Institute of experimental Medicine and Surgery,
University of Montreal, Montreal (Canada)

ON THE STRUCTURAL CHANGES PRODUCED IN THE BRAIN AND HYPOPHYSIS BY PARENTERAL ADMINISTRATION OF HIGHLY HYPERTONIC SOLUTIONS

By HANS SELYE

In the course of our studies concerning the production of adrenal changes by the parenteral administration of highly hypertonic solutions (*Selye*, in press), we incidentally observed certain peculiar structural changes in the brain and hypophysis, which we believe deserve special attention.

Rats given intraperitoneal injections of highly hypertonic solutions of various substances almost immediately develop an extraordinarily pronounced venous and capillary congestion of the entire cephalic region, especially the brain and hypophysis. This is often accompanied by actual hemorrhages into the meninges, the anterior pituitary, and the hypophyseal cleft. Such lesions were consistently observed in all our animals treated with a variety of hypertonic fluids (e.g., urea, glucose, NaCl). The parenteral administration of 10% NaCl, however, unlike our other hypertonic solutions, caused—in addition to this purely vascular change—a peculiar lesion in the nuclei of nerve cells throughout the brain. Neither our own search of the literature nor our discussions with a number of experienced neuropathologists have given us any indication that such lesions have ever been observed before.

Although the conditions under which these changes occur are rather extreme, we wish to describe them here, as they may furnish a lead for further studies concerning the response of nervous and hypophyseal tissue during certain types of extreme stress.

Experimental Animals and Materials.

Forty female Wistar albino rats, having a body weight of 150 to 175 g. were subdivided into four groups of eight animals each, as follows: Group I, normal untreated controls; Group II received 4 cm^3 of a 20% urea solution intraperitoneally; Group III, 4 cm^3 of 60% glucose solution intraperitoneally; Group IV, 4 cm^3 of a 10% NaCl solution intraperitoneally.

The concentration of urea, glucose and NaCl in the four groups was so adjusted as to yield approximately equally hypertonic solutions. In this manner we felt that the changes due directly to the hypertonicity of our solutions should be equal in all three groups, and any qualitative differences could therefore be ascribed to the specific actions of the three compounds employed.

