Cortisone and Roentgen Radiation in Combination as Synergistic Agents for Production of Lethal Infections.* (19543)

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This paper reports a highly efficacious method for the production of rapidly progressive lethal infections. Cortisone and x-ray when employed in combination for the preparation of test animals alter their resistance to enhance remarkably the pathogenicity of a variety of infectious microbiological agents. To date studies in this laboratory have applied successfully this method for the enhancement of the pathogenic effects of poliomyelitis virus, type 2, Lansing strain(1); Coxsackie virus, subgroup A, type 4, Minnesota strain(2); *Blastomyces dermatitidis,* yeast phase(3); *Candida albicans*(4); *Streptococcus pyogenes hemolyticus,* group A, type 28(5); and *Mycobacterium tuberculosis,* var. hominis(5). We record this supplementary note at the present time to permit without delay the application of the method to laboratory studies of exotic agents and infectious agents of limited host receptivity. The ready applicability of the method is illustrated in this report by the results that were obtained in 4 experiments.

Cortisone and x-ray, when employed singly, have been reported to exert an enhancive effect upon the severity of infection when it is induced by any of a variety of infectious agents. Measurable alterative effects of cortisone to the disadvantage of the host are recorded for a wide range of viruses, bacteria, and a single fungus. The viruses include influenza A(6), influenza B(6), mumps(6), poliomyelitis, type 2, strain MEF-1(7), pneumonia virus of mice(8); Coxsackie virus, subgroup B(9); vaccinia(10), West Nile(11), Ilheus(11) and Bunyamwera(11). The bacteria studied were *Staphylococcus aureus*(10), group A streptococci(12-14), *Mycobacterium tuberculosis* (15) *Diplococcus pneumoniae*(16), *Brucella spp.*(17) and *Treponema pallidum*(18). The fungus was *Trichophyton mentagrophytes.* The variable effects of x-irradiation on infection and immunity were ably reviewed recently by Taliaferro and Taliaferro(19).

Materials and methods. Infectious agents. The microorganisms used were: 1) poliomyelitis virus, type 2, Lansing strain, derived from a sample originally given us by Dr. J. E. Salk; 2) Coxsackie virus, subgroup A, type 4, Minnesota 1 strain which was isolated in this laboratory; 3) *Candida albicans,* the Asbury strain which was kindly supplied by Dr. J. R. McGrath; 4) *Blastomyces dermatitidis* isolated from a patient with blastomycosis. These infectious agents were prepared for injection as follows: 1) The test suspension of Lansing virus employed for intracerebral inoculation, 0.03 ml, was the supernatant fluid prepared by centrifugation at 2000 r.p.m. for 10 minutes of a 10% mouse brain suspension in 0.85% NaCl. 2) The inoculum of the Coxsackie virus, 5 million LD_{50} per 0.1 ml, was the Seitz-EK filtrate of the supernatant fluid derived by centrifugation at 4000 r.p.m. for 20 minutes from a 10% tissue suspension. 3) *Candida albicans* was injected intraperitoneally, 0.5 ml, as cells which had been grown for 48 hours at 30°C on Sabouraud's dextrose agar and made ready for injection by diluting them, 1:250, in 0.85% NaCl. 4) *Blastomyces dermatitidis* was grown on Francis' blood dextrose cystine agar at 37°C for 4-5 days, centrifuged horizontally at 1500 r.p.m. for 5 minutes and resuspended in saline, 1:200 for injection, 1.0 ml, intraperitoneally. *Mice.* Swiss albino mice, CFW strain, were employed in groups of 5 to 25 for test and for titration. The age of the mouse was determined by the

