TABLE II. Effects of X-Irradiation and Testosterone Propionate on Body and Seminal Vesicle Weights and Nitrogen Content of Seminal Vesicles of Castrate Rat.

Animals per group	Treatment Irradiation, r	Hormone daily, μg	Body wt at castration, g	Body wt at irradiation, g	Body wt 60 hr after treatment, g	Seminal vesicles wt, mg	N/gland, mg
13	0	0	305 ± 5*		347 ± 6*	107 ± 4*	1.8 ± .1*
13	0	500	304 ± 6		344 ± 7	233 ± 8	5.5 ± .1
6	80	"	293 ± 9	337 ± 7*	337 ± 6	207 ± 7	4.9 ± .1
6	160	"	294 ± 3	351 ± 6	347 ± 7	222 ±10	5.1 ± .2
4	320	"	270 ±10	326 ±11	324 ±12	209 ± 3	5.2 ± .1
8	640	"	303 ± 3	334 ± 7	318 ± 5	193 ± 9	4.7 ± .2
5	1000	"	312 ± 4	342 ± 5	320 ± 4	197 ± 4	4.6 ± .1
5	1500	"	296 ± 1	348 ± 3	312 ± 3	180 ± 5	3.6 ± .3
5	2000	"	285 ± 7	334 ± 7	298 ± 7	159 ± 8	3.9 ± .4
5	3000	"	284 ± 3	337 ± 5	301 ± 3	166 ± 5	4.0 ± .2

* Mean ± S.E.

trated and accessory organs regressed 20 days before X-irradiation and/or hormone treatment. Androgen-treated castrates received daily 500 μg of testosterone propionate in oil, total amount 1500 μg. Animals were sacrificed 60 hours following initial treatment. The mitotic index with hormone was 8%, whereas following androgen and irradiation at 80, 160, 320 and 640 r it was 14, 20, 17 and 15% respectively. At levels of 1000, 1500, 2000, and 3000 r these values were 5, 4, 3 and 1%. These data indicate inhibition of mitotic activity at levels of 1000 r and above. It is suggested that irradiation between 80 and 640 r caused a delay in mitotic response of secretory epithelium and as a result the mitotic index was greater than in those which received androgen alone. X-irradiation had no significant effect on cell height. There was a decrease in seminal vesicle weights of animals treated with hormone and increasing amounts of irradiation.

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Effect of Malonate on Selected Body Defenses.* (22463)

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Previous work from this laboratory(2-7) has shown that repeated injections of sublethal doses of malonate reduce survival time of mice infected with *Salmonella typhimurium* from 64-84 hours to 8-24 hours. Survival time is also reduced by injections of fluoroacetate, arsenite, citrate, and succinate. Bacteria multiply more rapidly in malonate treated mice than in saline treated mice as revealed by total viable pathogen counts in homogenates prepared from mouse carcasses (7). Survival time seems dependent, therefore, upon time required for microorganisms to reach a fairly constant lethal number. The greater rate at which this lethal number of pathogens is reached in mice, given malonate, may be attributed to one or both of the following conditions: (a) decreased rate of destruction due to impairment of animal's body defenses or (b) more rapid rate of reproduction due to more favorable nutritive environ-

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ment. Whether the increased susceptibility associated with injections of malonate can be correlated with impairment of selected phases of the defense systems is the subject of the present report.

