mine (or allithiamine) for 5 test organisms. Some of these results are reported in Table 1. The yields obtained with 0.1 μg of allithiamine and thiamine are included for the purpose of comparison.

Aqueous extracts, prepared from the allithiamine and thiamine-mycelium were treated with alkaline ferricyanide and cyanogen bromide. The thiamine activity of the treated extracts was destroyed equally for 2 test fungi: *E. fimbriata* and *P. blakesleeanus*. Since control experiments under the same conditions had shown that thiamine was inactivated while the activity of allithiamine was only slightly diminished, it was concluded that allithiamine is converted into thiamine by *P. blakesleeanus*.

*Experiments with albino rats.* Four weanling albino rats were fed a thiamine-free diet until two of them died. At that time, the 2 surviving rats were transferred to a diet containing allithiamine, but otherwise the same as the thiamine-free diet. After 2 wk on allithiamine, they were placed on a diet containing thiamine instead of allithiamine. The results for both rats were essentially the same. Figure 2 shows the results

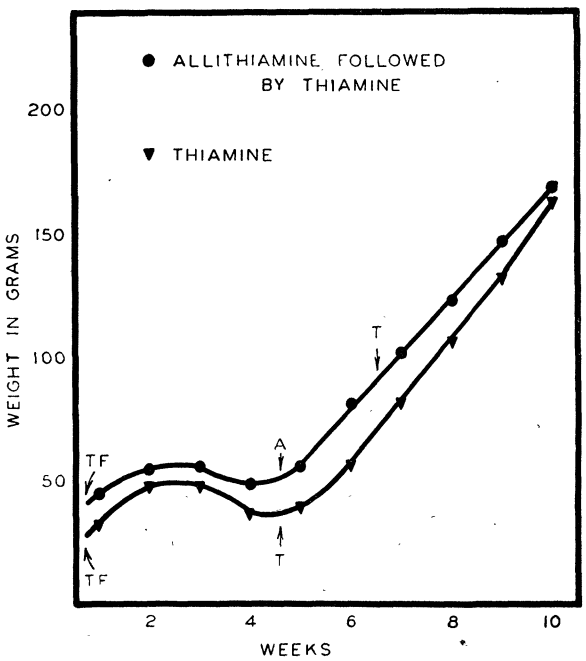


FIG. 2. Growth curves for 2 male rats kept on the thiamine-free diet for 4 wk, followed by allithiamine for 2 wk for 1 rat (circles) and by thiamine for the other (triangles). Time of change of diet is indicated by arrows: TF, thiamine-free diet; A, thiamine-free diet plus allithiamine; and T, thiamine-free diet plus thiamine.

for 1 of the 2 rats. Note that, after being placed on the thiamine-free diet, the rat gained weight during the 1st and 2nd wk, leveled off during the 3rd wk, and lost during the 4th wk. When transferred to the allithiamine diet, the rat gained rapidly and continued to gain after being transferred to the thiamine diet. For comparison, a typical growth curve for another rat from a series of experiments in which the rats were changed from the thiamine-free diet directly to the thiamine diet is also shown. On the basis of the above results, one may conclude that allithiamine may replace thiamine in the diet of the rat.

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## The Effect of Cortisone upon the Therapeutic Efficacy of Antibiotics[1]

Ernest Jawetz and Elizabeth R. Merrill

*Departments of Microbiology, Medicine, and Pediatrics, University of California School of Medicine, San Francisco*

It is well established that cortisone enhances various infections in animals and man and aggravates their severity (*1*). This property is attributable to a depression of the antimicrobial defenses of the host, rather than to a direct promotion of microbial growth or invasiveness (*2*). To protect a patient receiving cortisone against dissemination of latent or active infection, antibiotics are often administered. The question arises whether cortisone might also impair the therapeutic effectiveness of antimicrobial agents. If certain of these drugs acted in conjunction with normal antimicrobial defenses of the host then the depression of these defenses by cortisone might result in measurable impairment of the curative effect of these drugs. The experiments reported here were undertaken to explore this possibility.

The following laboratory model was used. White Swiss mice (15–19 g) were infected intramuscularly with a virulent strain of *Klebsiella pneumoniae*. The $LD_{50}$ of this strain consisted of 50–100 organisms injected into the thigh muscle, in a volume of 0.1 ml. After infection with 10–500 $LD_{50}$, all animals died in 3–5 days with positive heart blood cultures. The antibiotics, aureomycin hydrochloride[2] and streptomycin sulfate were dissolved in suitable concentration in saline, and each dose was administered intraperitoneally in a volume of 0.2 ml. Antibiotic treatment was started 6 hr after infection, two doses were administered on the next day, and a single daily dose on the following 3 days.

Cortisone acetate was suspended in saline and a daily subcutaneous dose of 10 mg/kg was administered for 5 consecutive days, beginning 24 hr before infection. This dose of cortisone was not harmful to the animals (*3*) and permitted normal growth and weight gain. Alternate groups of mice received cortisone as shown in Table 1.

[1] Supported by a grant (E214) from the National Institutes of Health, U.S. Public Health Service.

[2] Supplies of aureomycin hydrochloride were made available by Dr. Stanton Hardy, Lederle Laboratories.

