amounts to only 0.1 g or less. In feeding diets *ad libitum* we employ weighed amounts of food about 10% in excess of the expected consumption. The feeders may be constructed with varying numbers of compartments but when the food intake is being restricted below the *ad libitum* level, they should at least equal the number of mice in the cage. The compartment feature gives reasonable assurance that all of the mice in a group have an equal chance to feed when the food intake is restricted. In this regard, the practice[1] of dividing the animals in any group into smaller groups of narrower weight ranges is useful. To prevent spillage and contamination of the food with excreta the compartments must allow the entry of only the fore part of the animal; they should not accommodate the whole animal. The use of similar feeders, with compartments and wire mesh of the appropriate size, would appear entirely feasible for weanling mice or for rats of varying size.

[1] Tannenbaum, A., *Cancer Research*, 1945, **5**, 609.

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The Effect of Exercise on Mortality of Animals Poisoned with Diphosgene.

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Absolute rest for victims of pulmonary irritants has until recently been the rule of thumb. The clinical consensus against exercise is clearly represented in the directions for first aid treatment of lung irritant casualties given in a U. S. War Department Field Manual dated September 7, 1942:[1]

"Patients suspected of having breathed lung irritant gas should be given absolute rest. All casualties known or suspected of having been seriously exposed should be considered as litter cases . . . when he has been carried to a place free of gas, the gas mask is removed, his clothing loosened and the man kept completely still, relaxed and warm with blankets."

Strict adherence to these regulations precludes any attempt by the gassed soldier to make his way to an aid station during the interval between exposure and the appearance of pulmonary edema. The present study was designed to test the validity of prohibiting exercise during the clinical latent period. Similar work has been done independently and at about the same time by I. de Burgh Daly and co-workers, who found that exercise on an endless carpet had no injurious effect on rats[2] and dogs[3] after exposure to L Ct-50 concentrations of diphosgene or on dogs gassed with phosgene.

Preliminary Experiments. Swimming was used as a controllable and relatively constant form of vigorous exercise requiring virtually no apparatus for mice, rats or dogs. Its disadvantage, temporary change to a water environment, was controlled by experiments testing the effects of wetting and temperature changes immediately after gassing.

While normal mice tolerated prolonged wetting (standing in a shallow pan of water for 42 hours) with water and air at 29°C, but not at 17°C, gassed mice did poorly under prolonged wet conditions both at 28°C and at 15°C (Table I). There are no figures for "dry cold" survival of normal mice but

* This work was done as part of a contract, recommended by the Committee on Medical Research, between the Office of Scientific Research and Development and the University of Chicago.

[1] *Defence Against Chemical Attack*, U. S. Government Printing Office, Washington, D.C., September 7, 1942, p. 28.

[2] Daly, I. de Burgh, and Ritchie, A. O., personal communication.

[3] Daly, I. de Burgh, Fegler, J., and Ritchie, A. O., personal communication.

TABLE I.
Influence of Wetting and Temperature on Normal and Diphosgene Gassed Mice.

Gas exposure		Condition*				
Mg/L.	Min.	(temp. °C)	No. of mice	% alive at 36 hr	X^2	P
Normal controls		42 hr wet at 29°C	20	100	10.80	< .01
		42 " " " 17	20	50		
0.45	10	42 " dry " 28	25	70	9.80	< .01
		42 " " " 15	25	20		
		42 " wet " 28	25	0		
		42 " " " 15	25	0		
0.43	10	1 " dry " 30	20	20	0.322	>7.50
		1 " " " 14	20	30		
		1 " wet " 30	20	25		
		1 " " " 14	20	15		

* Low temperatures (14°-17°C) maintained in constant temperature room.

TABLE II.
Influence of Swimming on % Survival of Gassed Mice (7 experiments, 420 animals).

	% surviving at 36 hr						
Gas conc'n (mg/L):	0.33	0.40	0.42	0.42	0.46	0.46	0.49
Dry control	70	45	30	40	20	15	0
Shallow water control	45	65	—	20	—	20	0
Moderate early swim	65	70	50	60	35	—	10
Heavy early swim	30	65	—	—	—	10	—
Swim at 1.5 hr	—	—	—	15	—	—	—
Swim at 3.0 hr	—	—	—	20	—	5	—
X^2	6.77	3.22	1.66	9.13	1.14	1.07	4.17
X^2 at P = 5%	7.81	7.81	3.84	9.49	3.84	7.81	5.99

TABLE III.
Pooled % Survival of Gassed Mice in 5 Treatment Classes.

