CELLULAR CHANGES IN THE HUMAN SUPRAOPTIC AND PARAVENTRICULAR HYPOTHALAMIC NUCLEI IN DEHYDRATION* †

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The supraoptic hypothalamic nuclei (SOHN) and the paraventricular nuclei (PVN) have excited much interest because of their dual neural and endocrine roles (1, 2). In some experimental studies, systemic osmotic overload induced by hypertonic saline or by dehydration has been used to stress the antidiuretic hormone function. When so stressed, the SOHN and PVN neurons showed marked temporary morphological changes with the appearance of cytoplasmic vacuoles in neurons (3–8).

Cytoplasmic vacuolization of human SOHN and PVN neurons has been described as an incidental finding at autopsy (9–11), in non-specific trauma and aging (12), and in retrograde changes following hypophysectomy (13). In a study of 73 autopsies, we found neuronal cytoplasmic vacuolization associated with dehydration.

MATERIALS AND METHODS

A. Cases: Brains from 73 autopsies, 5 from the Infectious Diseases Hospital, Madras, India and 68 from the Johns Hopkins Hospital, were used in this study. Eight of the autopsies were from cases with the clinical diagnosis of terminal dehydration, and the remaining 60 autopsies were randomly selected. The 5 brains from India included 3 cases dying of cholera and 2 dying of smallpox.

B. Preparation of Slides: All brains had been fixed in 10 per cent formalin for periods of at least 1 month. The brains were washed, and blocks of the hypothalamus including the SOHN and the PVN were embedded in paraffin. Twenty micron thick sections were stained with hematoxylin and eosin or with cresyl violet. Selected sections were also stained with phosphotungstic acid hematoxylin (PTAH) or with aldehyde fuchsin.

C. Microscopic Methods: As the slides were completed, the authors independently and then together examined the SOHN and PVN. Each nucleus was examined over its entire cross section and compared to the corresponding contralateral nucleus. Primary attention was focused on neuronal morphology where clear round cytoplasmic vacuoles were found as

*From the Department of Pathology and Division of Laryngology and Otology, The Johns Hopkins University School of Medicine, Supported by Research Grants No. NB 8196 and NB 5664 and by Career Fellowship Award NB 5963 from the National Institute of Neurological Diseases and Stroke, and by the John A. Hartford Foundation, Inc.

†This paper was presented in part at the Meeting of the American Academy of Neurology, Washington, D. C., April 24, 1969.

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a variable feature. These were found in neurons of the SOHN or PVN, and when present showed considerable variability in size. In order to grade this variation, the vacuolization was scaled along the following two parameters: 1. Twenty neurons in each nucleus were examined and the proportion of neurons showing cytoplasmic vacuolization was expressed on the following 4 point scale: 0 to 25 per cent—1+; 26 to 50 per cent—2+; 51 to 75 per cent—3+; and 76 to 100 per cent—4+. 2. The size of the cytoplasmic vacuoles in involved neurons was measured. The diameters of the largest vacuoles clearly within cells were measured and graded on the following scale: less than 5μ—small; 5 to 10μ—medium; greater than 10μ—large.

On 10 random SOHN neurons from each case in groups I and II, the following measurements were taken: 1. The average of the neuronal body length and width, 2. the nuclear diameter, and 3. the nucleolar diameter. Only cases over 7 years of age were included in the final averages.

*Medical Records:* In each case the medical records were obtained. Each admission was reviewed, and the references to hydration status as well as main clinical features were noted. From these clinical records, cases were grouped into 1 of 3 categories based on hydration. Group *I* consisted of clinically dehydrated cases. Here the clinical records of the terminal admission indicated dehydration on admission which was not corrected prior to death. Group *II* was comprised of those cases regarded as adequately hydrated. The clinical records of the terminal admission gave positive evidence of good hydration. Group *III* consisted of those cases in which the clinical records of the terminal admission showed incomplete or missing entries or a fluid imbalance precluding an accurate estimate of terminal hydration. Those cases rehydrated during their terminal admission were also included in this group because of the uncertain temporal relationship between clinical course and observed morphology.

