinoma of stomach, 2; embryonal carcinoma of testicle, 2; and one each of recto-sigmoid and uterine carcinoma, synovioma, lymphosarcoma, Hodgkin's disease, acute and subacute lymphatic leukemias, and chronic myelogenous leukemia.

Crystalline chymotrypsin in saline was given in daily intramuscular doses ranging from 4 to 60 mg for periods of 21 to 135 days, with an average of 64 days. The usual dose was the recommended one of 30 mg. Three patients could not tolerate more than 4 to 6 mg without intense pain in the most involved organ (liver, stomach and spleen) accompanied by chills and fever 12 to 24 hours after injection. Six cases developed pronounced allergy to the drug, forcing reduction in dose or discontinuation of treatment. Two had generalized urticarial reactions, 2 exhibited anaphylactic shock, and 2 had extreme local erythema and edema at the site of injection. Two patients died in congestive failure about a week after onset of precordial pain, ankle edema and dyspnea. Electrocardiograms were normal before death and autopsy failed to reveal significant cardiac pathology. Whether or not these deaths were related to treatment is debatable.

The sedimentation rate was doubled during chymotrypsin therapy. There were no other consistent laboratory findings. The serum level of chymotrypsin inhibitor was not altered detectably, which is understandable in view of the fact that there is sufficient inhibitor in the circulation alone in many such patients to inactivate 25 to 50 g of pure chymotrypsin.

In no case was the clinical course of the patient influenced by chymotrypsin therapy. Nine patients have died and a study of autopsy material has not revealed any unusual degenerative changes in the neoplasm.

Summary. Treatment with crystalline chymotrypsin did not influence the course of neoplastic disease in experimental animals or in fifteen selected cancer patients.

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17153. Action of Chloromycetin on Salmonella.

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As yet there exists no effective remedy to prevent or cure Salmonella infections in man and animals. Any new antibiotic with selective activity against gram negative organisms, therefore, is worth a trial against Salmonella infections. Chloromycetin, the antibiotic produced by Streptomyces Venezuelae[1] possesses these qualities in addition to its marked effect against Rickettsia. Clinical experiments indicate some success in the treatment of human typhoid fever (a Salmonellosis.)[2]

In vitro tests with a number of gram negative bacilli were performed by Smith *et al.*,[3] and revealed remarkable sensitivity, usually in a range below 1 μg of the drug. These results were obtained by a special turbidity assay method developed by Joslyn and Gal-

[1] Ehrlich, J., Bartz, Q. R., Smith, R. M., Joslyn, D. A., and Burkholder, P. R., *Science*, 1947, **106**, 417.

[2] Woodward, T. E., Smadel, J. E., Ley, H. L., Jr., Green, R., and Mankikar, D. S., *Ann. Int. Med.*, 1948, **29**, 131.

[3] Smith, R. M., Joslyn, D. A., Gruhzit, O. M., McLean, I. W., Jr., Penner, M. A., and Ehrlich, J., *J. Bact.*, 1948, **55**, 425.

braith.[4] A strain of *Sh. sonnei* No. 04628 was used as the test strain, and inhibited by .2 μg in this set-up. In our experiments* the same test strain was employed in a simplified serial dilution method. Sensitivity was measured by the smallest amount of chloromycetin that completely suppressed growth after 24 hours. With this method the test strain was regularly sensitive to 1 μg.

Sixty-eight gram negative strains were examined; they included *B. coli* and other coliform organisms, *aerobacter aerogenes, B. proteus, Pseudomonas aeruginosa, Shigella* types, 2 strains of *S. typhosa* and 21 other Salmonella types. Most strains of *Pseudomonas aeruginosa* and some coliform organisms were remarkably resistant to chloromycetin and required concentrations from 20-100 μg per ml for inhibition. Of the 23 Salmonella types, 19, including *S. typhosa,* were sensitive to 2 μg, four to 4 μg, none higher. No relation existed between streptomycin resistance and chloromycetin resistance; no absolute resistance to chloromycetin was encountered in any strain.

For animal experiments (oral and parenteral treatment) solutions of chloromycetin in 20% propylene glycol were used; emulsions in acacia, as recommended, were not practicable because of the small amount of the material to be ingested.

Crystallized chloromycetin is not readily soluble in water; suitable solutions however, can be produced by use of 20% propylene glycol. Final concentrations of 10 mg per ml were obtained by heating for 10-20 minutes in a water bath at 56°. At room temperature needle-shaped crystals precipitated. They disappeared on repeated immersion into the water bath at 56°. The antibiotic activity of these solutions was not impaired by repeated exposure to 56°. Mice, 20 g of weight were fed with chloromycetin solution by introduction through a smoothly blunt 18 gauge needle attached to a tuberculin-syringe. For 4 consecutive days 2000 μg (contained in .2 ml) were given twice daily, at 9 A.M. and 4 P.M. The total dose amounted to 16 mg or 800 mg per kg mouse. No toxic effects were observed.

