

hibiting "aggressive" and another group exhibiting "passive" behavior were studied during and after a period of high cholesterol and fat feeding. Although the "aggressive" groups exhibited more pronounced evidences of androgen activity, their plasma and aortic cholesterol contents were perhaps less than those of the "passive" group. The extent and degree of coronary atherosclerosis was the same in the two groups.

We wish to express our thanks for the technical assistance of Clarence Omoto and Warren Hayashi.

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Received July 15, 1957. P.S.E.B.M., 1957, v96.

Altitude Stress: Its Effect on Tissue Citrate and Salmonellosis in Mice.* (23444)

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Mice exposed to simulated altitude of 20,000 ft for 3 weeks or for 6 weeks show a uniform percentage decrease in tissue citric acid content and in their susceptibility to salmonellosis(2). Attempts to verify these findings in guinea pigs native to the Andean altiplano compared to those native to the coastal plain of Peru were not convincing for tissue citrate because of the wide range of individual values found within groups(3). The present communication presents results of studies on mice subjected to simulated altitude for a period of 3 to 4 months along with additional measurements designed to elucidate possible causes for the observed changes in tissue citrate.

Methods. Mice were exposed to simulated altitude in decompression chambers according to the schedule and technic previously described(1) with one important modification. Rather than control pressure within the chamber by a relay, an adjustable needle valve was substituted. The pressure of a desired altitude could be maintained during continuous operation of the pump with a variation in 24

hrs of approximately 3 cm of mercury ($\pm$ 1,000 ft). Daily, the chambers were brought to atmospheric pressure while the cages were cleaned and fresh food and water were added. After about 15 to 20 minutes the animals were again at simulated altitude. The method of Ettinger *et al.*(4) was used for citric acid assays on blood, liver, spleen, kidney, duodenum, diaphragm, heart, and lung. Each analysis was done on the pooled tissues of 3 mice. Animals were killed by decapitation. The blood for assay was collected in a paraffined watch glass and transferred by tuberculin syringe to tungstic acid, the protein precipitant. The tissues were dissected quickly from each animal in 75 to 150 mg amounts and placed directly on dry ice. They were weighed in the frozen state, returned to the dry ice until all tissues were collected, and then they were homogenized in cold tungstic acid using a teflon in glass homogenizer. CF 1 female mice were used in all experiments. They were fed Purina dog chow *ad libitum* and water was available at all times.

Results. *Citric acid in tissues of control mice.* The mean values of citric acid (μ g/g wet wt) for each of the tissues are given in column 2, Table I. These agree closely with

* This study was supported at Bryn Mawr College by funds provided under Contract with School of Aviation Medicine, USAF, Randolph Air Force Base, Texas.

TABLE I. Citric Acid Content of Tissues of Normal Mice, of Mice Exposed for 3-4 Months to a Simulated Altitude of $20,000 \pm 1,000$ Ft, of Mice Injected Intraperitoneally with 1 mg Cortisone Acetate Daily for 2 Weeks, and of Mice Starved for 17 Hours. Each value in the table is the mean $\pm$ stand. dev. of 8 separate determinations, each on the pooled tissues of 3 mice.

Citric acid content (γ /g wet wt $\pm$ stand. dev.) of tissues from				
Tissue (1)	Control mice (2)	Mice kept at altitude 3-4 mo (3)	Mice given 1 mg cortisone acetate daily for 2 wk (4)	Mice starved 17 hr (5)
Blood	36 ± 6.6	23 ± 2.5	36 ± 3.2	130 ± 9.4
Diaphragm	86 ± 6.2	55 ± 8.1	79 ± 4.8	134 ± 10.4
Duodenum	84 ± 6.7	68 ± 6.9	91 ± 4.0	129 ± 10.6
Heart	55 ± 4.7	36 ± 3.2	54 ± 4.8	97 ± 6.7
Kidney	63 ± 5.3	41 ± 3.3	96 ± 6.1	72 ± 8.3
Liver	76 ± 6.6	58 ± 6.4	79 ± 5.6	87 ± 9.6
Lung	68 ± 4.3	52 ± 6.0	72 ± 5.6	148 ± 7.0
Spleen	92 ± 7.6	62 ± 5.6	79 ± 5.6	167 ± 13.2

values already published(2) except for a statistically significant difference in duodenum. The present value is lower by about 20 γ per g. The 2 experiments were carried out, however, more than 3 years apart.