**RESULTS**

*Morphology:* Microscopic examination of the SOHN showed a characteristic neuronal morphology that appeared in all nuclei and in many was the only form present (fig. 3). Ganglion cells were usually stubby, fusiform in shape ranging from oval to spindle and averaging 13 by 21 μ in size. The Nissl substance was coarsely granular and aggregated toward the cell periphery, sometimes clumped at the poles of the cell, within a homogeneous or slightly granular cytoplasmic matrix. Neuronal nuclei were round to slightly oval in shape averaging about 8 by 10 μ in size, frequently located slightly eccentrically. In each nucleus was a fine chromatin network and a single slightly eccentric nucleolus measuring about 2.5 μ in diameter. The parenchyma was loose with numerous capillaries closely approximating all ganglion cells. Astrocytes were fusiform with processes tending to parallel the small vessels. Oligodendroglia and microglia, although present, were not prominent.

In a few cases some neurons of the SOHN showed a marked departure from the preceding description (figs. 1, 2). The cytoplasm of these neurons contained clear, structureless, generally round vacuoles ranging from 2 to 14 μ in diameter. In these nuclei there were usually some cells with the normal morphology, while the remaining cells showed a variable degree of vacuolization.

The first vacuoles to appear in minimally involved cells were small and were found peripherally, usually in contact with the cytoplasmic membrane apparently displacing the Nissl substance. As this vacuolization increased in severity, the amount of Nissl substance decreased until, in severely involved cells,
Fig. 1. Neurons of the SOHN in Group I, showing cytoplasmic vacuolization. a. Case 1, b. Case 2, c. Case 3, d. Case 4, e. Case 5, f. Case 6. Cresyl violet stain; ×1000.
Fig. 2. Neurons of the SOHx in Group I, with cytoplasmic vacuolization. Cresyl violet stain; ×1000. a. Case 7, b. Case 8, c. Case 9, d. Case 10, e. Case 11; ×500.
no Nissl substance was recognizable (figs. 1, 2). In these more extreme cases vacuoles tended to become confluent producing a frothy or Swiss cheese appearance. As the vacuolization increased, neuronal outlines became distorted developing irregular and bizarre shapes with obscure cytoplasmic boundaries and the cells measured up to 50\(\mu\) in diameter. In these cases the nuclei varied both in the percentage of vacuolated cells and in the size of vacuoles in involved neurons. There was, however, a generally linear relationship between an increasing percentage of vacuolated neurons and an increasing vacuole diameter.

Rarely these vacuoles had a light magenta hue in cresyl violet-stained sections. Similarly, the hematoxylin and eosin-stained sections occasionally revealed a faintly cosinophilic vacuole. When so stained, the vacuolar contents appeared as an amorphous substance filling the entire vacuole. Sometimes there was a linear gradient of staining intensity across the vacuolar diameter, but there was no indication of any internal structure. These vacuolar contents were noted only in these two stains. PTAH and aldehyde fuchsin failed to further substantiate these observations.

In contrast to the marked changes in the cytoplasm, the nuclei of vacuolated neurons showed remarkably little change. In mature cases the nuclear size and staining characteristics were similar in groups I and II, although nuclei tended to be pushed peripherally in vacuolated cases. Nuclear outlines were well defined in all cases. However, the neuronal nucleoli were significantly enlarged in group I neurons, averaging 3.5\(\mu\) in diameter, in contrast to group II neuronal nucleoli which averaged 2.5\(\mu\) in diameter.

The supporting tissue was somewhat looser in the vacuolated cases. There was a questionable increase in astrocyte size in two of the vacuolated cases, but no general astrocytosis was noted. Oligodendroglia showed no changes. Microglia were not increased.

These changes described in the SOHN were also present in the PVN. The latter nuclei, however, had generally smaller neurons and did not show as extreme vacuolization, there being both fewer and smaller vacuoles than in the corresponding SOHN. Neurons outside both the SOHN and PVN but in the same section showed no similar vacuolization.