Treatment class	No. of groups	No. of mice	Mean % survival
Dry controls	7	135	31.4
Shallow water controls	5	79	30.0
Moderate early swim	6	115	48.3
Heavy early swim	3	53	35.0
Late exercise	3	48	13.3

$$F \frac{\text{between means}}{\text{within classes}} = 1.27;\ n_1 = 4,\ n_2 = 19$$

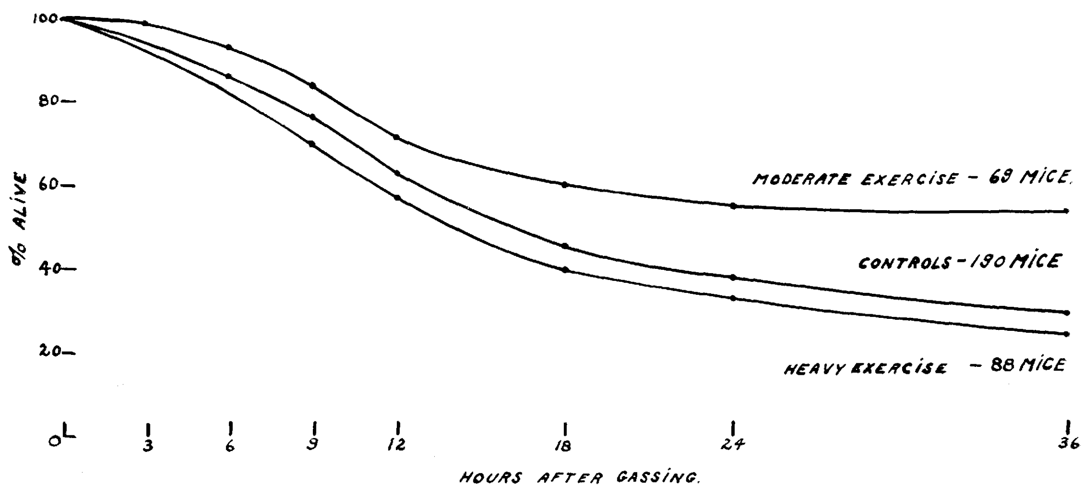
$$F \text{ at } 5\% = 2.89$$

gassed mice kept dry at 15° showed a mortality more than twice as great as a comparable group at 28°C. Reducing the exposure to wet and cold to one hour, beginning half an hour after gassing, eliminated their effects; mortality of gassed mice then being the same under dry cold, wet cold, dry warm or wet warm conditions (Table I). Experiments were therefore planned with a swimming period of not longer than an hour at a water temperature between 25°C and 30°C and with the animals dried afterwards. Floats were placed in the water from time to time to give mice and rats short rests. All diphosgene exposures for mice were within a range of 0.33 to 0.49 mg/L for 10 minutes, resulting in a mortality of from 25 to 80% at 36 hours.

Results of Exercise Experiments. A. Mice. Four hundred and twenty mice were exposed to diphosgene in 7 experiments. In each, the mice were divided into a dry control

FIG. 1.

SURVIVAL OF MICE EXERCISED AFTER GASSING.



group of from 10 to 25 mice and one or more (up to 5) experimental groups containing 9 to 25 animals. In 5 of the experiments a second control group was kept standing in a pan of shallow water during the swimming period of exercising groups. The results of these experiments, expressed as per cent surviving at 36 hours for the individual groups, are shown in Tables II and III and Fig. 1. Moderate exercise consisted of a 20- to 40-minute swim with one or two 5-minute rests; heavy exercise, a one-hour swim with 3 evenly spaced 5-minute rests. This latter taxed animals to the limit; without rest periods, they would have drowned. Exercise was begun within the first half hour after the end of gassing except, as indicated in the last 2 rows of Table II, when 40- to 60-minute swims were begun at 1½ and 3 hours after gassing to test the effect of later exertion.

It is apparent from Table II that the populations of each of the 7 experiments are homogeneous. Differences between the mean survival percentages of the several treatment classes (Table III) are also not significant, as shown by analysis of variance. There is, however, a significant difference between the mean of moderate early and late exercise classes considered apart from the other treatment classes ($F = 6.47$; $n_1 = 1$; $n_2 = 7$). This difference is due either to the inconclusiveness of the 5% point in statistical analysis or to inadequate sampling. If the latter, more experiments might conclusively demonstrate a beneficial effect of moderate, early, postgassing exercise (Table II and Fig. 1) or a deleterious effect of late exercise, or both. In any event, heavy or moderate early exercise, as defined in these experiments, was noninjurious to mice gassed at the levels indicated.

B. Rats. Forty rats were exposed to a 0.52 mg/L concentration of diphosgene for 10 minutes. Twenty were dry controls and 20 swam immediately after gassing, for 20

TABLE IV.
Influence of Swimming on Gassed Dog Survival Time.

Treatment	No.	Mean survival time (hr)	$\frac{\sigma}{\sqrt{n}}$
A. Controls	15	14.1	2.31
B. Moderate swim	6	18.0	4.41
C. Heavy swim	8	9.9	1.17

Significance of Differences Between Means

Groups	Observed t	t at 5% P
AB	0.78	2.09
AC	1.62	2.08
BC	1.77	2.18

TABLE V.
Terminal Lung-weight Body-weight Ratios of Control and Exercised Gassed Dogs.

Treatment	No.	Mean ratio lung wt/body wt %	Mean survival time (hr)	Mean ratio / Mean survival
A. Controls	15	3.82	14.1	2.71
B. Moderate swim	5	4.42	15.6	2.83
C. Heavy swim	8	4.80	9.9	4.84

Significance of Differences Between Mean Ratios.