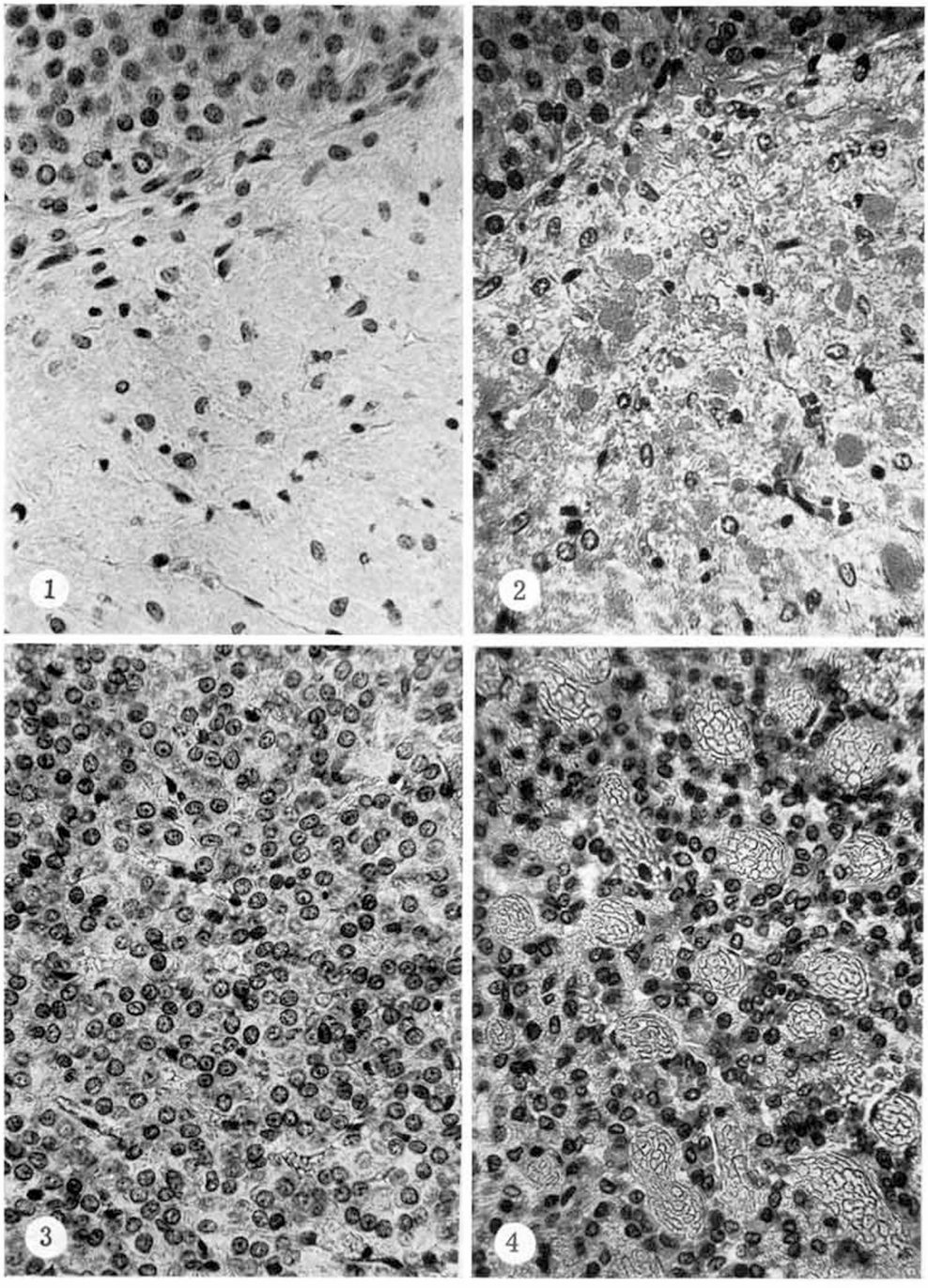
Observations.

Immediately following the injection of the hypertonic solutions, all animals which received intraperitoneal injections were greatly excited. The rats which received NaCl exhibited severe convulsions and subsequently coma; those treated with urea developed an intense trembling, somewhat reminiscent of the uremic syndrome in the rat; those given glucose were least affected.

All but two rats of the NaCl-treated group died or had to be killed in a moribund condition within the first hour. Two of the urea-treated animals and one of the glucose-injected ones died, while several rats in each of these last two groups became moribund during the two hours following the injection. They were killed at the end of the second hour.

Immediately after death, the brain, spinal cord, celiac ganglia, and pituitary were removed and fixed in Suza solution, in order to prevent any possibility of post-mortal autolysis. After fixation, these tissues were imbedded in paraffin, sectioned at 6 μ, and stained with hematoxylin and eosin.

Upon naked eye inspection, the most striking change (apart from the peritoneal irritation due to the local effect of the hypertonic solutions) was an extraordinary hyperemia of the brain, the meninges and the pituitary. This was very obvious and approximately of equal intensity in the animals of all the treated groups. In many rats, the venous engorgement was so extreme that it led to subdural hemorrhages of great intensity. These were usually visible even before the skull cap had been removed, since the massive accumulations of blood were readily visible through the translucent calvarium after ablation of the skin. Occasionally, hemorrhages occurred also into the pituitary cleft and distended the latter considerably.



Histologic examination revealed that, in all the treated groups the hypophyseal cleft was dilated either with serous fluid or with free blood. The posterior lobe showed intense imbibition with an eosinophilic edema fluid (Figs. 1 and 2). We had shown previously that the pituicytes of the posterior lobe may be stimulated to mitotic proliferation by more chronic overdosage with NaCl in the rat (*Selye* [1950]). Such mitotic proliferation was not observed in the present acute experiments.

