14. ———, *Cancer Research,* 1951, v11, 1.
15. Huseby, R. A., and Barnum, C. P., *Arch. Biochem.,* 1950, v26, 187.
16. Szafarz, D., *Biochim. et Biophys. Acta,* 1951, v6, 562.
17. Schneider, W. C., and Potter, V. R., *J. Biol. Chem.,* 1949, v177, 893.
18. Kennedy, E. P., and Lehninger, A. L., *J. Biol. Chem.,* 1949, v179, 957.
19. Hevesy, G., *Radioactive Indicators,* Interscience Publishers, Inc., New York, 1948.

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Virulence Enhancement of *Candida albicans* by Antibiotics and Cortisone. (20488)

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It was previously reported that the intraperitoneal injection of a non-pathogenic strain of *Candida albicans* produces a rapidly fatal disseminated infection if aureomycin is administered simultaneously by the same route (1). That this effect was not due to this special strain of Candida is evident from the fact that similar results were obtained with 9 other strains of Candida, 2 isolated from the oral cavity, 4 from urine, and 3 from stool of patients. With the exception of minor quantitative differences, the results with these strains were similar to those reported in the original communication. The death ratio varied from 60-100%; with an average of 76.6% (83 deaths among 107 mice). None of these strains alone was capable of causing death of the mice without concomitant intraperitoneal administration of aureomycin.

Routes of administration of aureomycin and fungus. When the drug was administered orally (2 mg aureomycin in 0.2 cc saline), and the fungus was given *per os,* subcutaneously or intraperitoneally, no death occurred; after the subcutaneous injection of aureomycin and oral or intraperitoneal infection, no deaths occurred; when the drug and fungus were mixed and given subcutaneously 2 out of 10 animals succumbed; after the intraperitoneal injection of aureomycin, oral and subcutaneous infection proved harmless. However, a mixture of drug and fungus administered intraperitoneally invariably proved fatal (Table I).

TABLE I. Various Routes of Drug and Fungus Administration.

Application of drug	Infection with Candida	Results with Aureomycin	Results with Terramycin
Per os	*Per os*	0/10	0/10
	Subcut.	0/10	0/10
	Intraper.	0/9	0/10
Subcut.	*Per os*	0/10	1/10
	Subcut.	2/10	4/10
	Intraper.	1/10	4/10
Intraper.	*Per os*	0/10	1/10
	Subcut.	1/10	2/10
	Intraper.	10/10	10/10

Legend: Doses of aureomycin, 2 mg in 0.2 ml; of terramycin, 3.3 mg in 0.2 ml. Doses of *Candida albicans,* 0.2 ml of an emulsion of packed cells in 2.5 ml saline (320 million living cells).
Numerator = Mice dead within 14 days of observation. Denominator = Total No. of mice.

All of 6 mice survived the intravenous administration of 1 mg aureomycin in 0.1 ml saline; 21 of 30 mice succumbed after the intravenous administration of 30, 40, or 80 million Candida cells in 0.1 ml saline, but only 5 of 21 mice succumbed when the same number of Candida cells were suspended in 0.1 ml (1.0 mg) of aureomycin. It is evident that intravenously introduced *Candida albicans* is pathogenic for mice and that the pathogenic effect of the fungus is remarkably reduced when combined with aureomycin. These results contrast with all other combinations of routes of administration.

Tests with other antibiotics. In another series of experiments other antibiotics were

employed in place of aureomycin. The test (simultaneous injection of Candida and the antibiotic intraperitoneally) was performed with penicillin G (up to 10,000 units in 0.2 ml saline), streptomycin (10 mg), bacitracin (up to 200 units), and chloromycetin (up to 5 mg in 0.2 ml solution) in propylenglycol, 50% acetamide or 50% N,N-dimethylacetamide. The aureomycin results were not duplicated. Some animals succumbed in the course of the experiment, but most of the deaths were late occurring from 7 to 14 days after treatment. These findings could not be compared with the immediate action of aureomycin. Of 54 mice treated with the various antibiotics 18 died, 12 in this belated fashion. The recovery of Candida from the organs of several of these cases indicates that some interaction between drugs and fungus may have taken place. In control experiments 12 mice received 0.2 ml Candida emulsion intraperitoneally and no drug. The animals were sacrificed after 1, 5, 9, 10, and 14 days. Cultures from kidneys were all negative for Candida. The yeast cells obviously were excreted under these "normal" conditions with no permanent damage to the renal tissue.

