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Influence of Adrenal Hormones on Lactic Acid Content of Rat Brain Tissue. (23958)

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An increase in lactic acid content of brain tissue in hypoxic animals has been established by various investigators(1-4). When brain tissue is analyzed one to 3 hours post mortem the lactic acid level may increase 4- to 5-fold (4). This probably is the result of endogenous enzymatic activity on lactic acid precursors such as glycogen, glucose or other carbohydrate material. There is considerable evidence to show that adrenal steroids are involved, in a permissive manner, in carbohydrate metabolism(5). This fact coupled with the almost exclusive use of carbohydrate in normal brain metabolism, suggested a study of the lactic acid content in brain tissue as influenced by hypoxia and adrenal hormones.

Methods. Male Sprague-Dawley rats weighing about 350 g were used. They were divided into 2 main groups; intact and adrenalectomized. All animals received Purina Laboratory Chow and water *ad libitum*, with the adrenalectomized animals receiving physiological saline in place of water. Four days post-operatively, the animals were placed into 2 subgroups; a ground level group which will be referred to as the "non-hypoxic" group and an altitude group which will be designated as the "hypoxic" group. Hypoxia was produced by taking the animals to a simulated altitude of 30,000 feet (225.6 mm Hg) for a period of 105 minutes in a decompression chamber. This time and altitude were found to give a large increase in lactic acid content of brain tissue in intact control animals. At the end of experimental or control period, the animals were decapitated in their respective environments; *i.e.*, at ground level or at altitude. The decompression chamber was routinely returned to ground level within 5-6 minutes following decapitation. All heads were left intact at room temperature (23°-27°C) for a period of 3 hours, after which the brains were removed, blotted of excess blood, and homogenized in glass homogenizers with 10% trichloracetic acid (10% homogenate). The lactic acid content of the whole brain was determined by the method as described by Van Fossan(4). Lactic acid content of brains from intact and adrenalectomized animals was determined under non-hypoxic and hypoxic conditions to obtain control data. In comparison, brain lactic acid content of adrenalectomized animals receiving the following adrenal hormones and dosages was determined: (a) epinephrine, 0.15 mg in sesame oil given subcutaneously 15 minutes prior to ascent to altitude; (b) corticosterone, 1.0 mg in saline given subcutaneously at 4 two-hour

TABLE I. Brain Lactic Acid of Intact and Adrenalectomized Rats.†

Treatment	Intact N	Intact X̄ S.E.	Adrenalectomized N	Adrenalectomized X̄ S.E.
Non-hypoxic	10	115 ± 4	10	113 ± 5
Hypoxic	10	216* ± 11	10	136 ± 12
" + sesame oil			4	131 ± 3
" + epinephrine	9	240* ± 10	9	217* ± 11
" + saline			4	106 ± 17
" + hydrocortisone	5	217* ± 10	9	220* ± 10
" + corticosterone			9	243* ± 8
" + cortisone			9	210* ± 18
(Acclimatized to 18,000′)				
Killed at ground level	5	124 ± 4		
" " 18,000′	5	137 ± 8		
" " 30,000′	8	171* ± 16		

* $P < .01$ in reference to non-hypoxic values. † mg of lactic acid/100 g of tissue.

intervals prior to exposure to altitude; (c) hydrocortisone, 0.33 mg in saline given as in b above; (d) cortisone, 0.5 mg in saline given as in b above. In addition, the brain lactic acid content of rats that had been maintained at a simulated altitude of 18,000 feet for a period of 12 weeks was also determined after killing at ground level, 18,000 and 30,000 feet.

Results. Brain lactic acid content of intact and adrenalectomized nonhypoxic animals was similar, 115 ± 4 and 113 ± 5 mg/100 g of brain tissue respectively. When similar groups were made hypoxic, the brain lactic acid content of adrenalectomized animals was considerably less than that of intact animals, 136 ± 12 and 216 ± 11 mg respectively. This difference was statistically significant at the $P < .01$ level (Table I). However, lactic acid level in hypoxic adrenalectomized animals was not statistically different from control value of intact animals ($P > .05$). Hypoxic adrenalectomized animals given adrenal hormones as outlined above, had an elevated brain lactic acid content similar to that of the intact hypoxic animals. Adrenalectomized animals given sesame oil only and made hypoxic, had lactic acid values similar to hypoxic adrenalectomized controls (131 *vs.* 136 mg).

