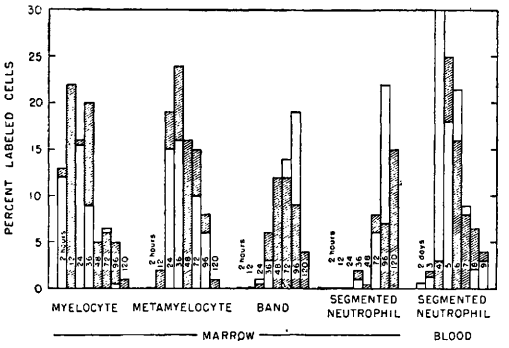

FIG. 2. Time relationship between inj. of tritiated thymidine and appearance of label in the various cells of the neutrophil series. (Hatched bars, male beagle; open bars, female beagle.)

during aspiration, which influences the percentage of labeled segmented neutrophils in marrow. When this factor is considered, it is clear that the true peak in marrow occurs about a day earlier than in the periphery. A similar sequence of segmented neutrophils in marrow and blood has been seen in the mouse with C^{14}-labeled adenine(5). It is of interest to note that labeled myelocytes may be detectable in marrow for several days. It can be inferred from this that some labeled neutrophils are released to the circulation for about an equivalent length of time, although in decreasing numbers. This conclusion is supported by an apparent decrease in grain counts of the peripheral neutrophils with time. The overall sequence of events is depicted in Fig. 2. Comparable results were obtained in both dogs.

There is, in general, a reasonable correspondence between these results and the indirect approximations of neutrophil balance reported previously(6,7). It would appear from these data that the time for differentiation of a myelocyte to a segmented neutrophil in the dog is between 2 and 3 days. Another 2 to 3 days are spent in the segmented form. More detailed counting and more frequent sampling is necessary, however, to establish the precise chronology of neutrophil maturation and the life cycle of the proliferating elements.

Summary. The pattern of neutrophilic granulocyte development has been studied in 2 dogs by high resolution radioautography with tritiated thymidine. Two to 3 days are required for differentiation from the myelocyte to the segmented neutrophil. The half time for disappearance of the latter in the periphery appears to be of the order of 2 days.

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Effectiveness of Gluconate, Chloride, and Other Sodium Solutions in Treatment of Experimental Burn Shock.* (24191)

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There is adequate experimental(1,2,3) and clinical(4,5,6,7) evidence for the effectiveness of oral sodium solutions in prevention and treatment of burn shock. For other types of shock the experimental evidence(8,9,10,11) is just as good, but clinical confirmation is meager. There is relatively little evidence on

* Dr. R. Carl Millican and Dr. Sanford M. Rosenthal of N.I.H. made their laboratory available for preliminary experiments. Dr. Stanley Levenson, Walter Reed Army Inst. of Research, furnished encouragement and criticism.

optimum electrolyte composition of solutions for oral use. In particular, there has been no systematic approach to the design of an oral sodium solution which will offer a great improvement in palatability with minimal, if any, loss of effectiveness. Palatability of currently employed solutions has been no great problem in hospital use, but it may be of major importance in treatment of burns *en masse*. Acceptability of iced solution in hospital environment might be expected to be better than acceptability of the same solution in tepid water on battlefield or in temporary facilities of a disaster-stricken city. Our study approached the determination of an effective, but more palatable, oral sodium solution by comparing effectiveness of solutions of a number of sodium salts, singly and in various combinations; and by appraising the influence on effectiveness of various ancillary measures for improving palatability.