Materials and methods. Survival tests. Rats weighing between 50-65 g were infected intraperitoneally with 0.5 ml of a saline suspension of *S. typhimurium* containing approximately 45 x 10^6 cells. Bacteria were cultured 15 hours in brain-heart infusion broth (Difco). Three groups of rats were employed. Two groups were infected and 30 minutes later the first of 8 hourly injections of either 0.5 mg malonate per g of rat contained in approximately 0.5 ml of sterile saline or comparable volume of saline alone was administered. The third group, not infected, was given injections of malonate and served as toxicity control. *Phagocytic measurements.* Human blood was defibrinated and added to an equal volume of saline. Rat blood, obtained by cardiac puncture with the animal under light ether anesthesia, was added to equal volume of saline containing 0.5 mg heparin per ml. Heparin at this concentration has no observable effect on phagocytic activity of neutrophiles(1). Malonate was introduced into the test systems by adding to 0.5 ml blood an equal volume of disodium malonate (Eastman) dissolved in sterile saline or by administering a series of 8 hourly injections of 0.5 mg of malonate per g body weight. The animals were bled 15 minutes after the last injection. The method of Cottingham and Mills(8) was followed for phagocytic tests. One ml blood-saline mixture was brought to 37°C by gentle agitation in water bath. Agitation was continued for 15 minutes after addition to each tube of 0.2 ml suspension of *Staphylococcus aureus,* density adjusted in a Coleman Spectrophotometer, Model 11, to give a light transmittance of 70% at wave length of 650 mmu. Smears were made on 2 cover slips and stained with Wright and Giemsa stains. Phagocytic activity is expressed as percent of neutrophiles showing engulfment of one or more bacteria determined by microscopic inspection of 100 to 200 cells. All slides were evaluated without knowledge of groups to which they belonged. *Serum bactericidal activity.* Blood of 3 or 4 rats was defibrinated and pooled. Following centrifugation, 3 ml of serum was brought to temperature in a 37°C incubator, during which time a suspension of *S. typhimurium,* cultured for 15 hours in brain-heart infusion broth (Difco), was prepared by serial dilutions with sterile saline. To the serum, 0.1 ml of the suspension was added. At hourly intervals thereafter, 0.1 ml of serum-bacteria mixture was pipetted off, diluted with saline, and 0.1 ml cultured in triplicate on SS agar. Number of colonies times dilution factor gave the number of viable bacteria per ml of mixture. The effect of malonate on bactericidal activity of serum was tested by bleeding rats one-half hour after administration of the second of 2 hourly injections of 0.5 mg malonate per g body weight. For experiments requiring addition of zymosan, this compound was prepared, with some modifications, from fresh yeast according to Pillemer and Ecker(9). Zymosan was suspended in saline for addition to serum according to Pillemer, *et al.*(10). Final concentration was 3 mg zymosan per 1 ml serum. Zymosan and serum were incubated at 37°C for one hour prior to addition of bacteria. *General.* Male and female white rats (Carworth Farms) were used. The rats were fed Purina dog chow checkers and water was available at all times.

Results. Survival time of infected rats given malonate. In 2 experiments survival time of rats infected intraperitoneally with *S. typhimurium* and then administered a series of 8 hourly injections of 0.5 mg malonate per g body weight was significantly less than that of control rats similarly infected and given injections of saline. The average survival time of the group given malonate was 48 hours compared with 114 hours for the controls ($P < 0.05$ according to rank order test(12)) in the first experiment, while in the second, values were respectively 8.5 hours and 118 hours ($P < 0.001$). None of the uninfected rats given the same number of injections of malonate, died. These results compare favorably with those previously reported for mice(2,4).

Phagocytic measurements with human neu-

TABLE I. Phagocytic Activity of Human Neutrophiles Expressed as Percent of Cells Ingesting Bacteria. All values in each row are averages of 2 separate determinations made on the same sample of blood.

	A .09% saline			B .30% saline			C .90% saline			D Control		
	1	2	3	1	2	3	1	2	3	1	2	3
	15 mg*	5 mg*	1 mg*	15 mg*	5 mg*	1 mg*	15 mg*	5 mg*	1 mg*	sal.	sal.	sal.
	%											
	62	66	—	66	78	89	62	65	88	89	97	79
	68	83	87	70	75	87	—	71	88	84	84	82
	66	71	86	60	73	92	57	72	88	86	92	91
	56	72	83	67	88	85	63	89	84	75	83	85
Avg	63	73	85	66	79	88	61	74	87	84	89	84

* Concentration of malonate/ml blood-saline mixture.

trophiles. The percent of active phagocytes obtained with human neutrophiles is listed in Table I. Sections A, B, and C represent values obtained with 3 different concentrations of saline to make the final solution containing malonate approximately isotonic with blood. Thus 0.09% saline and 15 mg of malonate, 0.30% saline and 5 mg malonate, and 0.9% saline and 1 mg malonate have similar calculated tonicities. It is evident, however, that no significant difference in phagocytic activity can be attributed to osmotic effects.