Simultaneously with these changes in the cleft and the posterior lobe, there occurred a marked dilation of the anterior-lobe sinusoids, which sometimes gave the pituitary the aspect of a cavernous body (Figs. 3 and 4).

The above-mentioned changes were relatively non-specific in that they were produced by any of the hypertonic solutions we used. On the other hand, rather singular and presumably specific changes were observed in the brains of rats treated with NaCl. In all these animals, without exception, a large number of nuclei throughout the grey matter of the cerebral cortex, the cerebellum, and the basal ganglia underwent a peculiar change which we had never observed before under any other experimental condition. The nuclei first became somewhat vesicular, and the chromatin appeared to rearrange itself in the form of filaments. The nucleolus did not participate in this rearrangement and tended to remain essentially unchanged. At this time, the nuclei remotely resembled the aspect usually exhibited during the prophase of mitotic division.

Although adult nerve cells do not normally undergo mitosis, the possibility of such proliferation does appear to exist, at least in vitro (*Pomerat, C. M.* [1951]). It will be recalled, furthermore, that quite recently *Overman* and *Dortch* [1951] found mitotic figures in the retina, brains and optic nerves of rabbits infected with Semliki Forest virus.

We wish to emphasize that, in our NaCl-treated rats, these "pseudo-mitoses" only resembled mitotic proliferation, but did not

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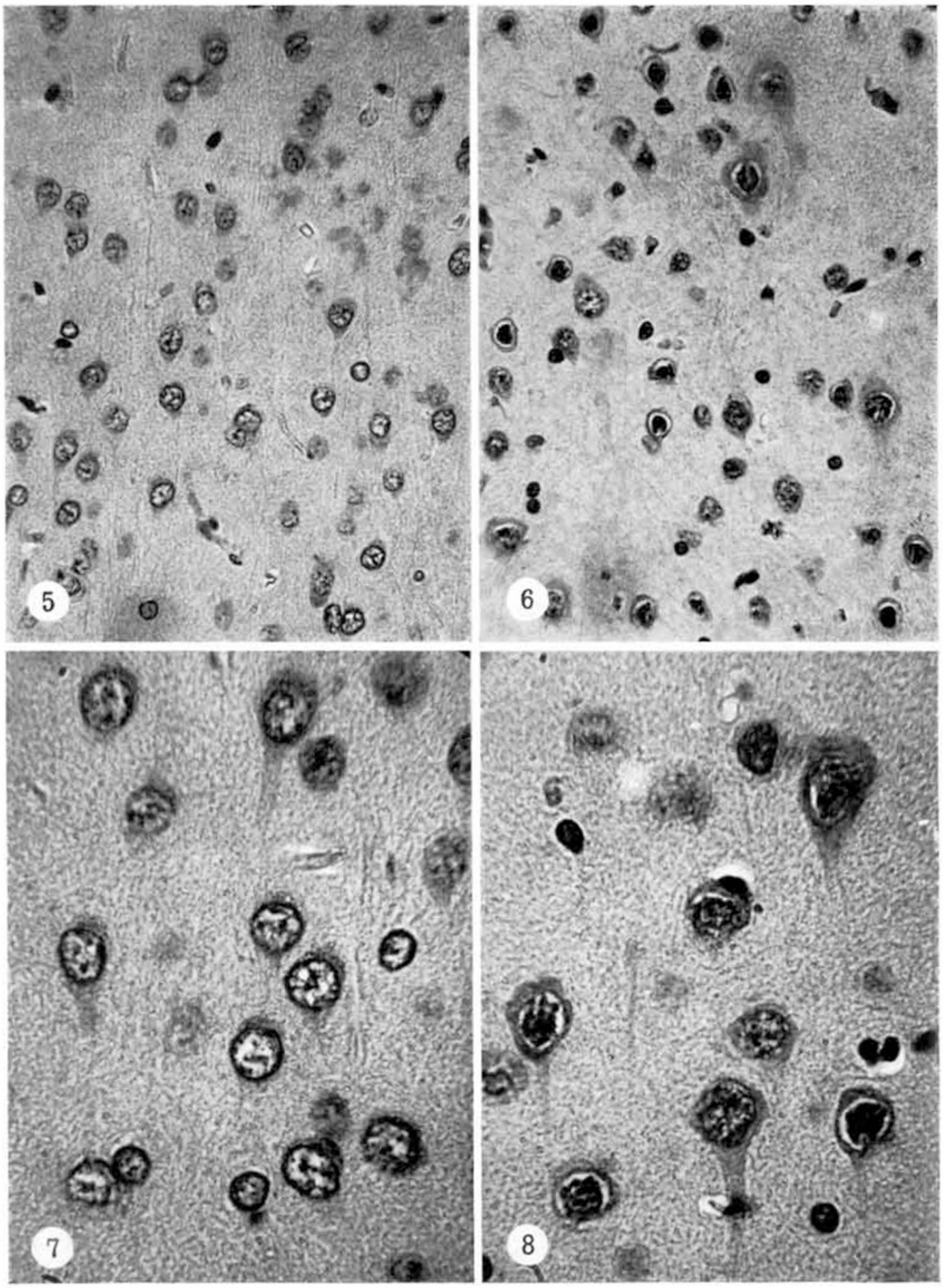
Fig. 1. Posterior lobe (with a small portion of the intermediate lobe near the upper edge of the field) of an untreated control rat. – *Fig. 2.* Edematous posterior lobe of an NaCl treated rat. – *Fig. 3.* Anterior pituitary of a normal control rat. – *Fig. 4.* Anterior pituitary of a rat which had received the hypertonic NaCl solution. Note the extraordinarily intense hyperemia as manifested by the dilatation and engorgement of the sinusoids; these take up the major part of the field and tend to compress the glandular cells situated between them.



