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Succinic Dehydrogenase and RNA in the Supraoptic Nucleus and Hypothalamic Anterior Area in Dehydrated Rats.* (29526)

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The purpose of this investigation was to develop a quantitative method for determining the degree of activity of neurohumoral cells in the hypothalamus of rats. Succinic dehydrogenase activity (SDH) and ribonucleic acid (RNA) content were chosen as possible indicators as it seemed feasible to combine known analytical methods for these substances into a procedure that would give quantitative results for both from the same tissue. The supraoptic nucleus (SON) was selected for study since dehydration is known to increase the ribonucleic acid (RNA) content(1) of the nucleus and an increase in SDH has also been reported in these neurons (2). However, conflicting reports exist concerning SDH in the SON as some investigators have found little or no activity there(3). The anterior area of the hypothalamus (AA) was included in the present study for comparative purposes.

Materials and methods. Eighty female rats (Charles River Breeding Laboratories) weighing approximately 250 g were divided into 8 groups of 10 each. Half of the groups were deprived of water for 7 days while the others served as normal controls. The animals were killed in groups of 10 by decapitation, the hypothalamus removed, immediately frozen and sectioned in a cryostat at 32 μ thickness. The sections were affixed to 75 mm $\times$ 25 mm glass slides by warming to room temperature for 30 minutes. The slides were then placed in Coplin jars and incubated for 15 minutes in a succinate-tetrazole mixture at room temperature. This mixture was prepared according to the method of Nachlas *et al*(4) with the exception that 2-(p-iodophenyl)-3-nitrophenyltetrazolium chloride (INT) was substituted for the Nitro-BT of the original. The slides were then dried overnight in a vacuum desiccator at 9°C. Under a dissecting microscope the SON and the AA were removed by scraping. The material from each rat in a group was pooled and analyzed for formazan, RNA and DNA.

The analysis procedure was that of Scott *et al*(5) for RNA and DNA. The procedure was modified to the extent that the ethanol washing of step 4 and the ethanol-ether mixture of step 5 were saved as the formazan was extracted in these washings. The formazan was measured in these solutions in a Beckman DU spectrophotometer with a Lowry-Bessey attachment at 500 mμ.

Results and discussion. Table I summarizes

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TABLE I. Succinic Dehydrogenase (SDN)/DNA Ratio and RNA/DNA Ratio (See Text) for Supraoptic Nucleus (SON) and Anterior Area (AA) with Results of t-Test with P = <.05 Considered Significant.

		Normal	Dehydrated	P	% Increase
SDH/DNA	SON	1.91 ± .68	2.58 ± .85	>.30	35
RNA/DNA	SON	2.23 ± .85	4.42 ±1.26	<.05	98
SDH/DNA	AA	2.66 ± .55	6.41 ± .35	<.001	140
RNA/DNA	AA	2.35 ±1.11	9.25 ± .61	<.001	293

the results. Using DNA as a base line for tissue amount the ratio of SDH and RNA was calculated for each group and the average of these is given in the Table. A t-test was applied and a P value of less than 0.05 was considered as significant. From the Table it may be seen that while SDH increased 35% in the dehydrated animals this was found to be not statistically significant. It would appear, therefore, that the Krebs cycle does not play an important role in production of the antidiuretic hormone by the SON. RNA was significantly increased as might be expected. The surprising finding, however, is that a very large increase in both SDH and RNA occurred in the AA. Since other hypothalamic nuclei were not analyzed nor other brain regions it is not known whether this increase is peculiar to the AA. Why dehydration should increase activity in these neurons is not clear.

Summary. A quantitative method that combines an analysis for succinic dehydrogenase activity (SDH) and ribonucleic acid (RNA) content in nerve cells was applied to the supraoptic nucleus (SON) and the anterior area (AA) of the hypothalamus of normal and dehydrated rats. Dehydration resulted in a significant increase in RNA in the SON but not in SDH. In the AA both SDH and RNA increased significantly.

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Nitrogen Balance in Radiation Chimeras.* (29527)

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Mice exposed to lethal doses of total-body irradiation can recover if they are given normal bone marrow cells after irradiation(1). If the injected cells are genetically different from the irradiated host, the animals may subsequently die because of a secondary syndrome not related to the acute effects of irradiation. This syndrome is presumed to be a consequence of immunological reaction between the grafted cells and the host animal. It has been suggested that secondary disease is associated with a "metabolic starvation" (2) because food intake of mice given homologous marrow is adequate and comparable to that of normal or isologous marrow-treated mice. We have previously reported results

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