The average phagocytic activities determined in presence of 15 mg malonate per 1 ml blood-saline mixture and in 3 concentrations of saline, are 63%, 66%, and 61%. The corresponding average for phagocytic activity, in the absence of malonate, is 84% (column D_1). Data presented in columns A_2, B_2, and C_2 were obtained from phagocytic tests employing 5 mg malonate. Control values are shown in column D_2. Average percentages of phagocytic activity of experimental groups are 73%, 79%, and 74%, while control values average 89%. In the presence of 1 mg malonate (columns A_3, B_3, and C_3) average percentages are 85%, 88%, and 87%. Control values average 84%.

The data of Table I were subjected to statistical analysis, using the rank order test. Comparisons were made between all values obtained with each concentration of malonate regardless of strength of saline in which it was dissolved. All differences are highly significant (P values of 0.01 or less) except for data obtained with 1 mg of malonate and with controls, where the P value is greater than 0.05.

TABLE II. Phagocytic Activity of Rat Blood Neutrophiles Expressed as Percent of Cells Showing Ingestion of Bacteria. Each value was obtained with blood from a different rat.

	A		B	
	1	2	1	2
	1 mg malonate *in vitro*	Saline *in vitro*	8 inj. of 0.5 mg malonate per g body wt	8 inj. of saline
	%			
	85	84	83	89
	73	88	90	—
	84	—	86	—
	77	91	84	95
	84	—	87	—
	92	81	95	—
			81	69
			84	79
			56	85
Avg	83	86	83	83

Phagocytic measurements with rat neutrophiles. Phagocytic activities of rat neutrophiles are presented in Table II. In section A, the procedure employed for tests with human blood was duplicated. Malonate was added to give concentration of 1 mg malonate per ml. Activity of malonate treated blood averages 83%, the control averages 86%. In agreement with tests on human blood, 1 mg malonate fails to alter the activity of blood phagocytes. For section B of Table II, blood was drawn from rats that had previously received 8 injections of malonate. Both experimental and control values average 83%.

Bactericidal activity of blood serum. Table III summarizes experiments designed to determine the bactericidal effect of rat serum. In section A the average bacterial counts of 3 experiments employing normal serum are re-

TABLE III. Number of Viable *Salmonella typhimurium* Present in 3 ml Serum at Times Indicated following Addition of Bacterial Suspension. Section A—normal serum. Section B—serum from rats previously given 2 hourly inj. of 0.5 mg malonate/g body wt. Section C—serum after addition of 3 mg zymosan/ml.

	No. of bacteria/3 ml serum		
	A	B	C
	Avg counts of		
	3 exp.	2 exp.	3 exp.
Hr	Control	Malonate	Zymosan
0	11,111	12,500	12,444
1	2,487	2,200	19,817
2	4,123	3,835	79,333
3	4,267	3,800	255,000
4	5,087	3,900	796,667
5	8,900	5,838	2,076,667

corded. In 1 hour, approximately eight-tenths of the bacteria were destroyed. A slow increase in number is noted between 1 and 4 hours, this increase being more rapid after 4 hours.

The effect of malonate injections on the bactericidal properties of serum is shown in section B. These results are similar to those of control experiments.

Section C gives average bacterial counts from 3 experiments employing addition of zymosan to rat blood serum. An exponential increase in bacterial numbers was obtained throughout the period of observation, indicating absence of any effective antimicrobial property.

Discussion. On the basis of these results, it seems permissible to conclude that malonate, at concentration of 1 mg/ml phagocytic mixture, fails to alter the phagocytic capacity of human or rat blood neutrophiles. At concentrations of 5 and 15 mg malonate/ml phagocytic mixture, an increasingly significant inhibition is observed with human neutrophiles. Taking dosage into consideration, rate of absorption from the peritoneal cavity and rate of excretion of malonate, the concentration of 1 mg malonate/ml phagocytic mixture would seem most closely to approximate conditions present when 8 injections of 0.5 malonate/1 g body weight are administered. With these injections, the capacity of phagocytes to ingest bacteria is not impaired but survival time of rats injected with *S. typhimurium* is significantly reduced. Phagocytic cells must destroy ingested bacteria, if their role in defense is to be fulfilled, but our experiments offer no evidence of the effectiveness of this important process. Only the initial step in cellular defense is evaluated and that appears unimpaired by malonate in agreement with earlier observations on absence of effect of malonate on uptake of thorotrast by liver, spleen, and lungs of mice(7).