Methods. Mice were subjected to standard thermal injury, and were treated with various sodium solutions. Mortality measured at 24 hours was chosen as criterion of effectiveness of the solutions, after recommendations of Hamilton *et al.*(12), Rosenthal and Millican (13), and Allen(14). The 24 hour point appears to offer a separation between early deaths, due to shock, and late deaths, due to other factors(15). The anions chosen for study included chloride, because it is the experimental standard; bicarbonate, lactate, citrate, and acetate, because they have been used clinically; gluconate, because it is quite palatable;† and succinate, in spite of its impalatability, because it has been used experimentally with recognition of its influence on respiration of damaged tissue(16,17). Solutions were administered in 2 doses; the first given orally, the second either orally or intraperitoneally. Intraperitoneal injection was selected for the second dose as a matter of convenience. Rosenthal(1) and Millican *et al.*(18) found no difference between the effect of oral and intraperitoneal administration. After preliminary experiments, a total dosage of 18% of body weight was selected. This volume approaches the tolerance of animals for rapid oral administration, and a large dose was desired to increase the probability of detecting any deleterious effect from excessive dosage of anions. Female albino mice were lightly anesthetized with ether and immersed to the axilla in water at 70°C. The hair was not clipped, current practice in Rosenthal's laboratory. The mice were of 2 different strains and weights: 1500 were W. R. Bagg mice of 12-14 g; 1215 were CWL mice of 14-18 g. In individual experiments all mice were of same strain, weight, and nutritional status. Period of immersion was adjusted to strain and weight of animals to obtain in untreated animals a 24 hour mortality of 80% to <100%. All W. R. Bagg mice were immersed for 7 seconds. CWL mice were immersed 8 seconds or 8.5 seconds; 0.5 second was added to immersion time for groups of mice which appeared unusually vigorous and active. In 2 experiments (A, Tables I and II) mortality of untreated animals fell outside the range 80% to <100%. These experiments were repeated (B, Tables I and II). For experiments involving W. R. Bagg mice environmental temperature was 23.5°C ± 1.5°; for CWL mice, 22.8°C ± 0.6°. Immediately after immersion, the first dose was administered by gavage. The second dose followed at 2 hours. With 2 exceptions, all solutions contained sodium as the only cation, and in concentration of 140 meq/l. The Lactated Ringer's Solution was the USP product, and one of the multiple ion formulations contained 145 meq/l of sodium, the additional 5 meq/l being in a flavoring agent. Confidence limits (95%) of percentage mortality were computed by the formula:

$$C.L. = p \pm \left(t_{(\infty)} \times \sqrt{\frac{pq}{n}} \right).$$

Probability (p) figures for validity of differences in mortality were obtained from standard t tables, with t computed by the formula:

† Dr. Joseph M. White and Miss R. Millard called my attention to the palatability of sodium gluconate.

$$t_x = \frac{p_1 - p_2}{\sqrt{\frac{p_1 q_1}{n_1} + \frac{p_2 q_2}{n_2}}}.$$

All p values quoted are for mathematically independent comparisons of data involving the same immersion times, and animals of same strain, weight, and nutritional status.

Results. Standard solutions. Following treatment with sodium chloride, Lactated Ringer's Solution, and the customary (6,7,19) chloride-bicarbonate mixture, mortality was approximately the same (Table III). Mortality following treatment with sodium chloride is much higher than that reported by Millican *et al.* (2) in similar, but not exactly comparable, experiments. "However, the significant criterion is the deviation from control which occurs as a result of treatment . . ." (20).

TABLE I. Mortality following Treatment with Lactate and Chloride as Related to Untreated Mortality.

Solution*						
Chloride	None	140	105	70	35	
Lactate	None		35	70	105	140
Exp. A						
†	(.70)	(.23)	.33	.33	.13	.33
n	(30)	(30)	30	30	30	30
Exp. B						
†	(.97)	(.55)	.73	.43	.67	.77
n	30	30	30	30	30	30‡

Strain, W. R. Bagg; wt, 12-14 g; immersion, 7 sec.; method of admin., 6% body wt by gavage, 12% intraper.

( ) = Data utilized in Table III.
n = No. of animals.
* Figures = Anions in meq/l.
† Mortality at 24 hr.
‡ Tetany observed.

TABLE III. Mortality following Treatment with Standard Solutions.

Solution*	None	Cl 140	Cl 93 HCO_3 47	Lactated Ringer's
†	.84	.28	.24	.31
.95 C.L.	± .033	± .041	± .039	± .042
n	120	120	120	120

Strain, W. R. Bagg; wt, 12-14 g; immersion, 7 sec.; method of admin., 6% body wt by gavage, 12% intraper.

n = No. of animals.
.95 C. L. = 95% confidence limits.
* Figures = Anions in meq/l.
† Mortality at 24 hr.

Single anions in concentration of 140 meq/l. Under our conditions, some anions (lactate, acetate, bicarbonate, citrate) are far less effective than chloride in lowering mortality below that of the control (untreated) group (Table IV). That this lack of effectiveness may be the result of active toxicity is suggested by appearance of tetany in some groups of animals treated with these anions alone or in high concentration with chloride (Tables IV, V). Tetany was never seen in animals treated with gluconate, and only rarely with succinate. The number of animals used in simultaneous comparison, presented in Table IV, is too small to give validity to differences between lactate, acetate, bicarbonate, and citrate, which showed a mortality range of 77-97%. Succinate was significantly more effective than lactate ($p < .02$). In this experiment no valid difference

TABLE II. Mortality following Treatment with Chloride and Gluconate if Injury Is Equal to or Greater Than LD_{100}.

