physiological balance of growth medium may lead to imbalance of wall synthesis and cell lysis. Related effects may be occurring in those intracellular systems which prevent multiplication of Brucella, thereby rendering the bacteria more accessible to intracellular enzymes. The results of these experiments suggest that *in vitro* attempts to reconstitute intracellular conditions must take into account the necessity for establishing the proper physiological test environment; that death of cells may be a stepwise series of reactions; and that omission of any of the necessary components may result in failure to demonstrate the factors concerned. Operation of a mechanism similar to that produced by glycine in monocytes may be involved in natural resistance to infection. It is non-specific and perhaps dependent upon day-to-day variations in physiological levels of at least 2 agents.

Summary. A lysozyme-like material from rabbit monocytes is described. It acts on glycine-treated brucella to cause lysis and death. When parasitized monocytes containing this agent are treated with low concentrations of glycine, intracellular growth and yields of smooth *Brucella melitensis,* Rev Is is suppressed. The theory is proposed that intraphagocytic death involves a stepwise alteration of the wall, followed by enzymatic degradation of the mucopolysaccharide component responsible for structural integrity of the bacteria.

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Effect of Metabolic "Stress" on Development of Tumor Following Inoculation of Walker Carcinosarcoma Cells.* (25875)

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The effect of dietary deficiency in production of spontaneous tumors has been well described by Tannenbaum and Silverstone(1) who demonstrated that prolonged restricted caloric intake and malnutrition in laboratory animals will diminish induction and growth of spontaneous tumors. However, to our knowledge the influence of acute metabolic changes has not been investigated in relationship to increased susceptibility of rats to inoculation of transmissible malignant tumor cells. In this present investigation the effect of "acute" metabolic changes, such as starvation and dehydration, which can be considered a form of metabolic "stress," have been studied in relation to the animals' susceptibility to inoculation of Walker 256 carcinosarcoma.

Method. Female Holtzman white rats weighing between 215 and 230 g were divided into 5 groups and housed in the same room with identical diet (Purina lab-chow) for 3 weeks. They were weighed every day to be certain a similar weight gain was obtained in all animals before experiment was started. At end of this period, the groups were treated as follows: In *Group 1* water bottles were removed for 48 hours but "chow" feed was still available; in *Group 2* food was taken away

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TABLE I. Effects of Dehydration and Starvation on "Takes" of Walker 256 Carcinosarcoma in Rats.

	No. of rats*	% "Take"	Loss or gain in wt
1. Dehydration, 48 hr	43	77%	Loss, 39 g
Control	23	60%	Gain, 5 g
2. Complete starvation, 48 hr	75	89%	Loss, 35 g
Control	50	76%	Gain, 3 g
3. Starvation and dehydration, 48 hr	21	69%	Loss, 35 g
Control	21	71%	Gain, 3 g
4. Starvation, 7 days	44	80%	Loss, 50 g
Control	42	57%	Gain, 12 g

* 25,000 cells ("aged" 12 hr to reduce "takes" below 100%) inoculated subcut. over the abdomen —half of each group at commencement of experiment; the other half at end of deprivation. There was no significant difference between the 2 groups.

for 48 hours but water bottles were left; in *Group 3* animals were deprived of food and water for 48 hours; *Group 4* was kept without food for 7 days but water was still available. The *5th Group* was fed and watered in the normal way and used as control animals. A suspension of Walker 256 carcinosarcoma was made as described by Talalay *et al.*(2) and "aged" (Chan *et al.*) to diminish virulence of tumor suspension, and thus produce a lower percentage of "takes." A suspension containing 25,000 cells aged 12 hours was injected subcutaneously over the abdomens of half of each group at commencement of the period of experiment and the other half at the end of period of deprivation. Control groups of rats were also inoculated at the same time as experimental groups with the same tumor suspension and same number of cells. Following period of deprivation of food and water the rats were fed the identical diet given previous to experiment. Tumors developed in 14-21 days in rats in which "takes" occurred. The rats in which tumors did not develop were kept for 3 months to safeguard against late development of tumors, but none developed. The effect of "takes" in rats during growth periods was investigated in 5 groups of female Holtzman rats of ages 28 to 150 days, when they can be considered mature; all rats were inoculated at the same time with the same suspension of "aged" Walker 256 carcinosarcoma cells subcutaneously on their abdomens. The rats were then kept under same conditions and fed same diet. At end of 3 weeks number of "takes" was counted and rats in which no tumor developed were observed for 3 months.