TABLE 1

IMPAIRMENT OF ANTIBIOTIC EFFECTIVENESS BY CORTISONE

| No. of *Klebsiella pneumoniae* injected intramuscularly | Antibiotic | | Cortisone total dose, mg† | Mortality of mice | | $P$‡ |
|---|---|---|---|---|---|---|
| | Drug | Dose, mg* | | Dead/total | Per cent | |
| 20.000 | Aureomycin | 0.5 | 0 | 25/25 | 100 | |
| 20.000 | Aureomycin | 0.5 | 1.0 | 25/25 | 100 | — |
| 20.000 | Aureomycin | 1.0 | 0 | 17/25 | 68 | |
| 20.000 | Aureomycin | 1.0 | 1.0 | 24/25 | 96 | 0.03 |
| 20.000 | Aureomycin | 2.0 | 0 | 3/25 | 12 | |
| 20.000 | Aureomycin | 2.0 | 1.0 | 18/25 | 72 | 0.005 |
| 20.000 | Aureomycin | 4.0 | 0 | 0/25 | 0 | |
| 20.000 | Aureomycin | 4.0 | 1.0 | 5/25 | 20 | 0.05 |
| 1000 | Aureomycin | 1.0 | 0 | 2/24 | 8 | |
| 1000 | Aureomycin | 1.0 | 0.75 | 16/25 | 64 | 0.001 |
| 1000 | Aureomycin | 2.0 | 0 | 1/20 | 5 | |
| 1000 | Aureomycin | 2.0 | 0.75 | 1/24 | 4 | — |
| 50.000 | Streptomycin | 0.06 | 0 | 24/30 | 80 | |
| 50.000 | Streptomycin | 0.06 | 0.75 | 29/30 | 97 | 0.1 |
| 50.000 | Streptomycin | 0.18 | 0 | 1/30 | 3 | |
| 50.000 | Streptomycin | 0.18 | 0.75 | 10/30 | 33 | 0.009 |
| 50.000 | Streptomycin | 0.6 | 0 | 0/29 | 0 | |
| 50.000 | Streptomycin | 0.6 | 0.75 | 0/30 | 0 | — |
| 1000 | — | — | — | 48/48 | 100 | — |
| — | — | — | 1.0 | 1/46 | 2 | — |
| — | — | — | 0.75 | 0/54 | 0 | — |

* Injected intraperitoneally in 5 equal doses, beginning 6 hr after infection.

† Injected subcutaneously in 5 equal, daily doses, beginning 24 hr before infection.

‡ Probability of chance occurrence estimated by chi-square test. A figure of 0.05 or less indicates statistical significance of the difference.

Groups of 25–40 mice were infected and treated with various dosage levels of antibiotic. The animals were carefully observed for the development of the local lesion in the thigh which always preceded the systemic illness and death. Deaths and survival times were recorded and the differences in mortality rates subjected to the chi-square test for determination of statistical significance. The results of representative experiments are summarized in Table 1.

The single death among 46 control mice receiving 1.0 mg of cortisone must be attributed to an accident: there was no evidence of infection or gross abnormality. The average survival time of infected animals with and without cortisone did not differ significantly.

The antibiotic doses were so adjusted as to cover a range from complete protection to none. It can be seen from Table 1 that over much of this range the cortisone treatment of animals materially depressed the rate of cure which could be achieved with any given dose of the antibiotic. This interference with the curative effects of the antibiotic applied not only to the bacteriostatic drug, aureomycin, but also to the bactericidal drug, streptomycin. Other experiments, to be reported elsewhere, revealed similar effects of cortisone in a variety of bacterial infections treated with a number of antibiotic agents. In all instances a large excess of the antibiotic drug overcame this effect of cortisone and resulted in cure in spite of cortisone administration.

These experiments suggest that defenses of the host may materially aid the direct antimicrobial action of antibiotics (*4–6*). When cortisone depresses these host mechanisms, the manifest outcome appears to be an impairment of the therapeutic effect of the antibiotic. This observable end result is most pronounced with barely curative amounts of the antibiotic. With much larger doses of antibiotic the contribution of host defenses in overcoming the infection is less essential and consequently the cortisone effect is not readily demonstrable. The mechanism of these contributory host defenses is currently under study.

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## Resumption of Heartbeat in the Rabbit Embryo after Exposure to Low Temperatures[1]


M. C. Chang

*Worcester Foundation for Experimental Biology, Shrewsbury, Massachusetts*


Since the discovery of glycerol as a protective agent for the vitrification of spermatozoa at low temperature by Polge, Smith, and Parkes (*1*), deep-frozen bull semen has been successfully used in artificial insemination (*2*). Revival of mammalian ovarian tissue (*3*), revival of chick heart, and development of the chick embryo (*4–6*) after deep freezing have been reported. This paper reports a series of preliminary experiments on the resumption of the heartbeat in early rabbit embryos after exposure to various low temperatures.

Ten-day-old rabbit embryos were removed from the uteri under a stereoscopic microscope. Equal volumes of buffered Locke-Ringer solution and rabbit serum were used as a medium for the embryos before treatment and for their culture in Carrel flasks after treatment.

For treatment at 10° or at 0° C, the embryo was placed in a Carrel flask containing 5 ml of serum-Ringer fluid and kept either in a constant temperature bath at 10° C or in a Thermos flask containing ice for 1 day. After storage, the flask was attached to a rocking device (*7*) in an incubator at 38° C.

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