NEUROPATHOLOGICAL CHANGES IN NITROGEN TRICHLORIDE INTOXICATION OF DOGS*

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Bread is the staff of life for a large portion of the world’s population. Hence, its composition has been a major concern of physician and government alike, particularly since we became vitamin conscious. The advantages of the various brands of so-called wholewheat bread have been widely advertised; but the consumer, by and large, has been little impressed by these nutritional considerations. The public in general continues to request white bread and the housewife, specifically, white flour—as had her ancestors as far back as can be traced. In addition, yellow wheat meal is notoriously hard to bake and has a tendency to become sticky and unattractive because the dough does not rise so well. The baker, therefore, referred the consumer’s desire to the miller, with the result that the treatment of wheat flour with a bleaching and softening agent has become the universal custom in the wheat-consuming Anglo-Saxon countries [Dunlap (1)]. The agent most used for this purpose during the last 25 years has been Nitrogen Trichloride (NCl₃), commercially called “agene.” There is no case on record of any untoward effects on human beings from bread so treated.

In 1946, Sir Edward Mellanby (2) of the British National Research Council—on the suggestion of a dog-minded miller—began a systematic investigation of the possible connection between agene-treated flour and a disease of dogs which had troubled their owners in Great Britain during the last score of years, viz. “running fits”, also called “dog hysteria”. He demonstrated that this disease could be produced in growing dogs by including in their diet flour that had been improved and bleached by NCl₃, the agene process. The same flour when untreated did not produce the nervous condition. “Affected dogs returned towards normal, and the typical hysteria and fits stopped when the agenized flour was removed from the diet and replaced by unimproved flour of the same grist.”

The problem was immediately taken up and elaborated in various laboratories of the U. S. A. It turned out that the action of NCl₃ was species bound and neurotoxic only to dogs, ferrets and rabbits, but not to guinea pigs, cats, monkeys and men [Pollock and Kark (3)]. The electroencephalogram of dogs suffering from “canine hysteria” or “running fits” was similar to that of epilepsy in man. Progressive EEG changes terminating in seizures could be produced by feeding or injecting one of the proteins of flour such as gliadin, glutamin, casein, after treatment with NCl₃ [Silver et al (4)]. However, no noxious effects were obtained in men even when enormous doses of the various agenized proteins were given. Twenty persons, some of them known epileptics, were placed on a diet containing 20 times the usual amount of agenized flour for 2

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to 18 weeks. Numerous EEG records were taken and no pathological EEG seen [Erickson and Gilson (5)].

Nevertheless, the Federal Food and Drug Administration in cooperation with the Food and Nutrition Board of the National Research Council requested that NCl₃ should not be used as a bleaching and softening agent and should be replaced by a chemical which is harmless even for dogs. Consequently, a new agent (ClO₂) has been developed, and the Federal Food and Drug Administration agreed on the use of it after extensive experiments had shown that no clinical, physiological, electrical, or pathological phenomena could be produced in any species of animals even after feeding them flour so treated in quantities many times the normal content of bread.

The experimental effect of NCl₃ treated flour in the dog’s diet is almost immediate. In one or two days the animal becomes sullen, withdrawn and leans against a corner, probably because of the high degree of ataxia and disturbance of equilibrium. Suddenly, the dog runs forward some ten or fifteen feet until it falls. This episode may be followed by twitching of muscle groups and eventually by a convulsion lasting up to two minutes. There may be one or two of these attacks a day or a real status epilepticus may develop. If the agenized flour is removed promptly from the diet, the animal may return to its usual health: if not, the animal succumbs, as a rule in a short time.

Neuropathological examinations of the central nervous system of dogs with this type of epilepsy after being fed NCl₃ treated flour, in comparison with the brains of animals which had been on a concentrated ClO₂ diet, are interesting from various points of view. The findings in all animals examined are fairly uniform and differ only quantitatively from one another.

The cerebral cortex of dogs who had suffered from fits after having been on a NCl₃ diet shows severe disturbances in both the grey and the white matter. The most severe alterations are concentrated in the deeper layers of the cortex where large areas have a lace-like appearance (fig. 1) with the nerve cells pyknotic or absent. In some areas capillaries are sprouting, in others the blood vessel walls are also in degeneration. In other words, the cortex presents the picture of a patchy necrosis with beginning liquefaction. The arrangement of the lesions is not visibly connected with the course of blood vessels. This impression—gained on the basis of slides stained with phosphotungstic acid hematoxylin—is confirmed by other staining methods.

Many nerve cells show signs of pyknosis with their apical process deeply stained in corkscrew fashion. The cortical architecture is maintained despite the patchy loss of nerve cells (fig. 2). The cell degeneration appears to have taken place in a precipitous manner since there are no macrophages or compound granular corpuscles, no products of disintegration, and only an abortive reaction of the astrocytes is visible.