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infectious agent under study: Lansing virus, 21 days; Coxsackie virus, either 1-2 days or 5-6 weeks; *Candida albicans* and *Blastomyces dermatitidis*, 8-12 weeks. *Method of radiation.* X-radiation was administered as a single massive dose to the whole body at a target-skin distance of 60 cm. The Roentgen rays were generated by a current of 15 milliamperes having a 250 kilovolt peak. They were filtered through 0.5 mm of copper and 1.0 mm of aluminum (HVL 1.35 mm Cu). The size of the field was 14.0 cm in diameter. The output, as determined by a Victoreen r-meter, was 41.5 r per minute in air. *Cortisone acetate.* Cortisone acetate, 25 mg per ml, (Cortone, Merck) was employed directly, or by diluting it in saline to provide a concentration of 1.0 mg per 0.1 ml, for injection subcutaneously. *Experimental plan.* The experimental plan was to test the enhancive effect in single doses of cortisone, of x-radiation, or of these two agents in combination upon the pathogenicity of selected microorganisms upon injection within the next 24 hours. Each agent, and combination of agents, was titrated in groups of from 5 to 30 mice for determination of the LD_{50}, as calculated by the method of Reed and Muench (20). The animals were kept under observation daily for 30 days. For control purposes cortisone was tested in mice 5-6 weeks of age in a dosage range of from 0.1 mg to 9 mg; x-radiation in a range of 50 r to 800 r. When evidence for toxicity was obtained for cortisone in a dosage greater than 6 mg, for x-radiation in excess of 450 r, and for cortisone and x-radiation in combination in excess of 3 mg and 400 r, respectively, the test doses of these agents were limited to from 2 to 4 mg for cortisone and kept within a range of 200 to 400 r for x-radiation, whether employed singly or in combination. For mice 8 to 12 weeks of age as employed routinely for fungi, the uninfected control mice in groups of 16 over a period of thirty days showed no ill effects from cortisone, 4 mg, or from x-radiation, 400 r, singly or in combination.

Results. Lansing virus. The results of Exp. 1, as presented in Fig. 1, are representative of the findings that were obtained when the Lansing strain of poliomyelitis virus was employed. The LD_{50} titer (Fig. 1a) that resulted in animals which had been prepared by the administration of cortisone, 2.0 mg, and x-radiation, 200 r, was $10^{5.5}$ as contrasted with an LD of $10^{3.9}$ for the control animals that received virus only. Evidence for enhancement of infection also was obtained for Roentgen radiation to yield an LD_{50} of $10^{4.5}$ and for cortisone to result in an LD_{50} of $10^{5.2}$. A more graphic depiction of these findings in Fig. 1b makes it apparent that 7586 LD_{50} for normal mice is enhanced in pathogenic effect approximately a) 4-fold (31,630 LD_{50}) when tested in mice prepared by x-radiation, b) 20-fold (147,900 LD_{50}) by cortisone and c) 40-fold (316,200 LD_{50}) by the two agents in combination.

Coxsackie virus. Since Coxsackie virus (A4, Minn. 1) is highly infectious upon transfer to newborn mice, evidence for enhancement by the agents under study was sought by employing for test an insusceptible host, the adult mouse. A series of experiments involving 250 mice was set up to measure the enhancive effect of cortisone and x-radiation in combination by assay of the LD_{50} for each of the agents singly and by block titration. Fig. 2 depicts the results of LD_{50} titrations based upon the employment of a constant amount of x-radiation and incremental amounts of cortisone within a dosage range of 0.1-4.0 mg a) at 10 days after infection and b) at 30 days after infection. Conversely, Fig. 3 makes known the results at 10 and 30 days of LD_{50} titrations when the dose of cortisone was kept constant and x-radiation was employed in a dosage range of from 50 r to 400 r. For evaluation of these results and for control purposes, it was established that cortisone or x-radiation singly, or cortisone and virus in combination, was without apparent effect upon the recipient mice when observed at either the 10- or 30-day period. Moreover, it was learned for the higher dosage ranges of either agent and virus that 3.7 mg, or more, of cortisone were required in combination with virus to establish an LD_{50} titer and that 321 r, or more, of x-radiation in combination with virus were required to establish an LD_{50} titer. Fig. 2 and Fig. 3 present findings summarily which show that