Citric acid in tissues of mice adapted to altitude for 3-4 months. The results listed in column 3, Table I are about 30% (19% to 34%) lower than the corresponding control values. Duodenum shows the minimum percentage change. In each tissue the difference between means is significant at less than the 0.1% level as estimated by the rank order test of White(6). In no tissue was the largest value from altitude adapted mice as large as the smallest value for control mice. These data extend, therefore, the results previously reported for mice kept at altitude for shorter periods of acclimatization(2).

Citric acid in tissues of mice injected with cortisone acetate daily for 2 weeks. The possibility that the change in tissue citrate associated with acclimatization to altitude is mediated through a glycocorticoid from the adrenal was considered. As a means of testing this hypothesis, daily intraperitoneal injections of 1 mg of cortisone acetate (Nutritional Biochemicals) for 2 weeks into a group of normal mice preceded the assays for tissue citrate. The hormone was suspended in isotonic sodium chloride solution with a drop or two of Tween 80 as stabilizing agent. The desired amount of cortisone was contained in 0.5 ml of suspension. The number of injections was based on the previously observed fact that 2 weeks exposure to simulated alti-

tude results in a statistically significant drop in citrate concentration in all tissues examined(2). The dosage level was arbitrary but an amount was desired that might reasonably be expected endogenously. The adrenals from several mice were weighed in the freshly frozen state. Total wet weights varied from about 8 to 11 mg per animal. To administer an amount of hormone equal to about 10% of total gland mass seemed, therefore, to be a more realistic dose than some that have been employed in mice for other experimental purposes as reported in the literature(5). The results of these tests are summarized in column 4, Table I.

It can be seen that the citric acid content of all tissues is not significantly different from that of control animals, with 2 exceptions. Kidney of cortisone-treated mice has more citric acid than in normal mice while spleen shows the reverse change. Under the conditions of the experiment, therefore, cortisone fails to produce tissue citrate changes of the general type found in animals acclimatized to simulated altitude.

Citric acid in tissues of mice starved for 17 hours. Blood sugar of mice exposed to simulated altitude for 3 weeks is approximately 30% lower than that of control animals (108 mg% vs. 149 mg%). Thus the altitude exposed animals have decreases in both blood sugar and tissue citrate of comparable magnitude. This suggests the possibility of a correlation between the two. An experiment was designed accordingly. Blood sugar was lowered by withdrawing food from otherwise

TABLE II. Survival Time of Mice Infected Intraperitoneally with *10⁸ Salmonella typhimurium*. The altitude mice were exposed to a simulated altitude of 20,000 $\pm$ 1,000 ft for 3-4 mo but were removed from the decompression chambers for the post-infection period.

Time post-infection (hr)	No. of mice surviving in group of	
	Altitude mice	Control mice
0	10	10
47	9	10
49	8	10
100	4	9
103	3	9
119	2	9
124	2	8
143	1	5
148	1	4
149	1	3
167	0	2
191		0
Mean survival time	103 $\pm$ 44	150 $\pm$ 27

normal animals for a period of 17 hrs. At the end of this time blood sugar was 75 ± 13 mg% and the tissue citrate was found to be that listed in column 5, Table I. In every case the citric acid is above the corresponding control value. The smallest percentage changes are seen in kidney and liver but these are significantly larger (*P* between 0.05 and 0.01) by rank order test(6). All other values, compared to controls, are significant at less than 0.001 probability.

Survival time of altitude acclimatized mice infected with Salmonella typhimurium. Table II presents the results obtained when mice exposed to simulated altitude for 3 to 4 months are infected intraperitoneally with 0.5 ml of a saline suspension of a 15-hour brain-heart infusion broth culture of *Sal. typhimurium* containing about 100,000 cells. The animals were kept at atmospheric pressures for the entire postinfection period. Their survival is compared with control mice similarly infected. The altitude mice are more susceptible as revealed by the lower survival time (*P* is approximately 0.01 by "t" test), even for this small sample.

Discussion. The earlier suggestion(2) that mice at altitude establish a lower steady state concentration of tissue citric acid seems to apply to animals maintained in the decompression chambers for periods up to 4 months.

