tance in determining the number of counterions available at exchange sites. Or in other words, provided the water is not structured around these sites, the lower the salt concentration the greater will be the proportion of free water in the interlamellar spaces and the more easily will a counterion fit into these spaces, regardless of its size.

Summary. The water content of pellets of a myelin extract of bovine optic nerve decreased with increasing salt concentration of the medium in which they were spun down. This water content was always less when Ca was the medium cation than when either equiequivalent Na or K was present. The pellet water cation concentration was always greater than that of the medium for the above 3 cation species. It is concluded that the extracted material carried a net negative charge. Extract Ca could not be completely exchanged by Li, Na, K, Rb, Cs, or Mg. At higher salt concentrations the ease of exchange of Ca by monovalent cations was in the same sequence as their hydrated diameters, the smallest counterions exchanging most readily.

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Thyroxine Biosynthesis and Thyroidal Uptake of I^{131} in Rats at The Onset of Hypoxia Exposure.* (31022)

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Based on early observations that hypoxia tolerance can be increased by thyroidectomy or injections of antithyroid drugs(9,17) there have been sporadic attempts to determine the extent to which hypoxia exposure influences thyroid function in the intact animal. The

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available evidence, however, remains somewhat contradictory since both a stimulation and depression of certain aspects of thyroid functions have been attributed to acute hypoxia exposure. For example, some groups have noted a significant increase in the thyroidal uptake of I^{131} during the onset of hypoxia exposure(5,8); others report a depression(15,16) or no alteration(14) in thyroidal I^{131} content under similar conditions of exposure. Significantly, none of these reports offers data on whether the biosynthesis of thyroid hormone may be altered in hypoxic animals.

The present study was undertaken to determine changes in the distribution of various I^{131} labeled iodoaminoacids in thyroid gland homogenates from rats exposed to moderate hypoxia for 2-60 hours in order to cover the critical stages of altitude acclimation. During the course of this work data were also obtained on thyroidal uptake of I^{131}.

Materials and methods. A total of 107 male, albino rats (CFN strain, Carworth Farms, N. Y.) weighing 180-250 g was used in this study. Groups of 5 to 16 rats were placed in a well ventilated, walk-in decompression chamber maintained at a reduced pressure of 380 mm Hg (18,000 ft) for 2, 4, 8, 16, 32 and 60 hours, respectively. Corresponding control groups were maintained at an ambient pressure of 725 mm Hg (1,200 ft). Exposures were continuous except for brief periods (*ca* 20 min daily) necessary to remove experimental animals from the chamber or to give them food and water. All rats were fed Purina Laboratory Chow and water *ad libitum*.

To determine the effects of hypoxia on the synthesis of thyroxine and on uptake of I^{131}, rats were injected i.p. with 10 μc of NaI^{131} and immediately placed into the decompression chamber. Hypoxic rats and ambient pressure control groups were autopsied after the designated intervals of exposure (2-60 hours) for radioanalysis of the thyroids. The routine procedure for measuring uptake of I^{131} consisted of excising thyroids from rats kept under light ether anesthesia, weighing the glands and counting with a well-type scintillation counter.

In addition to the total radioactivity counts of the thyroid, glands from 71 rats were used for analysis of I^{131} incorporation into thyroidal iodoaminoacids. Each pair of glands was homogenized in ice-cold Krebs-Ringer bicarbonate buffer, pH 8.0, and hydrolyzed according to the methods of Rouche *et al*(11) using crude pancreatin. Hydrolysis was carried out in Krebs-Ringer bicarbonate buffer, pH 8.0, for 48 hours at 37°C. The I^{131} labeled iodoaminoacids present in a measured volume of the hydrolysate (30-60 μl) were separated by unidimensional chromatography in a collidine-H_2O-NH_4 solvent system on Whatman No. 1 paper. Radioactive spots representing I^{131} labeled monoiodotyrosine (MIT), diiodotyrosine (DIT), thyroxine (T_4) and radioiodide were located autoradiographically, cut from the chromatogram, placed into a shell vial and counted.

Results. The effects of hypoxia on uptake of I^{131} are summarized in Table I. These data show that the mean counts of I^{131} per minute per milligram of thyroid tissue are significantly increased in altitude exposed rats 8 hours after injection. Radioiodine uptake in the thyroid reached a maximum of about 42,000 to 56,000 cpm/mg thyroid following 16 to 60 hours exposure to hypoxia. The maximal uptake of thyroidal I^{131} in control rats was only about half that in the rats exposed to altitude. These data suggest that hypoxia stimulates thyroid gland activity. Furthermore, stimulation of this aspect of thyroidal activity is elicited within 8 hours of hypoxia exposure.

The data obtained on the effects of altitude exposure on thyroid hormone synthesis are summarized in Table II. Chromatographic analyses of thyroid gland hydrolysates revealed that hypoxia exposure results in changes in the relative distribution of I^{131} labeled components. Specifically, the most striking effect of hypoxia on hormone synthesis was the shift observed in the relative distribution of I^{131} labeled MIT and DIT, as shown in Table II. For example, after 17-32 hours of exposure the relative amount of labeled MIT in thyroids of hypoxic rats is significantly higher ($P<.01$) than that in corresponding controls. Conversely, the amount

TABLE I. Thyroidal Uptake of I^{131} in Rats Exposed to Reduced Pressure.