**CLINICAL CORRELATION**

**Group 1:** Based on the review of clinical records there were 11 cases with definite clinical dehydration conforming to the Group I clinically dehydrated cases.

Case 1 was a 13-month-old boy with transposition of the great vessels. He had numerous admissions with recommendations of cardiac surgery which the parents refused. The child was repeatedly and terminally noted to be dehydrated due to difficulty feeding. The infant died in surgery on the second day of admission. Histologic study of the SOHN showed 4+ large vacuolization (fig. 1a).

Case 2 was a 20-year-old Indian woman dying of cholera after an 8-day hospital course. Microscopically the SOHN showed 4+ large vacuolization (fig. 1b).

Case 3 was a 22-year-old Indian boy dying of cholera 4 days after admission. There was 4+ large vacuolization of the SOHN on histological examination (fig. 1c).

Case 4 was a 50-year-old man with carcinoma of the base of the tongue. He had a loss
of thirst with marked weight loss on admission. A neck dissection was done, but the patient was uncooperative and was not rehydrated. There was 4+ large vacuolization of the SOHN on microscopic examination (fig. 1d).

Case 5 was a 76-year-old man with aspiration pneumonia. He was admitted cachectic and severely dehydrated, but because of congestive heart failure, rehydration was delayed and never completed. Microscopically the SOHN showed 4+ large vacuolization (fig. 1e).

Case 6, a 7-year-old boy, entered the emergency room with bacillary dysentery, severe diarrhea, dehydration, and in shock. He died one half hour after arrival. Microscopically the SOHN had 4+ large vacuolization (fig. 1f).

Case 7 was a 62-year-old man with multiple pulmonary emboli. The patient had anorexia, nausea, and vomiting for 2 months resulting in weakness and inability to feed himself. He was severely dehydrated and cachectic when seen terminally in the emergency room. Microscopic examination revealed 4+ large vacuolization of SOHN (fig. 2a).

Case 8, a 30-year-old man with metastatic rectal carcinoma, had anorexia, nausea, and vomiting beginning 6 months prior to admission resulting in severe terminal dehydration. He died 2 hours after admission. Microscopically, there was 3+ medium vacuolization of the SOHN (fig. 2b).

Case 9, a 72-year-old woman with a carcinoid tumor of the small intestine and pulmonary emboli, was admitted in shock after nausea and vomiting for 1 week. Fluid intake was a problem and rehydration was never achieved. Microscopically, there was 4+ large vacuolization of the SOHN (fig. 2c).

Case 10 was a 19-year-old Indian man with cholera. During the 1 day hospital course, marked vomiting, diarrhea and dehydration were noted. Microscopic examination revealed 4+ large vacuoles in the SOHN (fig. 2d).

Case 11 was a 65-year-old man with pancreatic duct adenocarcinoma. The patient had had dysphagia, vomiting, and diarrhea continuously during the previous 6 months resulting in hypoglycemic coma and dehydration which were only partially corrected. The microscopic finding in the SOHN was 3+ medium vacuolization (fig. 2e).

Group II: Group II consisted of adequately hydrated cases and served as a control for normal hydration. Based on clinical records there were 19 cases definitely belonging to this group.

Case 12 was a 67-year-old woman with metastatic uterine carcinoma. There was no indication of dehydration. Microscopically, the SOHN showed 1+ small vacuolization (fig. 3a).

Case 13 was a 51-year-old man in chronic renal failure. Fluid balance was carefully watched throughout the 17-day terminal admission with no indication of dehydration. Microscopically there was no vacuolization in the SOHN (fig. 3b).

Case 14 was a 36-month-old boy with tricuspid valve atresia, and atrial and ventricular septal defects. Corrective surgery was attempted on the 12th hospital day, but the patient died 1 day postoperatively. Fluids were noted to be well balanced with no indication of dehydration. Microscopically there was no vacuolization of the SOHN (fig. 3c).

Case 15 was a 70-year-old man with coronary vascular disease dying of a myocardial infarction 2 days after admission. Fluid balance was normal with no evidence of dehydration. Microscopically, there was no vacuolization of the SOHN (fig. 3d).