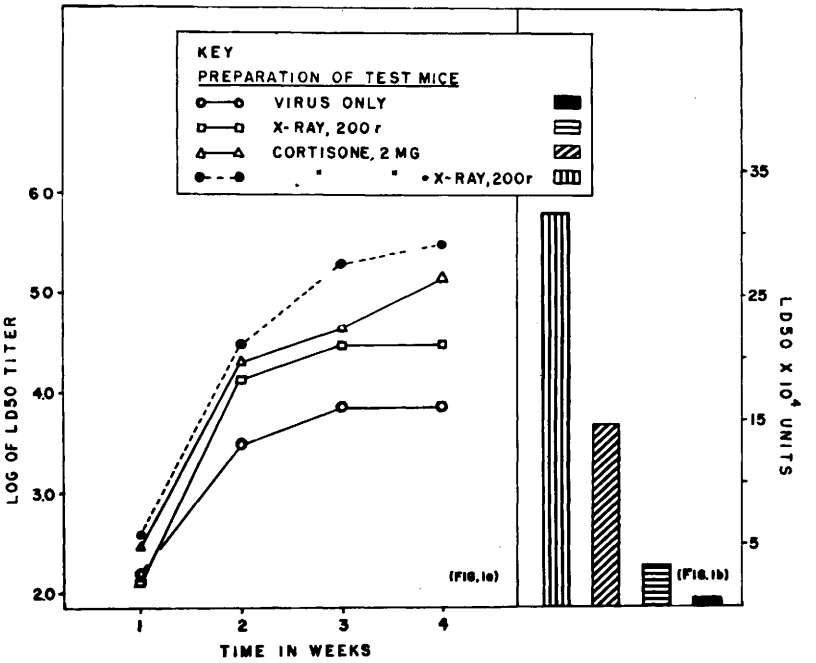

FIG. 1. The enhancive effect of cortisone and x-radiation, singly and in combination, upon the LD_{50} titer of poliomyelitis virus, type 2, Lansing strain.

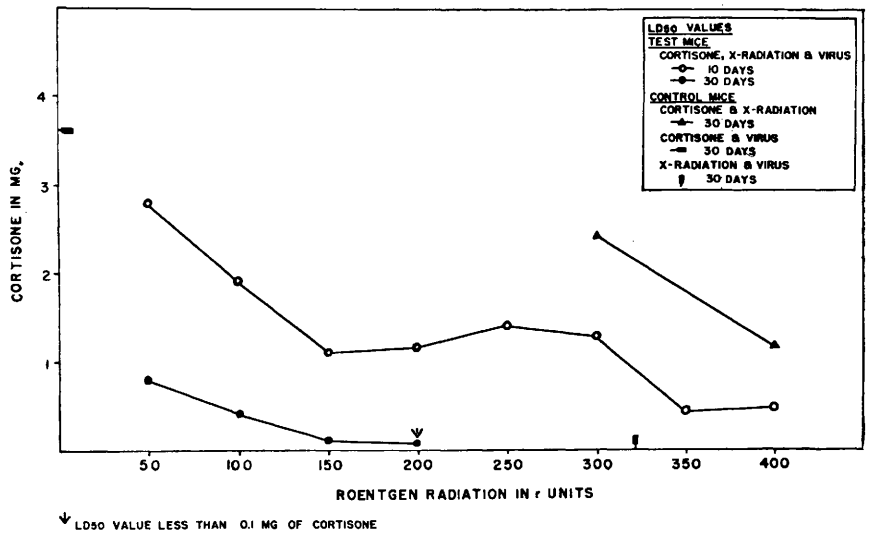

FIG. 2. The synergistic effect of cortisone and roentgen radiation on the pathogenicity of Coxsackie virus in resistant adult CFW mice as indicated by the LD_{50} value for a fixed amount of x-radiation in combination with graded doses of cortisone.

cortisone and x-radiation when employed in combination for preparative treatment of an insusceptible host, the adult mouse, operated synergistically over a wide range for each agent to render Coxsackie virus lethal. The synergistic enhancive effects were established as the LD_{50} titer over this wide range of dosages for each agent and over a limited range as the LD_{100} titer.

Candida albicans. Fig. 4 illustrates the synergistic enhancive effect of cortisone and x-radiation upon infection of mice by a yeast,