Results. Table I indicates that acute dehydration and acute starvation of 48 hours duration did not influence the "take" of Walker cells inoculated subcutaneously.

However, following starvation for 7 days, 80% of 44 rats developed tumors, compared to 57% of 42 control rats ($\chi^2 = 5.007$). There was no mortality during period of starvation or dehydration. Weight charts were kept on each group of rats for 24 days before and 10 days after starvation or dehydration (Table I).

The "takes" of Walker tumor cells inoculated subcutaneously into rats of varying ages (Table II) show no difference in the 4 groups varying between 145 and 250 g in weight. However, the incidence of "takes" in the young group with average weight of 85 g was considerably higher than the mature rats (94% vs. 68 to 73% ($\chi^2 = 4.97$)).

Discussion. Starvation and dehydration produce a disturbance in metabolism which might be considered a metabolic "stress." Anabolism is replaced by catabolism and there is a mobilization of body's reserves. Neither starvation nor dehydration nor combination of both for 48 hours produced any increase in susceptibility of animal to subcutaneous inoculation of Walker tumor. However, following prolonged starvation for 7 days, when there had been a loss of one-fifth of body weight, there was an increase in per-

TABLE II. Effect of Growth and Weight Changes on "Takes" of Walker 256 Carcinosarcoma in Rats.

Age (days)	Wt (g)	No. of rats*	% "Takes"
28	85	19	94
60	145	21	71
80	160	19	73
110	210	18	66
Mature	250	22	68

* 25,000 cells "aged" 12 hr were inoculated on same day in 5 groups of rats, of different age groups, using same suspension.

centage "takes" of experimental animals over normal control group. This leads to the belief that following marked catabolic stress conditions in tissues of experimental animal are more receptive to the implanted malignant cell than tissues of normal healthy animal.

At first glance the increased resistance of Tannenbaum and Silverstone's animals with chronic malnutrition to development of spontaneous tumors might appear to be at variance with our data showing that acute starvation reduces resistance of the rat to inoculated Walker 256 cells. However, in our estimation there is a marked difference in metabolic status of the 2 groups of animals. We believe that prolonged complete starvation (for 7 days) acts as a severe body "stress" whereas chronic malnutrition does not act as a stress, although the "soil" in malnourished animal's tissue is depleted or changed sufficiently to decrease incidence of formation of spontaneous tumors.

Our findings tend to support those of Buinauskas *et al.*(3) revealing increased susceptibility of animals having the stress of an operation (celiotomy) to inoculated Walker 256 cells.

Summary. The effect of acute starvation and dehydration in rats, on "takes" of Walker 256 cells following their injection subcutaneously has been investigated. Our results indicate that starvation and dehydration for 48 hours do not increase susceptibility of the rat to subcutaneous inoculation of Walker tumor, but if starvation is continued for 7 days there is increase in percentage "takes" of the tumor as compared to incidence in normal healthy animals. There is also an increase in "takes" of implanted tumor in young rats 28 days of age compared to more mature rats.

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Effects of Respiratory Inhibitors on Glucose and Protein Utilization and Growth in Strain L Cells.* (25876)

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Azide, urethan, arsenite and fluoride are well known respiratory inhibitors and have been used as such in innumerable investigations. Except for the work of Cailleau *et al.* (2) on azide, there have been no reports on effects of these substances on strain L cells. Westfall *et al.*(14) studied glucose utilization by strain L cells and although amino acid requirements have been ascertained to some extent, no measurements of total protein utilization by these cells have been reported. In the following report, results of experiments made upon strain L cells by exposing them to these several substances and noting the effects on glucose and protein utilization, as well as upon cell numbers and cell division, are recorded.

Materials and methods. Earle's medium, consisting of 40% horse serum, 40% balanced salt solution and 20% (1:1) chicken embryo extract was used. This medium was modified for control cultures by using 39% Earle's balanced salt solution and 1% triple distilled water. The test medium also contained 39% balanced salt solution and 1% of desired concentration of inhibitor solution. For these experiments, 1 ml of cell suspension containing approximately 1 million cells was placed in each of 20 Carrel D-3.5 flasks. The medium was removed 48 hours later and in each of 10 flasks, was replaced with 2 ml of fresh

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