actually progress to a point comparable to later stages in mitotic cell division. After the prophase-like rearrangement of the chromatin, the entire nuclear structure began to shrink away from the cytoplasm itself, and became separated from the latter by a clear space.

The significance of these widespread nuclear changes is obscure but the following points may deserve consideration:

1. The nuclear change appears to be specific for NaCl, since —at least under the conditions of these experiments—it was not duplicated by other solutions of comparable osmotic pressure.

2. There was a definite predilection for such "pseudo-mitoses" to occur in the cephalic part of the animal, which was also subject to the most intense hyperemia. Only in two instances have we seen comparable "pseudo-mitoses" in the ganglion cells of the adrenal medulla, and we never found them in the celiac plexus or in the spinal cord. This suggests that the change is not merely due to contact between any nerve cell and a hypertonic serum.

3. While the changes were most obvious in animals which actually died from NaCl overdosage, they also occurred in rats which—though in coma or convulsions—had to be killed at the end of the experiment. This shows that the change is not due to post-mortal decomposition, especially since in all instances the tissues were removed immediately after respiration stopped.

4. Finally, neither the nuclear changes nor the cephalic hyperemia are due to the particularly intense non-specific stress produced by NaCl, since they did not occur in any among a large number of experiments on rats (not recorded here in detail) in which equally intense and rapidly fatal stress was produced by the intraperitoneal administration of formalin, turpentine, mustard powder, croton oil, and other intense irritants.

Fig. 5. Section through the grey matter of the cerebral cortex of an untreated control rat. In this, as in all other photographs on this plate, the regions illustrated come from the convexity of the cerebral hemisphere, about 2 mm lateral from the sagittal sinus, on cross-section traversing the brain at the height of the tuber cinereum. Note normal appearance of ganglion cells. – *Fig. 6.* Ganglion cells in the cerebral cortex of a rat which had been treated with hypertonic NaCl. The stages in the development of characteristic nuclear changes can be followed by comparing the various cells in this field. The nucleus first begins to swell, then the chromatin rearranges itself in prophase-like fashion; eventually, the entire nuclear material shrinks away from the cytoplasm, thus becoming separated from the latter by a clear space. – *Fig. 7.* High magnification of fig. 5. – *Fig. 8.* High magnification of fig. 6.