Animals which had been maintained at a simulated altitude of 18,000 feet for 12 weeks and were decapitated at that altitude had lactic acid values similar to adrenalectomized hypoxic rats, 137 ± 8 mg *vs.* 136 ± 12 mg. When animals acclimatized to 18,000 feet were placed at 30,000 feet for 105 minutes the brain lactic acid content was 171 ± 16 mg/100 g of tissue. This value was statistically lower $P < .01$ than that of intact hypoxic brain tissue, and statistically higher $P < .01$ than brain tissue from adrenalectomized hypoxic rats. Altitude acclimatized animals, when returned to ground level for 2 hours, where found to have normal values of brain lactic acid (124 ± 4 mg).

Discussion. These experiments indicate that the adrenal hormones are involved in the accumulation of brain lactic acid following exposure to low oxygen tensions, for when the adrenals were absent, the typical increase in brain lactic acid concentration did not occur. Epinephrine as well as adrenal cortical hormones appeared to be equally effective in the dosages used. Exposure, of intact and adrenalectomized dogs maintained on cortisone or DCA, to a simulated altitude of 30-40 thousand feet, resulted in a moderate increase in blood sugar(6). If the same response occurred in rats in the present study, it may be concluded that the presence of this lactic acid precursor enabled the increase in brain lactic acid to occur following death in the intact and adrenalectomized animals treated with adrenal hormones. Exposure of adrenalectomized rats to hypoxia elevated the brain lactic acid level above that of adrenalectomized non-hypoxic rats which further supports the concept that exposure to hypoxia increased lactic acid precursor; thereby causing an elevated lactic acid level.

Acclimatized animals brain lactic acid level was essentially the same as in non-acclima-

tized animals after being returned to ground level and sacrificed. When killed at altitude to which the animal was acclimatized, however, brain lactic acid was significantly elevated over ground level control and when killed at higher altitude the brain lactic acid increased still further but was considerably below the level found in intact non-acclimatized animals exposed to the same altitude. It appears that acclimatization changed the usual response to altitude as far as brain lactic acid was concerned, probably by altering the lactic acid precursor level of the blood.

Summary. In this study, adrenalectomy prevented a rise in lactic acid content of brain tissue in hypoxic rats. When various adrenal hormones were injected, the brain lactic acid level of adrenalectomized hypoxic rats was elevated to the same extent as intact hypoxic rats. Animals adapted to a simulated altitude of 18,000 feet did not respond to the same degree as non-adapted animals when both groups were exposed to severe hypoxia.

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Labelling of Antibodies by 5-Dimethylamino-1-Naphthalene Sulfonyl Chloride, Its Effect on Antigen-Antibody Reactions. (23959)

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Histochemical technics using antibodies (Ab) labelled with fluorescent dyes have gained increasing application to immunological problems(1,2). The present paper deals with 5-dimethylamino-1-naphthalene sulfonyl chloride (DNS),† a fluorescent dye recently introduced by Clayton(3). Investigation of the usefulness of this compound for visualization of Ag-Ab reactions was undertaken because of the simple procedure for labelling proteins and the increasing knowledge about the nature of the protein-DNS union(4,5). An enzyme-antienzyme system, yeast alcohol dehydrogenase (ADH)-rabbit antiserum(6,7), was chosen to determine whether labelling of Ab with DNS has any influence on formation of Ag-Ab complex.

Methods. Chemical properties. DNS is a yellow substance which emits yellow fluorescence. It is almost insoluble in water, but readily soluble in organic solvents like ethanol, ether, ethyl acetate, acetone and chloroform. The corresponding sulfonic acid is formed slowly by hydrolysis but the dye can be used for a few months when kept dry and in the cold. The protein-DNS complex, however, is more stable. Labelled proteins can be precipitated with alcohol, acetone and ammonium sulfate without splitting of the protein-DNS bond. DNS and conjugates absorb light in 300-400 mμ with maxima about 320 to 355 mμ according to medium, pH and combination with different proteins. *Conditions for labelling proteins and DNS-protein ratio.* Proteins buffered to pH 7.4-8.0 are readily labelled by mixing with appropriate amount of DNS dissolved in acetone(4) or ethanol (Vinograd)‡. The suspended unbound dye can be removed by centrifugation while dis-

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