*Terramycin** was the only antibiotic that simulated the action of aureomycin. A dose of 2 mg was insufficient to produce constant results; but amounts of 3.3 or 5 mg in 0.2 ml saline solution were fully as effective as aureomycin. The technic previously described(1) was employed. With intraperitoneal injection of terramycin-Candida mixture, 26 out of 27 experimental animals died within 24-48 hours with a dose of 5 mg terramycin, and 19 out of 23 died when 3.3 mg terramycin was used. The terramycin solutions in these tests were standing from one to 3 days after preparation. No difference was found with freshly prepared solutions as compared with 3-day-old solutions, indicating a stability of the virulence enhancing factor which corresponds to the antibiotic stability(2,3). This contrasts with aureomycin which is much less stable when assayed for antibiotic activity as well as in the drug-fungus test; on standing the virulence enhancing and antibiotic capacities of aureomycin were lost after 1 or 2 days. Boiling, which destroys both these qualities of aureomycin, does not alter these same effects of terramycin. All of 31 animals succumbed after receiving boiled terramycin mixed with fungus. Numerous subcultures on Sabouraud's agar were made from the heart blood and from the kidneys of dead mice. Candida was recovered in 26 out of 27 kidney cultures and in 13 out of 22 heart blood cultures, indicating that a generalization of the monilia infection had taken place within the body of the antibiotic treated animals. Frequently grossly visible abscesses were observed in the kidneys.

Various routes of drug and fungus application. The various routes of administration of terramycin and *Candida albicans* in mice were tested as described for aureomycin in this communication. Terramycin appears to be slightly more active than aureomycin, as can be seen from Table I, but the overall results with both antibiotics are essentially alike: early death when drug and fungus are given intraperitoneally, and none or little action under other conditions (Table I). *Intravenous injection.* Results simulate those observed with aureomycin. Of 10 animals injected with 80 million yeast cells in 0.1 ml saline, one survived. Of 10 mice injected with 0.1 ml (1.7 mg) terramycin solution, 9 survived. Of 10 mice receiving 80 million Candida cells in terramycin (1.7 mg) 3 survived. The time factor in the occurrence of deaths may be seen from Table II. Thus terramycin as well as aureomycin reduce the pathogenicity of Candida on intravenous injection of the drug-fungus mixture, in contrast to all other forms of application.

Cortisone. It has been reported that this steroid exerts a definite influence on induced infection in mice, whether the infecting agent be either bacteria(4) or viruses(5). Dormant infections became manifest or sublethal doses developed fatal effects(6). Since these ob-

* Crystalline Terramycin hydrochloride intravenous was used. It is a yellow powder in mixture with sodium glycinate which, according to the C. Pfizer and Co. statement, retains its antibiotic potency for at least 12 months at room temperature. It is readily soluble in water and saline solution: this solution is undiminished in antibiotic activity after storage in the refrigerator for at least 3 days.

TABLE II. Result of Intravenous Injections.

Material injected	No. of mice	Surviving .... days after													
		1	2	3	4	5	6	7	8	9	10	11	12	13	14
0.1 ml Candida emulsion (80 million cells)	10	7	3	3	1	1	1	1	—	—	—	—	—	—	—
0.1 ml terramycin solution (1.7 mg)	10	10	10	10	10	10	10	10	10	10	10	9	9	9	9
0.1 ml terramycin-Candida mixture	10	8	7	7	6	6	5	5	5	5	5	5	4	4	3

servations are paralleled by our results with antibiotics and *Candida albicans*, similar experiments were set up with cortisone and Candida infection.

Intraperitoneal injection of Candida emulsion in cortisone (2.5 mg) as used in the antibiotic tests proved harmless; none of 7 treated mice became sick. When 5 mg cortisone was given subcutaneously and the fungus intraperitoneally at the same time, 3 out of 10 animals died. The kidneys of all 3 yielded *Candida albicans* cultures, one heart blood culture also was positive. In further experiments 2.5 mg cortisone was given subcutaneously 2 hours before fungus infection (2.5 mg intraperitoneally) and 2 hours after the infection, with striking results: 21 of 22 animals succumbed in the course of the 2 weeks following treatment. On autopsy numerous abscesses were found in all the internal organs, particularly liver, spleen, omentum, and kidneys. The latter organ was most heavily infected, being studded with numerous small abscesses. In 19 of the 21 cases *Candida albicans* was recovered from the kidneys, in 14 from heart blood.

Discussion. Previous experiments demonstrating virulence enhancing qualities of aureomycin on Candida were continued and expanded. Nine more strains of *Candida albicans* isolated from human sources reacted with aureomycin in the same manner as the original strain. Of the other antibiotics tested in the drug-fungus combination, only terramycin gave similar results. The similarity of action of the 2 drugs in growth inhibition and growth promotion in animals is well known (7). Their chemical structure, although different in details, shows a similar basic structure(8).