Hr after injection	cpm/mg thyroid (mean ± S.E.) Control (725 mm Hg)	Altitude exposed (380 mm Hg)	p*
2	9,300 ± 850 (5)†	10,400 ± 760 (5)	0.4
4	15,300 ± 2,500 (11)	22,600 ± 3,900 (11)	0.2
8	14,900 ± 3,000 (4)	27,500 ± 2,000 (9)	0.01
16	31,600 ± 2,800 (12)	42,100 ± 4,300 (12)	0.05
32	27,200 ± 3,300 (11)	45,800 ± 3,300 (16)	0.001
60	29,800 ± 2,600 (5)	56,000 ± 7,400 (6)	0.001

* All comparisons are between controls and experimentals at the same time interval. P was calculated using Student's t test.

† Numbers in parentheses represent No. of rats used.

S.E., standard error.

TABLE II. Radiochromatographic Analyses of I^{131} Labeled Iodoaminoacids in Thyroids of Rats Exposed to Reduced Pressure.

Hr after injection	n	Percent of I^{131} in thyroid gland (mean ± S.E.) DIT	MIT	T_4	I
		Control rats (725 mm Hg)			
2	5	49.2 ± 3.0	40.0 ± 2.1	7.1 ± 1.4	3.5 ± 1.4
4	4	53.2 ± 1.8	32.7 ± 1.9	7.1 ± 1.0	6.8 ± 1.0
8	4	58.9 ± 5.2	25.5 ± 3.6	7.8 ± 2.9	7.7 ± 4.4
17	4	59.8 ± 1.3	26.1 ± 1.2	6.3 ± 2.4	7.5 ± 1.6
32	5	61.8 ± .8	16.4 ± 2.1	14.3 ± 1.1	7.3 ± 1.0
60†	6	41.5	11.3	25.9	7.6 ± 1.0
		Hypoxic rats (380 mm Hg)‡			
2	5	47.5 ± 1.8	44.7 ± 1.1	6.2 ± 1.5	1.4 ± .7
4	6	51.9 ± 1.6	37.7 ± 1.3	3.7 ± .8	6.5 ± 1.0
8	10	53.4 ± 2.4	31.7 ± 2.0	9.4 ± 2.5	5.3 ± 1.2
17	6	54.3 ± .9*	30.9 ± 1.4*	7.8 ± 1.0	6.8 ± .3
32	10	55.0 ± 1.5*	31.0 ± 1.4*	9.8 ± 1.0*	4.0 ± .5*
60†	6	43.5	8.8	30.8	6.3

* Significant at a 1% level of confidence using Student's t test. Comparisons are always of hypoxic rats relative to control rats at corresponding intervals after injection.

† Determination based on a pool of 6 pairs of glands.

‡ Injected at time of introduction into the decompression chamber.

n = No. of rats used for each determination.

S.E., standard error.

of labeled DIT is reduced in hypoxic rats in relation to controls in the 17- and 32-hour exposed groups. This shift in the MIT and DIT fractions is better visualized in Fig. 1, which graphically illustrates alterations in the thyroidal I^{131} MIT/DIT ratio as a function of exposure time. These data also show that this response is transient since, after 60 hours of exposure, both the relative amounts of labeled MIT and DIT as well as the I^{131} MIT/DIT ratios of experimentals and controls are the same. The only change observed in the I^{131} thyroxine and radioiodide components occurred in the 32-hour exposed groups, which exhibited a decrease ($P<.01$) in the relative amounts of both these components (Table 2).

Discussion. It is well known that early metabolic adjustments such as depressed food intake, weight loss, water imbalance and reduction in body temperature occur during initial exposure to high altitude(12,13). Thus, it might be expected that functional activity of the thyroid is also altered after a few hours of exposure to high altitude. The present study clearly demonstrates this to be the case. Hypoxic rats exhibited a markedly increased thyroidal uptake of I^{131} after only 8 hours of exposure to 18,000 feet. That rats exhibit an increase rather than a depression

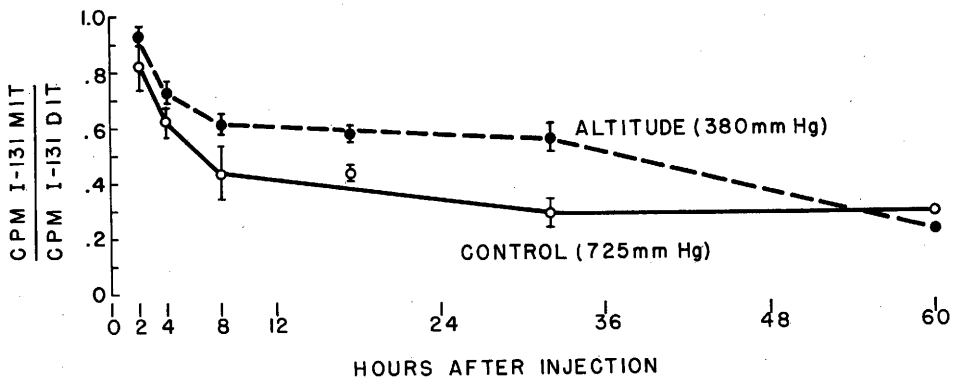

FIG. 1. Effects of reduced pressure on MIT/DIT ratios obtained from radiochromatograms of thyroid hydrolysates.