Solution*										
Chloride	None	140	122.5	105	87.5	70	52.5	35	17.5	
Gluconate	None		17.5	35	52.5	70	87.5	105	122.5	140
Exp. A										
†	1.00	.27	.47	.20	.67	.60	.73	.67	.93	.93
n	30	15	15	15	15	15	15	15	15	15
Exp. B										
†	.93	.13	.07	.13	.07	.13	.07	.07	.13	.47
n	15	15	15	15	15	15	15	15	15	15

Strain, CWL; wt, 14-18 g; immersion: A, 8.5 sec.; B, 8 sec.; method of admin., 6% body wt by gavage, 12% intraper.

* Figures = Anions in meq/l. † Mortality at 24 hr. n = No. of animals.

TABLE IV. Mortality following Treatment with Various Anions, 140 meq/l.

Solution	None	Cl	Gluc	Succ	Lact	Acet	HCO_3	Citr	Lactated Ringer's
†	(.93)	(.27)	.40	.47	.77	.77	.87	.97	(.40)
n	(30)	(30)	30	30*	30*	30*	30	30	(30)

Strain, W. R. Bagg; wt, 12-14 g; immersion, 7 sec.; method of admin., 6% of body wt by gavage, 12% intraper. ( ) = Data utilized in Table III.
n = No. of animals.
* Tetany observed. † Mortality at 24 hr.

between gluconate and chloride is demonstrated. However, when total experience with 140 meq/l gluconate solution is compared with experience with 140 meq/l chloride solution, the lesser effectiveness of gluconate is more striking: Cl 23% (n = 90), Gluc 51% (n = 90), no treatment 93% (n = 105); Cl *vs.* Gluc $p<.001$.

Mixtures of chloride and other anions. In spite of the relative ineffectiveness of non-chloride anions of sodium when used alone, the results from mixtures of these anions with chloride (Table V) indicate that potency of chloride is generally retained even when it is diluted half and half with a non-chloride anion. The data on gluconate-chloride mixtures (Tables II, V) indicate that even three-quarters of the chloride in a 150 meq/l solution might be replaced with gluconate without pronounced loss of effectiveness.

Effect of citrate, and free citric acid in mixtures. It was anticipated that citrate or free citric acid or both might prove deleterious on parenteral injection. Consequently, tests for effectiveness of some formulations containing citrate and citric acid included both oral and intraperitoneal administration. Citrate in concentration of 35 meq/l may be deleterious on intraperitoneal injection, but the mortality data are not conclusive: both doses oral, 20% (n = 30); second dose intraperitoneal, 63% (n = 75). However, these are composite figures from several different experiments. A single paired experiment did not demonstrate a significant difference (oral 40%, intraperitoneal 47%). It is clear that

TABLE V. Composite Mortality following Treatment with Graded Proportions of Chloride and Other Anions.

Solution		None							Lactated Ringer's
Chloride*			140	105	93	70	35		109
Non-chloride anion*				35	47	70	105	140	28
—		.91	.36						
		255	270						
Gluc	†			.23		.31	.30	.49	
	n			60		75	60	75	
Succ	†			.53	.50	.33	.43	.55	
	n			60	30	30	30	60‡	
Lact	†			.53	.20	.30	.31	.62	.31
	n			60	30	90	45	90‡	120
Acet	†			.40		.50	.51	.77	
	n			30		60	45	30‡	
HCO_3	†			.37	.29	.43	.53	.87	
	n			60	90	30	15‡	60‡	
Citr	†			.63		.60	.67	.97	
	n			75		30	15‡	30	

Data not suitable for independent mathematical comparisons. Composite results with W. R. Bagg 13 g mice immersed 7 sec., and CWL 16 g mice immersed 8 and 8.5 sec. Method of admin., 6% body wt by gavage, 12% intraper.
n = No. of animals.
* Figures = Anions in meq/l. † Mortality at 24 hr. ‡ Tetany observed.

TABLE VI. Mortality following Treatment with Solutions Containing Free Citric Acid.

Solution*	None	Cl 140	Cl 68, Glue 46, Citr 26					
Free citric acid, g/l		0	0	1.5	3.0	3.0	4.5	4.5
Method of admin.		P	P	P	P	O	P	O
†	.98	.40	.47	.53	.60	.27	.97	.42
n	60	60	15	15	15	15	60	135

Strain, CWL; wt, 14-18 g; immersion, 8.5 sec.
P = 6% of body wt at 0 hr by gavage; 12% of body wt at 2 hr, intraper. O = 9% of body wt at 0 hr by gavage; 9% of body wt at 2 hr by gavage. n = No. of animals.
* Figures = Anions in meq/l. † Mortality at 24 hr.

citrate is tolerated well at 35 meq/l and less on oral administration (Tables VI, VII). Free citric acid, 4.5 g/l, definitely increases mortality when given intraperitoneally (Table VI). Addition of a sweetening agent, and effervescence in the solution, do not appear to impair effectiveness after oral administration (Table VII).