Bactericidal tests with rat serum imply that decreased survival time cannot be correlated with impairment of this function. The destruction and reproduction of bacteria in serum from animals treated with malonate is similar to that from control animals. That all the *S. typhimurium* are not killed by the serum and subsequent multiplication ensues, indicates that the strain of bacteria employed is relatively resistant. The only evidence that the observed bactericidal activity of serum is due to properdin is loss of antimicrobial action that accompanies addition of zymosan. According to Pillemer, *et al.*(11), zymosan inactivates the properdin system.

Summary. Our data fail to implicate an alteration in capacity of blood neutrophiles to ingest bacteria or in the bactericidal property of blood serum as a possible explanation for the mechanism by means of which malonate injections reduce survival time of experimentally infected rats.

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Immersion Hypothermia: Effect of Glycine. (22464)

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Factors responsible for and methods of preventing ventricular fibrillation during hypothermia have been vigorously pursued of late. The anti-arrythmic effects of many pharmacologic agents have been investigated(1,2), but little attention has been paid to the use of metabolic stimulants in hypothermia. Such substances may not directly prevent the onset of ventricular fibrillation, but they may prolong the time required to approach a lethal hypothermic level by increasing heat production within the body. Thus the survival chances of individuals accidentally exposed to hypothermic conditions would be greatly enhanced. An effective metabolic stimulant would also be of therapeutic value during the rewarming process by its ability to aid in rapid reattainment of normal body temperature.

Proteins are known to exert a definite thermogenic effect due to high specific dynamic action of certain amino acid constituents, principally phenylalanine, tyrosine, and glycine(3). The practical applicability of this phenomenon was demonstrated by Gubner, DiPalma, and Moore in patients with peripheral vascular diseases(4). These workers observed that oral ingestion of glycine induced a marked rise in oxygen consumption, heat production, and blood flow. It therefore appeared that glycine might be able to alter rate of cooling and rewarming during and following exposure to acute immersion hypothermia. This present study was executed in an effort to demonstrate a possible thermogenic effort of glycine at low body temperatures.

Methods. Twenty experiments were carried out on 10 apparently healthy mongrel dogs of both sexes, ranging from 10-26 kg. All dogs were anesthetized with 30 mg/kg of intravenous pentobarbital and then immersed in iced water bath of 8°C in the manner described by Hegnauer, *et al.*(5). Shivering was inhibited by appropriate administration of 50-100 mg of pentobarbital. Animals were cooled to rectal temperature of 28°C at which point the cold water was drained. They were exposed then to room air until such time as the rectal temperature fell to 26°C. Rewarming was instituted by immersion in warm water bath of 42-44°C. Rectal, bath, and room temperatures in °C were recorded continuously by means of a Leeds-Northrup iron-constantan potentiometer. During cooling and rewarming the following measurements were made. Oxygen consumption was determined at 35-33°C, 30-28°C, 28-26°C, and during rewarming at 30°C and 35°C. Expired air was collected in a Tissot spirometer and oxygen content measured with Beckman oxygen analyzer. Heat production in Cal./kg/hr was then calculated from oxygen consumption data by the method of Weir(6). Carbon dioxide in expired air was determined with a Haldane-Guthrie gas analyzer and blood sugar levels were measured prior to cooling and at a rectal temperature of 26°C. Continuous electrocardiograms (lead 2) were recorded throughout the experimental procedure. Each dog was rendered hypothermic on 2 separate occasions, separated by 2 to 5 days. On first exposure 5 dogs received 500 cc of 5% glycine solution intravenously, the remaining animals received 500 cc of 5% glucose solution. On the second exposure the so-