of thyroidal activity at the onset of exposure is somewhat unexpected particularly in view of the known relationship between altitude tolerance and hypothyroidism(9,17).

In the present study attempts were also made to assess the effects of hypoxia on the biosynthesis of thyroxine. Radiochromatographic analyses of I^{131} labeled thyroidal iodoaminoacids revealed that hypoxia results in an increased MIT/DIT ratio due to a depression in the relative amount of radioiodide incorporated into the DIT fraction. However, since the observed increase in MIT/DIT ratio proved to be a transient response, and since there was an evident increased rate of incorporation of I^{131} into all of the thyroidal iodoaminoacids in hypoxic rats, it is suggested that the mechanisms governing biosynthesis of thyroid hormone are not impaired during early stages of hypoxia exposure. The most striking thyroidal change induced by hypoxia appears to be the enhanced uptake of I^{131}.

Some insight into the possible functional significance of the present findings is afforded from considering the elevated I^{131} uptake in light of what is presently known about the metabolic and endocrine state of animals exposed to hypoxia. There is considerable evidence that chronic exposure of animals to hypoxia elicits hypoplasia of the thyroid gland (6,8). Furthermore, the BMR remains unchanged during hypoxia(12) and serum TSH is reduced in relation to ambient pressure controls(7). These findings appear to be inconsistent with the suggestion(5) that an increased uptake of I^{131} during hypoxia is elicited through increased secretion of pituitary thyrotropin.

An alternative explanation may be that the observed thyroidal changes reflect temporary iodine insufficiency during the onset of exposure. There are several reasons for offering this rather than hyperthyroidism as an explanation for the hypoxia induced enhancement of I^{131} uptake. First, iodine insufficiency is the one condition in which both increased I^{131} uptake and an elevated MIT/DIT ratio occur at the same time(2,3,4,10). Injection of thyrotropin is reported to have no effect on the MIT/DIT ratio(2). Also, the reduced food intake and relative diuresis occurring during the first 24 hours of exposure(8) make conditions for the depletion of the iodide pool optimal(1). Furthermore, this mechanism may help to explain certain seemingly paradoxical reports in the literature. For example, when both hypoxic rats and ambient pressure controls are maintained on low iodine diets prior to exposure, I^{131} uptake is lower in hypoxic rats than in controls(14, 16). Conversely, if both hypoxic and control rats are maintained on diets containing an adequate iodine supply, the rate of I^{131} uptake in hypoxic rats (who do not consume food during the first 24 hours) is markedly elevated(5,8). It appears, therefore, that dietary alterations in hypoxic rats can affect the response of the thyroid gland to injected radioiodine.

If iodine lack is severe enough in hypoxic rats, one might speculate that this response

actually controls the biosynthesis of thyroxine. The result would be a short, but critical, period at the onset of hypoxia exposure during which elaboration of thyroxine and the subsequent utilization of oxygen by peripheral tissues are reduced.

Summary. Exposure of rats to a reduced pressure (380 mm Hg) simulating an altitude of 18,000 feet resulted in a markedly elevated I^{131} uptake in the thyroid, a relative increase in the amount of intrathyroidal I^{131} MIT and a lowered I^{131} DIT. The hypoxia induced alteration of thyroid hormone synthesis was found to be a transient response since the I^{131} MIT/DIT ratio returned to control levels after 60 hours of exposure. A possible mechanism which could account for these thyroidal changes is discussed.

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Toxicity of Malathion and Mercaptosuccinate to Growth of Chick Embryo Cells *in vitro*.* (31023)

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Much evidence indicates that many of the acute toxic effects of malathion (0,0-Dimethyl S-(1,2 dicarbethoxyethyl) phosphorodithioate) and other organophosphorus insecticides are due to their action on the nervous system, and are caused by their inhibition of the enzyme acetylcholinesterase(1,2). The phosphorus group in the insecticide molecule is believed to be responsible for this inhibition(2,3). The possible effects of other groups in the molecule have received little attention. Such effects would be difficult to investigate in the intact animal because the presence of the nervous system complicates studies of the responses of other cells and tissues to organophosphorus insecticides and their breakdown products. Isolated systems such as cell cultures seem appropriate for this kind of investigation. However, until recently(4,5,6) cell cultures have been little used in research on insecticide toxicity to vertebrates, although they have been utilized extensively in other areas of pharmacology such as the

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