Discussion. From a pharmaceutical standpoint, it is very difficult to alter, conceal, or compensate for the taste of chloride in concentrations of 90 meq/l or higher. It would be a great gain in palatability if the chloride component of oral sodium solutions could be reduced below this level. Such appears feasible, from the point of view of burn shock prevention and treatment, with the use of several non-chloride ions (acetate, citrate, lactate, gluconate), which in themselves are quite palatable. Of these, the safest appears to be gluconate, since it is the most effective in 140 meq/l solution, and has not shown a toxic effect under our conditions.

Additional gains in palatability can be obtained by effervescence with CO_2, free citric acid, and a sweetening agent.‡ To date, the best compromise I have found between effectiveness in burned mice and subjective palatability to the author's family and neighbors is:§

Sodium chloride	2.0 g
" gluconate	17.4
" bicarbonate	2.1
Citric acid, anhydrous	4.8
Sodium cyclohexylsulfamate	1.0

In one liter of water the solution offers a pleasant taste, an attractive fizz, and the following ionic composition:

Sodium	145 meq/l	
Chloride	35	
Gluconate	80	
Bicarbonate		(converted to citrate)
Citrate	25	
Citric acid	47	
Cyclohexylsulfamate	5	

This solution is comparable in effectiveness to sodium chloride in prevention and treatment of experimental burn shock in mice under our conditions (Table VII; Cl 42% *vs.* USG-20 39%, p = .7). If used in humans in dosage of 15-18% of body weight, the solution would supply approximately 1000 calories. The proportion of normal caloric requirement supplied to mice was considered negligible (2.5%). A solution containing a larger proportion of chloride (Table VI) might stand on better physiological grounds.

Conclusions. 1) Chloride appears to be the most important anion in oral sodium solutions used for prevention and treatment of experimental burn shock in mice. 2) Citrate, ace-

TABLE VII. Mortality following Treatment with Solution Containing Effervescence, Sweetening Agent, and Citric Acid.

Solution*	None	Cl 140	Cl 35, Glue 80, Citr 25‡	
Method of admin.		P	P	O
†	.91	.42	.70	.39
n	45	60	30	120

Strain, CWL; wt, 14-18 g; immersion, 8.5 sec.
P = 6% of body wt at 0 hr by gavage; 12% of body wt at 2 hr, intraper. O = 9% of body wt at 0 hr by gavage; 9% of body wt at 2 hr by gavage.
n = No. of animals.
* Figures = Anions in meq/l.
† Mortality at 24 hr.
‡ Plus free citric acid, 3.0 g/l, and sodium cyclohexylsulfamate, 5 meq/l; effervescent — citrate formed by interaction of citric acid and $NaHCO_3$.

‡ All such preparations were prepared through the courtesy of Mr. A. W. Taff, Emerson Drug Co.
§ Formulation USG-20, Emerson Drug Co.

tate, bicarbonate, and lactate are far less effective than chloride, and may be toxic in large doses. 3) There is no sanctity in the customary 93:47 or higher meq ratio between chloride and other ions in oral electrolyte solutions used in these experiments. 4) Gluconate appears to serve as a partial substitute for chloride, and it lends palatability; experimental and clinical studies are indicated on gluconate metabolism in man.

This article is not to be construed as reflecting the position of the U. S. Army or any element thereof. The author alone is responsible for data presented and conclusions drawn.

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Reversal of Respiratory Decline in Necrotic Liver Degeneration by Intraportal Antioxidants.[1] (24192)

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A specific metabolic lesion, respiratory decline, has been demonstrated in liver slices from rats maintained on a diet producing necrotic liver degeneration(1,2). Normal-appearing slices of such livers are unable to maintain respiration in the Warburg apparatus after initially normal O_2 consumption for the first half hour. The defect is characteristic for the latent period of the disease; it precedes the acute pathological lesion by several weeks. Respiratory decline is prevented by feeding of those factors which protect against liver necrosis, *i.e.*, cystine†. Vit. E. and Factor 3. The lesion is reversed within minutes after injection of Vit. E into the portal vein (3)‡, but not after that of Factor 3§. Pre-

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* These studies were performed during tenure of Brewer's Yeast Council Research Fellowship.

† Since submission of this paper it has been shown that Factor 3 is an organic selenium compound (Schwarz, K., Foltz, C. M., *J. Am. Chem. Soc.*, 1957, v79, 3292). The protective effect of L-cystine is caused by a trace contamination with Factor 3-active selenium.

‡ *In vitro* addition of α-tocopherol to deficient liver slices, either as an emulsion or in water-soluble forms, has no significant effect on metabolic lesion.

§ Relation of Factor 3-active selenium compounds to respiratory decline will be the subject of a separate report.