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.

References

1. THOMAS, L. *Ann. Rev. Med.*, **3**, 1 (1952).
2. GLASER, R. J., *et al. J. Lab. Clin. Med.*, **38**, 363 (1951).
3. ABERNATHY, R., and SPINK, W. W. *J. Clin. Invest.*, **31**, 947 (1952).
4. JAWETZ, E. *Arch. Internal Med.*, **77**, 1 (1946).
5. EAGLE, H., *et al. Proc. Soc. Exptl. Biol. Med.*, **82**, 201 (1953).
6. GOWANS, J. L. *Brit. J. Exptl. Pathol.*, **34**, 195 (1953).

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

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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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The media prepared for treatment of embryos at still lower temperatures were 5, 10, and 15% glycerol in serum-Ringer fluid. The embryos were suspended consecutively in these solutions for 10–20 min each, at a temperature of about 32° C. One or two embryos then were placed in a small Pyrex tube containing 15% glycerol and stoppered with a rubber bulb. For cooling at −10° C, the tube was kept at 10° C for 1 hr, 0° C for 1 hr, and then was transferred into a Thermos flask containing a freezing mixture (solid CO_2 in acetone and 95% alcohol) adjusted to −10° C for 1–2 hr. The embryos were then transferred into a Carrel flask for culture.

For deep-freezing at −75° C, cooling at different rates was performed as follows. For fast cooling, the tubes containing embryos at room temperature (20–25° C) were placed directly into a deep-freezing mixture at −75° to −79° C. This cooling took place in less than 1 min. For medium cooling, the deep-freezing apparatus devised by Polge and Lovelock (*8*) was used. Cooling from 20° to 0° C took place in about 15 min and from 0° to −75° C in about 30 min. For slow cooling, the tubes containing embryos were first placed in a bath at 10° C for 1–10 hr and then transferred into ice for 1–10 hr. The tubes were then placed either into the deep-freezing apparatus when its temperature was 0° C, and allowed to drop to −75° C, or into a freezing mixture at 0° C to which solid CO_2 was added gradually until the temperature dropped to −75° C, in 30–60 min. The tubes were kept at −75° C for 10–80 min and then transferred into a water bath at 40° C. Thawing took place in about 1 min. The embryos were transferred and cultured in Carrel flasks containing serum-Ringer fluid. Examinations by means of different magnifications under stereoscopic or compound microscopes were performed approximately every 6–12 hr during culture, and the number of heartbeats/minute was recorded.

From the summary of results in Table 1 it is evident that early rabbit embryos can resume their heartbeat after exposure to low temperatures, although the effects of different temperatures on the embryos may vary. After exposure to temperatures of +10° to −10° C for various lengths of time, the appearance of the embryos was quite normal. Resumption of the heartbeat occurred after from a few minutes to 2–4 hr in culture. Rhythmic contractions of the atrium and ventricle were obvious, though the rate dropped and irregularity of the beat (i.e., contractions of the bulb, atrium, and ventricle not in pace) occurred at the end of culture.

After the treatment at −75° C, before culture, the embryos appeared shrunken but otherwise normal. In culture, they did not recover their normal size and contractions of the heart region were observed only after 12–24 hr. Certain anatomical structures such as the somites, brain vesicles, ear and eye cups, and the different parts of the heart became indistinct, and in some cases the whole embryos appeared like masses of tissue. Although rhythmic contractions of the bulbar region in the inner part of the heart were observed

TABLE 1

RESUMPTION OF THE HEARTBEAT IN RABBIT EMBRYOS AFTER EXPOSURE TO LOW TEMPERATURES

Treatment of embryos	No. of embryos tested	No. of embryos which resumed heartbeat	Remarks on the embryos which resumed heartbeat*	
			Initial beats/min in culture	Beats/min after culture
No treatment, cultured at 38° C	7	6	80–96	19–52 after 1–3 days
At 10° C for 1 day	9	5	25–55	12–62 after 1–1.5 days
At 0° C for 1 day	9	5	35–70	18–82 after 1–1.5 days
At −10° C for 1–2 hr	3	3	42–88	38–72 after 1–1.5 days
At −75° C for 10–80 min	6	1		47 after 1 day under compound microscope
Fast cooling				
Medium cooling	18	5	57 (one embryo)	37–63 after 1 day: observed in 1 embryo under 32X and in 4 embryos under 72X
Slow cooling	16	5		35–55 after 1 day: observed in 5 embryos under 72X

* Individual embryos were cultured for various lengths of time. The range of these times and the range of beats/min are presented. In the series treated at −75° C, the heartbeat was recorded only after culture in most cases.

in the majority of the embryos that resumed their heart contraction, in only one embryo, after medium cooling, were contractions of the bulb and atrium observed.

Throughout this study, it was noticed that not all the embryos could resume their heartbeat even under the most favorable conditions, e.g., in culture. However, some were able to maintain their heartbeat for 2 to 3 days in culture, even though they underwent no obvious further development. In 10–15% glycerol at 32° C, the heartbeat of some embryos was observed, but only for a short time (1–2 hr). In 20–25% glycerol, it was observed for no longer than 5 min. Various percentages of ethylene glycol and glycerol with Cellosolve were tested, but no advantage was noticed. A few tests indicate that 10-day embryos can withstand low temperatures better than 11–13-day embryos.