The extent to which these animals are truly acclimatized to their new environment is difficult to assess but they give every evidence of being in good physical condition. How much the observed shift in citric acid concentration is to be attributed to altitude *per se* or to the daily compression and decompression necessary for renewal of food and water remains to be determined. Even if it is the latter, it must be assumed that animals adapt to the downhill-uphill stresses within a period of about 3 weeks and daily repetitions for many additional weeks lead to no further metabolic shifts.

The biochemical change responsible for the altered tissue citrate remains unknown. The fact that injections of cortisone acetate failed to imitate the effects of altitude on tissue citric acid is not conclusive. The choice of dosage, of number of daily injections, and of adrenocorticosteroid were all arbitrary. Under other experimental conditions the results might have been more revealing. The findings with starved mice clearly prove, on the other hand, that no direct correlation exists between blood sugar level and tissue citric acid concentration.

The results of the infection experiment serve to confirm previous observations on similar groups of mice except that the altitude animals were exposed for only 3 weeks at the time of challenge(1,2). In the earlier tests the survival time of the altitude group was about two-thirds that of the control group, in agreement with the data of Table II. Thus degree of susceptibility to salmonellosis accompanying hypoxic stress appears to remain approximately constant with time of acclimatization ranging from 3 weeks to 4 months. The correlation in time of change of tissue citrate with change in susceptibility to infection, previously noted(2), is now extended to include the 3- to 4-month period. That such a correlation between tissue citrate and susceptibility to infection is *not* to be expected in all situations leading to greater susceptibility is seen in the results with cortisone. Cortisone injections greatly reduce survival time of infected mice(5) yet fail to alter in all cases tissue citrate. This action of cortisone on infections has been confirmed with

our strain of *Sal. typhimurium* and CF 1 mice.

Summary. 1. Citric acid content (γ /g wet wt) of blood, diaphragm, duodenum, heart, kidney, liver, lung, and spleen was determined colorimetrically in the following groups of mice: (a) normal mice, (b) mice exposed to simulated altitude of 20,000 ft for 3 to 4 months, (c) mice given daily intraperitoneal injections of 1 mg cortisone acetate for 2 weeks, and (d) mice starved for 17 hours. 2. Mice of group (b) had 20-30% less tissue citrate than control mice. In group (c) there was no change in tissue citrate except in kidney, which increased, and in spleen, which decreased. Tissues from starved animals showed a significant increase in citrate in all tissues. Thus the change observed in group (b) cannot be correlated with cortisone injections nor

with a reduced blood sugar level induced by inanition. 3. Altitude exposed mice are more susceptible to *Sal. typhimurium* infection than control mice.

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Received July 15, 1957. P.S.E.B.M., 1957, v96.

Relation of Number of Transplants to Total Volume of Regenerating Adrenocortical Tissue.* (23445)

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Wyman and tum Suden(1) found that the total amount of adrenocortical tissue regenerating in intramuscular autoplasmic transplants in rats is fairly constant for each sex, irrespective of number of masses implanted or present, and suggested that a functional factor limits the growth of transplants. Eversole, Edelman and Gaunt(2) pointed out the considerable variability in amount of tissue regenerating from single grafts in different sites and suggested that the principle stated by Wyman and tum Suden would hold only for a given site. In the Wyman and tum Suden study the transplants were measured during the third month after operation or later. Komrad and Wyman(3) found that resumption of function of adrenal transplants, beginning about one week after operation, proceeds in a progressive or stepwise manner, presumably reflecting an increase in volume of the secreting tissue. In a study of the effect of x-irradi-

ation on growth of transplants(4) considerable variation in size of grafts was noticed at 14 days postoperation. An attempt was made, therefore, to assess the relation of number of implants or of surviving transplants to total volume of regenerating adrenocortical tissue in intramuscular grafts during the earlier stages of regeneration, and to find the time at which a limitation of growth appears.

Methods. Male rats of the Wistar strain, 35 days of age, weighing 80 to 120 g at operation, were maintained on Purina laboratory chow and tap water *ad libitum*. After operation saline drinking water was given for the duration of the experiment. The bisected adrenal glands were transplanted to the spino-trapezius muscle. Approximately equal numbers among the 56 rats used received one, 2, 3, or 4 implants of half a gland each. The rats were sacrificed at 9, 14, 21, or 90 days after operation and the number of regenerated transplants was determined. The fixed (Gilson's) tissues were serially sectioned in paraf-

* Supported in part by contract with U.S. Atomic Energy Com.