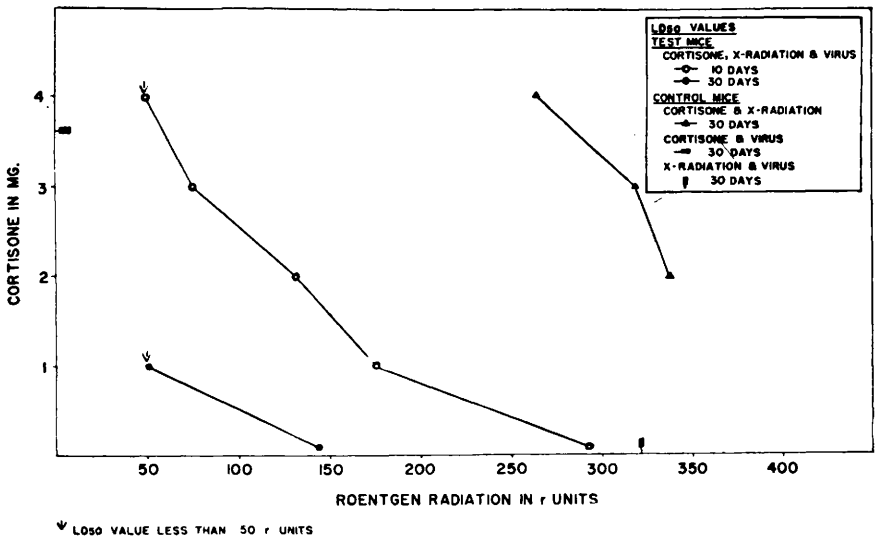

FIG. 3. The synergistic effect of cortisone and roentgen radiation on the pathogenicity of Coxsackie virus in resistant adult CFW mice as indicated by the LD_{50} value for a fixed dose of cortisone in combination with graded amounts of x-radiation.

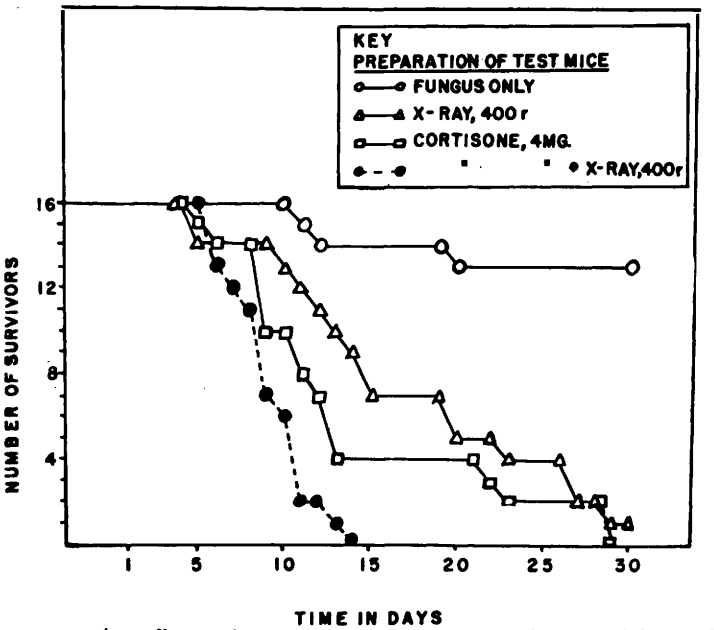

FIG. 4. The comparative effects of cortisone and x-radiation, singly and in combination, as agents for enhancement of the pathogenicity of *Candida albicans* for mice.

Candida albicans. It may be seen from Fig. 4 that a fulminant infection resulted in the mice which had been conditioned by cortisone, 4.0 mg, and x-radiation, 400 r. Twenty out of the 24 mice in the test group were dead within 6 days of infection and all 24 died within 9 days. In contrast to these findings, a) only 4 died of moniliasis of the 24 mice that received *Candida albicans* only; b) moderately effective enhancive effects were obtained from the use of x-radiation, 400 r, to result in death of 22 of 24 with an average