Summary.

Rats given intraperitoneal injections of intensely hypertonic NaCl solutions, exhibit a singular rearrangement of chromatin in the nuclei of their nerve cells, particularly those situated in the grey matter of the cerebral cortex, the basal ganglia, and the cerebellum. The nuclei assume an appearance remotely reminiscent of the first stage of mitotic division.

No lesions of this kind have been observed in rats given comparable doses of equally hypertonic urea or glucose solutions.

Intraperitoneal treatment with hypertonic NaCl solutions also causes an extraordinarily pronounced hyperemia of the brain, the meninges, and the pituitary. This is frequently accompanied by massive hemorrhage into the meninges or the hypophyseal cleft. Unlike the nuclear changes, the hyperemia of the brain can also be reproduced with other hypertonic solutions (glucose or urea).

Intraperitoneal injection of equally damaging doses of other irritants, such as formalin, turpentine, mustard, croton oil, etc., elicited neither hyperemia nor nuclear changes in the brain. Hence, the latter manifestations cannot be ascribed merely to non-specific peritoneal irritation.

Résumé.

Lorsque l'on injecte dans le péritoine du rat des solutions hypertoniques de NaCl, on peut constater une modification de la répartition de la chromatine des neurones, plus particulièrement dans le cortex cérébral, les ganglions de la base et le cervelet. Les noyaux offrent un aspect rappelant celui des premiers stades de la mitose.

Aucune modification semblable ne fut observée chez des rats ayant reçu des injections de glucose ou d'urée en solutions hypertoniques.

Des injections de NaCl en solution hypertonique déterminent de plus une hyperhémie considérable du cerveau, des méninges et de l'hypophyse, fréquemment accompagnée d'hémorrhagie massive au niveau des méninges ou du sillon hypophysaire. L'hyperhémie cérébrale peut être reproduite toutefois à l'aide d'autres solutions hypertoniques (glucose ou urée).

D'autres substances irritantes, telles que la formaline, la térébenthine, l'huile de moutarde ou de croton ne provoquent, après introduction dans la cavité abdominale, ni hyperhémie cérébrale ni

modifications nucléaires. Celles-ci ne peuvent pas être le résultat d'une irritation non spécifique du péritoine.

Zusammenfassung.

Wenn Ratten intraperitonaeale Injektionen von hypertonischen Kochsalzlösungen erhalten, so weisen die Kerne der Nervenzellen eine besondere Chromatinanordnung auf, ganz besonders diejenigen, welche in der grauen Substanz der Hirnrinde, der Basalganglien und des Kleinhirns gelegen sind. Die Kerne nehmen ein Aussehen an, das an die ersten Stadien der Mitose erinnert. Keine derartigen Veränderungen konnten beobachtet werden, wenn die Ratten entsprechend hypertonische Harnstoff- oder Glucose-Lösungen erhielten. Durch die hypertonischen Kochsalzlösungen wird auch eine sehr starke Hyperämie des Gehirns, der Meningen und der Hypophyse hervorgerufen. Sehr häufig findet sich auch eine Hämorrhagie der Meningen oder der Hypophyse. Die Hirnhyperämie kann auch mit anderen hypertonischen Lösungen (Glucose, Harnstoff) hervorgerufen werden. Intraperitonaeale Injektionen von Formalin, Terpentin, Senf, Krotonöl rufen im Gehirn weder eine Hyperämie noch Kernveränderungen hervor.

Acknowledgements.

These investigations were performed with the aid of the Fondation Rhéaume de l'Université de Montréal.

The author is indebted to Mr. *Kai Nielson* and Mr. *Robert Gangnon* for the preparation of the microphotographs.

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Received May 6th, 1952.