The rate of cooling, especially to −75° C, may play an important role in the subsequent resumption of the heartbeat in rabbit embryos, although in the present study, no striking difference in effect was revealed between the medium and slow rates of cooling. It appears that the effects of different temperatures on the dif-

ferent tissues vary. Growth of chorionic tissue was observed in every case and contractions of the heart region were observed in a few instances. However, distortion of the brain vesicles and eye cups was observed in every case after deep-freezing. It seems, then, that tissue of mesodermal origin may withstand deep-freezing better than that of ectodermal origin.

References

1. POLGE, C., SMITH, A. U., and PARKES, A. S. *Nature*, **164**, 666 (1949).
2. POLGE, C., and ROWSON, L. E. A. *Ibid.*, **169**, 626 (1952).
3. PARKES, A. S., and SMITH, A. U. *Proc. Roy. Soc. (London)*, **B140**, 455 (1953).
4. GONZALES, F., and LUYET, B. *Biodynamica*, **7**, 1 (1950).
5. ———. *Federation Proc.*, **10**, 52 (1951).
6. LUYET, B., and GONZALES, G. *Biodynamica*, **7**, 101 (1952).
7. SHAPIRO, H. *Science*, **90**, 308 (1939).
8. POLGE, C., and LOVELOCK, J. E. *Vet. Record*, **64**, 396 (1952).

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Lack of Protective Effect of Allyl Thiourea Against X-Irradiation[1]

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Alexander and Fox (*1*) recently found a correlation between the ability of various agents to protect animals against x-rays and the ability of these agents to protect polymethacrylic acid from radiation-induced loss of viscosity. The most effective agent they tested in their polymer system was allyl thiourea, and they pointed out that this chemical had never been tested biologically.

We have found that allyl thiourea does not protect mice from the lethal effects of whole body x-irradiation, and it therefore appears that the interesting polymer system of Alexander and Fox may not be used as an *in vitro* test of *in vivo* protective action.

TABLE 1

EFFECT OF ALLYL THIOUREA ON LETHALITY OF WHOLE BODY X-RADIATION TO MICE*

Expt.	Allyl thiourea (mg/kg)	X-rays (r)	Lethality	Av length of survival (days)
1	0	800	16/16	11.9
	250	0	0/16	—
	250	800	16/16	8.9
2	0	750	16/16	10.3
	35	750	16/16	7.0
	100	750	16/16	9.6
	250	750	16/16	10.7
	250	0	0/16	—

* Mice were Carworth Farm males, age approximately 60 days. Allyl thiourea freshly prepared for each use and injected within 15 min before beginning x-radiation. X-Ray factors: 250 KVP, 15 ma, ¼ mm Cu + 1 mm Al, 60 cm target distance. Rate: 60 r/min in expt. 1; 53 r/min in expt. 2. Lethality is expressed as the number of deaths over the total number tested.

[1] This study was supported by funds provided under contract AF 33(038)27353 with the USAF School of Aviation Medicine, Randolph Field, Texas.

Allyl thiourea is relatively nontoxic and soluble and may be given intraperitoneally to mice at a dose rate of 250 mg/kg without effect. In the experiments reported here we administered 750 or 800 roentgens of whole body x-radiation to our mice; these doses are 100% fatal, but appreciable percentages of mice may be saved from these doses by sodium azide, cysteine, or other agents shown by Alexander and Fox to be less effective than allyl thiourea in their polymer test system.

The results of two experiments are given in Table 1, which indicate no protective effect whatsoever of allyl thiourea. In fact, in some cases the drug, though without toxicity itself, seems to hasten the lethal result of the x-radiations.

Reference

1. ALEXANDER, P., and FOX, M. *Nature*, **170**, 1022 (1952).

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Photoperiodic Behavior of Medium-Early Varieties of Rice

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The effect of short days of 10 hr (8:00 A.M.–6:00 P.M.) on the flowering behavior of medium-early rice (*1*) has been studied. Three varieties, T.3 (a selection from Basamati of Dehradun), T.12 (a selection from Hanshraj of Unnab district), and T.21 (a selection from Chawal of Rampur State), grown in Uttar Pradesh, were used in pot-culture experiments. Pure seeds of these varieties, obtained from Nagina Rice Research Station, U.P., after a preliminary selection for uniformity by eye, were sterilized in 0.2% formalin, thoroughly washed in distilled water, and sown on June 18, 1949. Germination was complete in 5–6 days. Short-day treatment[2] was started in the seed bed with 7-day-old seedlings. Short days were given for periods of 3, 4, 5, and 6 wk to separate seed beds. The treatments

[1] Thanks are due to Shri Ranjan for his guidance and helpful criticism and for the facilities provided in the Department of Botany of Allahabad University for carrying out this investigation. I am grateful to C. M. Bastia for his help in the preparation of the diagram.

[2] Short-day treatment consisted of a daily 10-hr exposure to natural daylight in the open field, from 8:00 A.M. to 6:00 P.M. For the remainder of the 24-hr cycle, i.e., from 6:00 P.M. to 8:00 A.M. of the next day, the pots were removed to a well-ventilated dark room.

Long-day treatment consisted of a 24-hr continuous illumination obtained by supplementing the natural daylight with artificial illumination from a 1000-w gas-filled Osram bulb. The bulb was hung at a height of 5 ft, and the intensity of light falling on the surface of the soil, as measured by a Weston phototronic foot-candle meter, was 30–40 ft-candles. The pots were arranged in concentric circles on the ground and their respective positions were interchanged every day so that each pot received almost the same intensity of light.