Case 16 was a 60-year-old male chronic alcoholic with non-constrictive carcinoma of the esophagus and terminal hepatic pneumonia. He had lost 15 pounds over the previous 6 months, but was repeatedly noted to be well hydrated during his 3-week terminal admission. No vacuolization was present on microscopic examination of the SOHN (fig. 3e).

Case 17 was a 29-year-old woman with polymyositis dying of exsanguination from a tracheostomy site. Fluid balance and hydration were carefully maintained within normal limits. No vacuolization in the SOHN was seen (fig. 3f).

Case 18, a 51-year-old man with essential hypertension, had a terminal intracerebral hemorrhage, dying 8 days after admission. Fluid balance was normal throughout the terminal admission, and there was no indication of dehydration. Microscopic examination here revealed 2+ small vacuolization of the SOHN.
Fig. 3. Neurons of the SOHN in Group II. Absent or minimal neuronal cytoplasmic vacuolization. a. Case 12, b. Case 13, c. Case 14, d. Case 15, e. Case 16, f. Case 17. Cresyl violet stain; ×1000.
Case 19 was a 12-month-old infant with endocardial fibroelastosis and terminal bronchopneumonia. The child was followed for 1 day on an outpatient basis where fluid balance was noted as normal with no evidence of dehydration. There was 1+ small vacuolization of the SOHN.

Case 20, a 71-year-old man with a massive gastrointestinal hemorrhage and mesenteric venous thrombosis died 1 day after entry. There was no fluid imbalance and no indication of dehydration. Microscopically, the SOHN showed 1+ small vacuolization.

Case 21, a 67-year-old woman with rheumatic mitral stenosis and insufficiency, died of multiple pulmonary emboli. There was no indication of dehydration. There was 1+ small vacuolization of the SOHN seen on microscopic examination.

Case 22, a 57-year-old man, died of acute bacterial endocarditis. There was no indication of dehydration and microscopic examination revealed no vacuolization of the SOHN.

Case 23, a 50-year-old man with hypertensive arteriosclerotic cardiovascular disease, died of pulmonary emboli and a myocardial infarction. Fluid balance was normal with no indication of dehydration. Microscopically, there was 2+ medium vacuolization.

Case 24, a 63-year-old woman, had right heart failure with multiple pulmonary emboli. She was noted to be normally hydrated. Microscopic examination revealed 2+ medium vacuolization of the SOHN.

Case 25 was a 70-year-old woman with a dissecting abdominal aortic aneurysm. She was noted to be adequately hydrated. Microscopically, the SOHN contained 2+ small vacuolization.

Case 26 was a 50-month-old boy with ulceration and hemorrhage of the small intestine. He was specifically noted to be well hydrated despite diarrhea during his 3-day hospitalization. Microscopically, there was 1+ small vacuolization of the SOHN.

Case 27 was a 46-year-old woman with extensively metastasized cervical cancer. There was no indication of dehydration. Microscopically, the SOHN showed 1+ small vacuolization.

Case 28 was a 72-year-old chronic alcoholic male with pneumonia and pulmonary abscesses. Because of ulcer pain, the patient limited his intake to fluids in the 6 weeks prior to his 5-day terminal admission, losing 7 pounds but maintaining good hydration. There was no dehydration and no vacuolization of the SOHN.

Case 29 was a 82-year-old man with coronary vascular disease, a myocardial infarction, and pulmonary emboli. Fluid balance was carefully watched during the 20-day terminal admission with no indication of dehydration. Microscopically, the SOHN showed no vacuolization.

Case 30 was a 50-year-old man with coronary vascular disease. The terminal course following a myocardial infarction gave no evidence of dehydration. There was 1+ small vacuolization of the SOHN.