Virulence enhancing capacity of terramycin closely parallels its antibiotic activities. In contrast to the more labile aureomycin, terramycin is more stable and the virulence enhancing and antibiotic activity is retained after standing or boiling. Various routes of administration of drug and fungus produce similar results with both antibiotics. In both the intravenous route of drug-fungus injection did not lead to virulence enhancement, but on the contrary had some antifungal effect.

That cortisone lowers resistance in mice has been described repeatedly. This steroid, therefore, was tested in *Candida albicans* infected mice. When given subcutaneously in large amounts (2.5 mg) before and after i.p. fungus infection, death resulted regularly. A less rapidly fatal infection developed due to the invasion of the blood stream, with widely disseminated abscesses, particularly in the kidneys. *Candida albicans* was regularly found in pure culture from kidneys and frequently from the heart of the dead mice. Material taken from abscesses in spleen and liver likewise contained Candida organisms only. No gram positive rods (Corynebacterium) or gram negative organisms (salmonella) nor staphylococci were found in any of these organs.

In this and in the preceding communication(1) the term "virulence enhancement" has been used to indicate the increased morbidity and mortality. Perhaps this is a misnomer since virulence is not always a single quality, but represents the result of the aggressive forces of the infecting agent and the defensive power of the infected host. Therefore, enhancement of virulence may be conditioned by increased aggressiveness of the fungus or by modified resistance of the mouse. From the findings herein described it appears that aureomycin, terramycin, and cortisone

lower resistance of the mouse to infection. The same holds true for the action of mucin (9). No evidence could be found indicating a direct influence of the antibiotics on the fungus either *in vitro* or *in vivo*, pointing to a modification of the host's cell.

Summary. 1. Terramycin has the same virulence enhancing effect on *Candida albicans* as aureomycin. Other antibiotics failed to show this effect. 2. Terramycin due to its stability acts after standing or boiling while aureomycin, which is not stable on standing and is heat labile, does not. This parallels their antibiotic activities. 3. Only the intraperitoneal injection of either drug and fungus is effective in producing a fatal infection. All other routes of administration and combination were negative. 4. Intravenous injection of drug and fungus indicates a protective effect. 5. Cortisone administered shortly before and after infection with *Candida albicans* produces a generalized fatal infection. 6. Lowered resistance of the animal to infection is considered as the cause of "virulence enhancement."

1. Seligmann, E., PROC. SOC. EXP. BIOL. AND MED., 1952, v79, 481.

2. Regna, P. P., and Solomons, I. A., *Ann. New York Acad. Sci.*, 1950, v53, 229.

3. Gocke, T. M., Jackson, G. C., Wilcox, C., and Finland, M., *Ann. New York Acad. Sci.*, 1950, v53, 297.

4. Antopol, W., Glaubach, S., and Quittner, H., *Rheumatism (London)* 1951, v7, 187.

5. Southam, C. M., and Babcock, V., PROC. SOC. EXP. BIOL. AND MED., 1951, v78, 105.

6. Antopol, W., PROC. SOC. EXP. BIOL. AND MED., 1950, v73, 262.

7. Hobby, G., *Bact. Rev.*, 1953, v17, 36.

8. Regna, P. P., *Transact. New York Acad. Sci.*, 1952, Ser. II, v15, 12.

9. Olitzki, L., *Bact. Rev.*, 1948, v12, 149.

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Effect of Anterior Pituitary Growth Hormone on Certain Liver Enzymes. (20489)

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It has been reported(1) that growth hormone decreases the catabolism and excretion of amino acids rather than directly affecting the synthesis of body protein. In contrast, Russell(2) using nephrectomized rats given casein hydrolysate, found that the principal action of growth hormone is to increase amino acid uptake into tissues. This agrees with the results of Friedberg and Greenberg(3) who found an increased incorporation of isotopic methionine into skeletal muscle following growth hormone administration. Bartlett and Glynn(4) observed no effect of growth hormone on liver or renal aspartic-glutamic transaminase in the normal adult female rat though the hormone increased liver aspartic-glutamic transaminase in immature hypophysectomized rats. Fraenkel-Conrat *et al.*(5) have shown that the administration of growth hormone to plateaued female rats for 8 days at a level of 3 mg per day resulted in decreased fasting arginase activity in pooled liver samples. It seemed advisable to study the effect of growth hormone, given in acute dosage to normal rats, on the activities of certain liver enzymes involved in nitrogen metabolism.

Methods and results. Eighteen adult male rats of the Wistar strain which had been maintained on a stock chow diet and water *ad libitum* were divided into 2 groups of comparable average body weight (380 g; range 322 to 429 g). Following a fast of 22 hours, one group was injected intraperitoneally with an anterior pituitary growth hormone preparation (Connaught Medical Research Laboratories, Lot 200) in saline (made slightly alkaline) at a level of 3 mg per 100 g body