The intestinal flora was checked by stool cultures taken before the start of the experiment and after the 5th and 7th feeding. No growth difference was found; number and size of colonies (mostly *B. coli, B. proteus,* enterococci), remained almost unchanged during the course of the tests. In this respect the action of chloromycetin is different from that of streptomycin. Feeding of the latter drug results in a marked decrease of fecal organisms, even bringing about complete inhibition of growth.[5,6] Streptomycin remains practically unabsorbed in the intestinal tract, and is not destroyed in the intestines. Since chloromycetin is readily absorbed by the oral route,[3] no active material remains in the intestines for any length of time. The fecal flora, therefore, is not affected.

Salmonella infections. Virulent organisms used for infection of mice by the oral or parenteral route, as a rule, give rise to generalized septicemia with fatal outcome. The bacilli transgress the intestinal wall from both sides, from the intestinal into the general circulation as well as from blood and lymph system into the intestinal lumen. The failure, therefore, of chloromycetin given by mouth to influence the bacteria inside the intestinal tract does not preclude its possible activity against those Salmonella organisms that have entered the body system from there.

In the first experiment 40 mice of about 20 g body weight received orally .1 cc of an 18 hour broth culture of *S. typhi murium* 1908 known for its constant virulence. Twenty animals served as controls, 10 mice underwent oral treatment: 2000 μg twice a day for 4 days, once a day for 4 more days; total ingested chloromycetin was 24 mg or 1200 mg per kg. A second batch of 10 mice was injected subcutaneously with 2500 μg twice a day for 6 days and once a day for 2 more

[4] Joslyn, D. A., and Galbraith, M., *J. Bact.,* 1947, **54**, 26.

* Crystallized chloromycetin was supplied through the courtesy of Parke Davis and Co.

[5] Seligmann, E., and Wassermann, M., *J. Bact.,* 1946, **53**, 127.

[6] Seligmann, E., Barash, L., and Cohlan, S. Q., *J. Ped.,* 1947, **20**, 182.

TABLE I.
Oral Infection of Mice with 0.1 cc of Broth Culture of *S. typhi murium* 1908 and Treatment with Chloromycetin.

Treatment	No. of mice	Surviving after, days										
		1	5	6	7	8	9	10	11	12	14	21
None (controls)	20	20	16	12	9	6	3	1	0			
Oral	20	10	10	10	10	8*	7	7	5	5	2	1
Parenteral	10	10	10	10	10	9*	9	9	8	7	6	1

* Last day of treatment.

TABLE II.
Parenteral Infection of Mice with 0.1 cc of Broth Culture of *S. typhi murium* 1908 (1:60) and Treatment with Chloromycetin.

Treatment	No. of mice	Surviving after, days										
		1	5	6	7	8	9	10	11	12	14	21
None (controls)	20	20	7	5	4	0						
Oral	10	10	10	10	10	8*	7	7	5	5	2	1
Parenteral	20	20	20	18	15*	11	10	9	6	5	3	3

* Last day of treatment.

days. Total injected chloromycetin was 35 mg or 1750 mg per kg. Table I gives the results.

Treatment began immediately after infection and terminated on the eighth day. On the 11th day all controls were dead, while 5 and 8, respectively, of the treated animals were still alive. At the end of the experiment, however, (21st day) only one of each series of treated animals survived. Thus, oral infection with *S. typhi murium* could not be checked by oral or subcutaneous treatment with large doses of chloromycetin. In Table II similar experiments were performed after parenteral infection of the mice (.1 cc of an 18 hour broth culture of *S. typhi murium* 1908, diluted 1:60). The parenteral treatment with chloromycetin was performed for 7 days with a total of 25 mg or 1250 mg per kg; the oral treatment (8 days) with a total of 24 mg or 1200 mg per kg.

The results are almost identical with those of the first test. When all untreated animals had succumbed (7th day), a considerable number of treated mice were still alive. On withdrawal of the drug, however, and in the course of the next 2 weeks, most of the treated animals died. Only a few survived at the end of the experiment (21st day). *S. typhi murium* was recovered from the controls and the treated dead mice, often in pure culture. It was found in heart blood and intestines in both series. The chloromycetin sensitivity of the recovered strains had not changed. The original culture, as well as the strains isolated from the animals, still showed sensitivity to 2 μg.

Summary. Chloromycetin inhibits the growth of Salmonella organisms *in vitro* remarkably, but is not able even in large amounts to control Salmonella infections in mice. Neither the oral nor the subcutaneous form of treatment prevented the fatal outcome of the disease in most animals, although it prolonged the survival time. Its action in the animals was bacteriostatic and not bactericidal. Due to its absorption conditions, chloromycetin exerts no influence on the intestinal flora, when given orally.

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