Group III: Group III consisted of those cases with inconclusive clinical records with respect to hydration. As might be expected, this comprised the largest group and consisted of 43 cases. There was a wide variety of pathology at autopsy. Consequently, the microscopic findings in this group were variable, there being 3 cases of 4+ vacuolization, 3 with 3+ to 2+, and 29 with 1+. The vacuolization size showed a similar variation. It is perhaps significant that 2 of the 4+ cases had extensive burns and were receiving fluid therapy; the other 1+ case had incomplete records.

DISCUSSION

We found, in a clinically dehydrated group of cases, large cytoplasmic vacuolization in the majority of SOHN and PVN neurons. When vacuolization was absent or mild, we found no clinical evidence of dehydration. That dehydration was a factor in the etiology of the marked neuronal vacuolization in these cases is suggested by earlier animal studies in dehydration (3-8) and by our finding of dehydration as a common clinical experience.
A. Neuronal Changes in the SOHN and PVN in Experimental Animals

**Rats:** Hillarp found that an intravenous injection of 5 per cent saline to rats resulted in chromatolysis of neurons in the supraoptic and paraventricular hypothalamic nuclei (3). When given 1.5 to 2.5 per cent saline instead of water for 5 weeks, the author found neuronal and nucleolar enlargement involving only the SOHN and PVN.

Leveque gave 1.5 to 2.5 per cent saline to 20 adult rats for 1 to 5 weeks (4). Cytoplasmic vacuolation was noted in neurons of the SOHN and PVN. No changes were found in other parts of the brain. In a number of cells the nucleus was displaced to the periphery by a large cytoplasmic vacuole. Some cells appeared to have disappeared. The author, reviewing histological sections from the cerebral cortex, cerebellum, and medulla in these animals, found no neuronal abnormality. Leveque concluded that the SOHN cells progressed through a sequence of changes under saline stress. The Nissl substance first decreased in quantity, the nucleolus enlarged, and the neuronal cytoplasm became vacuolated. If further stressed, the neuron degenerated with disappearance of the cell.

Sloper and King subjected rats to 3 per cent saline for 14 days. Studying sections of the hypothalamus, they found an increase in size of neuronal bodies, nuclei, and nucleoli in the SOHN; this change was less obvious in the PVN (5). Nissl substance concentrated marginally, and neurosecretory material was sparser. The authors found also active uptake by these neurons of labeled methionine and cysteine, indicating active protein synthesis.

**Mice:** Stutinsky et al., studying neuronal ultrastructure in the mouse after injection of 5 per cent saline for 8 to 15 days, found numerous small lipid inclusions in the SOHN and PVN neuronal cytoplasm (6).

**Frogs:** Hikl studied the SOHN in the Rana esculenta (7). He found, in dehydrated animals, loss of neurosecretory substance with some empty zones.

**Dogs:** Bargmann produced marked vacuolation of neuronal cytoplasm of the SOHN in dogs by oral administration of 5 per cent saline (8).

Thus, in a number of species of animals, changes have been found in the SOHN and PVN neurons after stressing the animals with hypertonic saline or by dehydration. These changes included enlargement of the neuron bodies, dissolution of Nissl substance, neuronal nuclear and nucleolar enlargement, and neuronal cytoplasmic vacuolation.

B. Neuronal Changes in the SOHN of Man Described by Others

Scharrer and Scharrer described a wide variability in the morphology of the human SOHN that they had noted in their work during the previous decade (9). No attempts at clinical correlation were made.

Palay reviewed neuronal morphology in the human SOHN in cases with assorted terminal diagnoses (10). Among the neuronal changes were small cytoplasmic vacuoles; in one case there were large vacuoles displacing the nucleus peripherally. He stated that the vacuoles resembled myelin figures, and
suggested they may be post mortem artifact. No statement concerning the patients' hydration was made.

Lundberg described 3 different types of Gomori positive material in SOHN neurons (11). In a few cases he found prominent vacuolation which sometimes contained small amounts of Gomori staining material. Since this vacuolation was noted only in the SOHN and PVN he felt the vacuoles may be due to the dissolution of Gomori positive neurosecretory material in the fixation process. Again hydration status of any of the patients was not discussed.