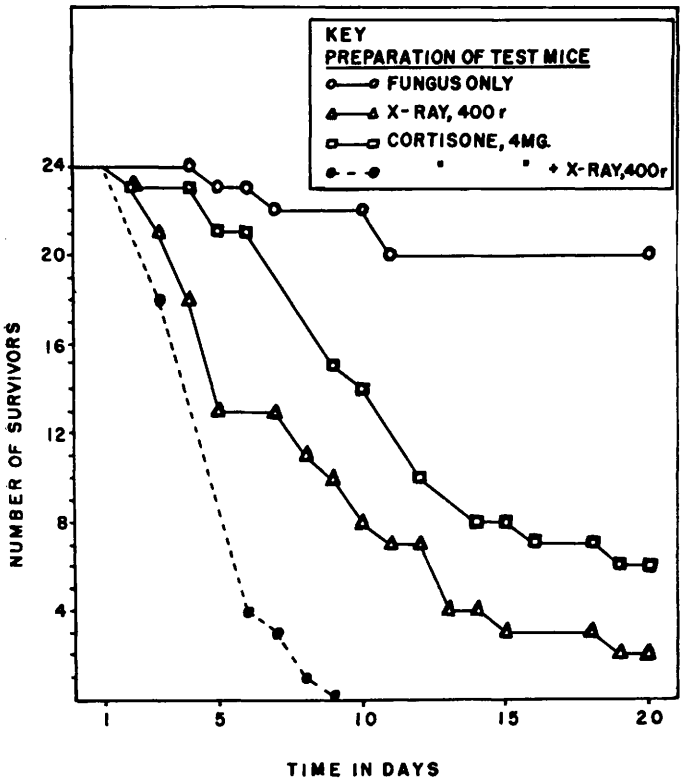

FIG. 5. The comparative effects of cortisone and x-radiation, singly and in combination, as agents for enhancement of the pathogenicity of *Blastomyces dermatitidis* for mice.

survival time to death of 7.5 days; and c) moderately effective enhancive effects resulted from cortisone, 4 mg, to kill 18 of 24 with a survival time to death of 11.7 days. Even though the lethality of the infection was markedly enhanced by the use of either cortisone or x-radiation, a prolongation in the time to death made either of these agents much less effective than the two in combination.

Blastomyces dermatitidis. Fig. 5 shows that the synergistic enhancive effect of the preparative treatment of mice by cortisone and x-radiation in combination upon infection by *Blastomyces dermatitidis* was essentially similar to that observed for *Candida albicans.* The treatment of mice by cortisone and x-radiation in combination before infection with *Blastomyces dermatitidis* resulted in death from blastomycosis of all 16 mice in the test group with an average survival time of 7.4 days. These findings are in contrast to the results that were obtained for the control group where death was limited to three of the 16 over a 30-day period. Finally, when preparative treatment was limited to either cortisone or x-radiation singly, an enhancive effect upon experimental blastomycosis similar to that observed for experimental moniliasis resulted in death of most of the recipients of the test inoculum over a period of 30 days, but the time to death was markedly prolonged.

Summary. A simple method is described for the production of rapidly progressive lethal infections. It consists of the administration of two readily available agents in combination, cortisone and x-radiation, to laboratory animals in preparation for test within the next 24 hours as recipients of a microorganism. Cortisone and x-radiation each over a wide dosage range act synergistically to potentiate the enhancive effect that may result from the employment of either agent singly. The result of this effect is a remarkable alteration

in the susceptibility of a test animal to infection by any of a variety of infectious agents, as evidenced by rapid weight loss, death and extensive histopathological lesions. The ready applicability of the method is illustrated in this paper by experiments that utilized four microbiological agents: poliomyelitis virus, a Coxsackie virus, *Candida albicans,* and *Blastomyces dermatitidis.* The experimental studies that employed these agents and the findings for other viruses, bacteria and fungi will be reported in detail elsewhere.

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Cortisone-Induced Metastases of Adenocarcinoma in Mice.* (19544)

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In more than 200 routine transplantations of a mammary adenocarcinoma in C3H mice no metastases were found(1). Local growth of this transplantable tumor was inhibited by cortisone as was also adenocarcinoma in another strain(2). But a new and unexpected phenomenon not yet described, to our knowledge, has been found in our work with cortisone: inhibition of local tumoral growth by cortisone was accompanied by the appearance of multiple metastases. Our finding offers the opportunity to study the mechanism underlying production of metastases and the influence steroids may have on the spread of metastases in the body and on resistance against malignant growth. This is why a more detailed study with transplantable adenocarcinoma in C3H mice has been undertaken.

Material and methods. Male and female C3H mice about 2 months old, weighing 16 to 17 g in the first experiment, and 19 to 20 g in the other 2 were used. A 0.1 ml saline suspension of mammary adenocarcinoma K7‡ was injected into the left flank. Treatment

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‡ This adenocarcinoma appeared spontaneously in our C3H mice and was maintained, previous to the present work, by 51 transfers in 200 animals. For our technic see 1,3.