Groups	Observed F	F at 5%
ABC(N_1 = 2, N_2 = 25	3.06	3.38
AC(N_1 = 1, N_2 = 20	5.88	4.35
AB(N_1 = 1, N_2 = 18	1.72	4.41

minutes with rests. Control mortality was 80%; swimmer mortality 65%. The 15% difference is statistically not significant ($X^2 = 0.502$), but again is in the direction of the beneficial effect suggested above.

C. Dogs. 1. Survival Time. Thirty dogs were exposed to 0.52 to 0.69 mg/L concentrations of diphosgene for 30 minutes, in groups of 6. Three control dogs from each of the 5 groups were wetted during the swimming periods of the exercise dogs. Moderate exercise was a 20-minute swim; severe exercise lasted 40 minutes. One 40-minute swimmer was completely overcome and 2 of the 20-minute swimmers were so exhausted as to be more properly classed with heavy exercises, leaving overall totals of 15 controls, 6 moderately exercised and 8 severely exercised animals. All but 4 dogs died within 30 hours after gassing. Of the 4 exceptions, one lived indefinitely and the other 3 from 43 to 105 hours. In the calculation of mean survival times these 4 dogs were treated as 30-hour survivors, deaths after about 30 hours usually being due to secondary causes.

The mean survival times of the 3 groups are shown in Table IV. The absence of a statistically significant difference between any 2 means indicates the indifferent influence of exercise on survival time.

2. Degree of Pulmonary Edema at Death. Estimates of the extent of pulmonary edema at death on the basis of lung-weight body-weight ratios, were homogeneously distributed, considering the whole dog population (Table V). There was, however, a statistically significant difference between the mean ratios of the controls and severely exercised animals considered alone; none between controls and moderate swimmers or between heavy and moderate swimmers. Considering average rate of lung water deposition (lung-weight body-weight ratio divided by survival time) suggests that the greater terminal ratio of moderate exercisers as opposed to controls, if shown to be significant, would probably be due to a longer mean survival time. Severe exercise, on the other hand, definitely favors increased water deposition. The latter may result from inability of the dogs to absorb whatever water they may aspirate during the swimming period, but one would rather suspect actual aggravation of the edema.

Conclusions. It is clear that the worst that can be said of swimming shortly after diphosgene gassing at mid- or somewhat above mid-lethal doses is that it had no influence on final mortality among mice and rats or on survival time in dogs. There was, in fact, some indication, especially from the finding of a significant difference between survival of moderate early and late exercised mice, that moderate early swimming was beneficial or late swimming deleterious or both. It is striking that in all of the pooled data (see tables), and very often in individual experiments, differences noted, though not statistically significant, are consistently in the direction of increased survival for moderate early exercise in all 3 species, and in the direction of increased mortality for moderate or heavy late exercise in mice and heavy early exercise in dogs.

The dog survival times and lung weights

suggest that "moderate" and "heavy" exercise as distinguished in these experiments are physiologically as well as quantitatively, of different orders of magnitude. Similarly, in the mouse experiments there is no indication of any effect, beneficial or deleterious, of heavy early swimming, as opposed to the indicated beneficial influence of moderate exercise.

Swimming seems a justifiable test of the effects of more natural types of exertion. The control experiments on mice show that the indifferent role of swimming or the perhaps beneficial effect of moderate early swimming does not represent an overcompensation of harmful exercise by a protective action of immersion in tepid water. If anything, swimming, as already indicated, may be responsible for the apparent ill effect of heavy "exercise" in dogs judged by the significantly greater pulmonary edema of heavy swimmers as opposed to controls. Furthermore, it may be valid to assume that, from the point of view of muscular, circulatory and respiratory mechanisms, swimming is at least as vigorous an activity as that envisaged for the soldier gassed at equivalent levels; we have here subjected exertion to a perhaps unduly rigorous test.

Finally, it may be pointed out that exercise by a diphosgene patient or other pulmonary irritant patient during the clinical latent period need not be expected *a priori* to be adding insult to injury. The water and blood demands of muscle during exercise could tend to delay the onset of edema. Once edema and attendant anoxaemia set in, however, increased oxygen demand becomes an undesirable feature of exercise. Since the time of onset of edema is variable, depending mainly on severity of exposure, which is in turn a function of more than just gas concentration and exposure duration, no particular time can be set as the limit of a safe period.

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Inositol Decholesterization in Old Hens.*†

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Inositol was found by Gavin and McHenry[1] to prevent the development of a special type of biotin fatty liver, but no such lipotropic activity was demonstrated against thiamin fatty livers. Abels *et al.*[2] found that inositol accounted for the lipotropic property of a lipocaic preparation when given to patients with gastrointestinal cancer. Inositol administered preoperatively, reduced the average concentration of fat to 46% of that of almost uniformly fatty infiltrated livers of untreated patients. Shay[3] could demonstrate no reduction in the size of fatty livers in diabetics nor any changes in the blood cholesterol by the daily administration of 1.2 g of inositol. However, Russakoff and Blumberg[4] reported reduction in the blood cholesterol and cure by inositol in doses of 1 g per day, in a patient with a dermatological condition associated with a disturbed fat metabolism.

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