Von Buttlar, studying neurons of the SOHN and PVN of man, concluded that with increasing age the neuron nucleus becomes peripherally located with a decrease in perinuclear Nissl substance (12). He noted cytoplasmic vacuoles in 3 cases not showing age dependence. The hydration status of these patients was not mentioned.

Maccabini and Van Buren counted neurons of the SOHN and PVN of 2 control subjects and 8 patients with hypophysectomies (13). Cell counts of the SOHN in the 2 control cases ranged from 56,000 to 76,000 neurons while neuronal counts in operated cases ranged from 670 to 37,480. The authors also found, in a specimen surviving hypophysectomy for 6 weeks, cytoplasmic vacuolation of the remaining neurons. In specimens surviving for a longer period, the neurons were normal or hyperchormatic and small.

Green et al. studied the SOHN in a case of hereditary diabetes insipidus and a case of idiopathic diabetes insipidus (14). They found a loss of neurons and an astrocytosis in the SOHN and PVN in these cases.

Thus, in man, neuronal vacuolation in the SOHN and PVN has been noted with varying suggestions made on their significance including post mortem artifact, dissolution of hormone, and possibly stress. Little attention has been given to the hydration status of these cases.

C. Neuronal Vacuolar Changes at Other Sites

Bullous neuronal vacuolation has been described in other conditions in man and animals. These changes have been found in anterior motor horn neurons of the spinal cord in poliomyelitis (15), in amputees (16), in the anterior horn and Clark's column neurons in pemphigus (17), in tellurium poisoning (18), in kuru (19), in serapic (20), in amyotrophic lateral sclerosis (21), in ultrasound injury (22), and in acute anoxic death (23).

D. Relationship of our Findings to other Observations in Man and Experimental Animals

Experimental studies on rats, mice, frogs, and dogs have shown several types of neuronal change in the SOHN and PVN associated with dehydration of hypertonicity. Similar neuronal changes including neuronal and nucleolar enlargement, some chromatolysis, and cytoplasmic vacuolation were also found in our human cases subjected to dehydration. However, the neuronal vacuolar change in our dehydrated cases was more severe than that noted in the ex-
perimental animals. There are two possibilities for this discrepancy: 1. that the stress in the human cases was different from that given the animals, resulting in more marked change, or 2. that the neuronal vacuolation was due to other factors such as post mortem artifact or other types of injury.

In the animal studies the terminal stresses, mode of death, and post mortem interval were under close experimental control. Human material is obviously not under such rigid control. Terminal stresses, except for anoxia and dehydration, have not been evaluated, and the mode of death is only rarely mentioned. A post mortem interval is inevitable and the artifact this introduces is difficult to evaluate. However, insight can be gained by: 1. examination of more cases in man with attention to clinical details including discussion with the clinician attending the patient terminally, and 2. subjecting experimental animals to a stress similar to that found in humans, i.e. prolonged dehydration and a post mortem interval before brain fixation.

Thus, we suggest, on the basis of experimental animal findings, and on the basis of our findings, that morphological changes do occur in neurons of the SONH and PVN of man in states of severe dehydration, and that these mild changes continue, after death, producing the more marked neuronal vacuolation.

**SUMMARY**

The supraoptic hypothalamic nuclei and the paraventricular nuclei were studied in brains from 73 autopsies. Eleven of the autopsies were from cases with severe terminal dehydration and 19 cases had a history of good hydration terminally. Neurons of the SONH and PVN in the dehydrated cases showed a marked cytoplasmic vacuolation in contrast to the normal neuronal morphology in the well hydrated cases. Neuronal and nucleolar size was also increased in the dehydrated cases, as compared to the control cases. Although experimental studies on mouse, rat, frog, and dog have generally shown some neuronal and nucleolar enlargement, vacuolar changes, although real, are less marked in dehydrated animals than in our cases. We suggest that dehydration has an effect on the neurons of the SONH and PVN, possibly as a severe stimulus to produce more ADH, resulting in mild morphological change as seen in experimental animals. After death these vacuoles may continue to enlarge as a post mortem artifact superimposed upon these cells.

**REFERENCES**

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