This process is diffusely distributed all over the cerebral cortex though changing in intensity from one convolution to the next. In some parts the cellular architecture changes abruptly, even within the same convolution, from normal to an area in which all nerve cells are shrunken, dark-stained or disintegrated. Empty spaces (probably filled with fluid during life) surround the cells and large vacuoles are encountered within the cell bodies. Such areas of liquefaction necrosis are often surrounded by a zone in which the edema is not conspicuous but in which the nerve cells have lost their ability to stain with basic dyestuffs to such a degree that, at first glance, the cells seem to be absent. However, hematoxylin-stained or silver-impregnated preparations reveal that quite a number of nerve
cells is still present. Such cells have dark, shrunken nuclei and fenestrated, pale cytoplasm, the outline of which is vague (fig. 3, middle). The appearance of these cells is sometimes so grotesque (fig. 3, right) that they could be regarded as artefacts due to poor fixation, were it not that these changes are confined to small clusters of cells around which the nerve tissue appears entirely normal.

![Image: Cerebral cortex. Patchy liquefaction necrosis in its deeper layers giving the tissue a lace-like appearance. Photomicrograph; phosphotungstic acid hematoxylin stain; X 50.](http://jnen.oxfordjournals.org/)

The statement that the process is a diffuse one requires some limitation, since in all animals the hippocampus is most severely hit. Both the pyramidal layer and the fascia dentata show a complete loss of architecture, all cells are shrunken and pyknotic, and many cells are lost. The cells of the pyramidal layer stain poorly, are fenestrated, and show a yellow substance (possibly lipochrom) in large vacuole-like spaces. The pericellular glial network is abnormally dense (figs. 4a and b). The cells of the fascia dentata consist of small, almost amorphous granules which barely resemble nerve cells (figs. 5a and b).
Fig. 2. Cerebral cortex. Focal loss of nerve cells. The remaining cells are pyknotic. Edema is present, indicating acuteness of the process. Photomicrograph; Phosphotungstic acid hematoxylin stain; \( \times 145 \).

Fig. 3. A—Nerve cell in dentate nucleus, showing extensive vacuolation; B— and C— Large nerve cells of third cortical layer showing hydropic swelling and degeneration; C— Three ghost (shadow) cells. Photomicrograph; Cresyl violet stain; \( \times 650 \).
Fig. 4 a. (Dog on ClO₂ treated flour). Orderly arrangement of the cells in the pyramidal layer of Ammon's Horn. Cell base toward lower margin of illustration, apex directed upward. Photomicrograph; Cresyl violet stain; × 660.

Fig. 4 b. (Dog on NCl₃ treated flour). Loss of cytoarchitecture of pyramidal layer in Ammon's Horn. The individual cells are shrunken and pyknotic, their nuclei are swollen, often devoid of chromatin. The cell processes are lost, and many cells have disappeared leaving free spaces. Photomicrograph; Cresyl violet stain; × 660.
The *cerebellum* is another part of the brain in which characteristic changes are found in all animals kept on a NCl$_3$ treated diet. The Purkinje cells are poorly preserved. They are pale, their Nissl substance is absent, as are their antler-like dendrites in the molecular layer, and the afferent nerve fibres normally ending around them. In animals showing less severe lesions, the dendrites of the Purkinje cells may be followed for quite a distance into

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**Fig. 5 a.** *(Dog on ClO$_2$ treated flour 100 times the normal concentration).* Normal arrangement of cells in granular layer of the fascia dentata. In the left upper corner is a large pyramidal cell. Photomicrograph; Cresyl violet stain; × 660.

**Fig. 5 b.** *(Dog on NCl$_3$ treated flour).* Severe damage of the nerve cells in granular layer of fascia dentata. The less damaged cells are swollen, their nuclei are pyknotic, and their processes are stained for abnormally long distances. The majority of the cells have disappeared, leaving behind spaces often filled with glial nuclei. In the upper left corner there is a degenerated cell of the pyramidal layer. Photomicrograph; Cresyl violet stain; × 660.
the molecular layer, but even here no climbing nerve fibres and no glia network are visible around them. In fact, the afferent nerve fibres are absent also in their course through the granular layer. The most conspicuous changes are found in the system of the basket cells,

![Image of cerebellar cortex](image_url)

**Fig. 6 a.** (Dog on ClO₂ treated flour). Cerebellar cortex. The nerve fibers of the afferent cerebellar tracts ascending from below the granular layer, and the horizontal fibers of the molecular layer interconnecting the Purkinje cells and ending around them in basket shape. Bodian's silver impregnation for axis cylinders; X 660.

**Fig. 6 b.** (Dog on NCl₃ treated flour). Cerebellar cortex. Absence of ascending nerve fibers in the granular layer. Their terminal arborization around the prolongations of the Purkinje cells is shrivelled and rarefied. The intercellular horizontal layer of fibers and their basket-shaped endings around the Purkinje cell bodies are barely visible. Bodian's silver impregnation for axis cylinders; X 660.

the axons of which run horizontally and give off basket-shaped arborizations to six and more Purkinje cells. These baskets are often poorly impregnated around well preserved Purkinje cells and entirely absent when the Purkinje cells have disappeared (fig. 6). Likewise, the horizontal layer of fibres interconnecting the Purkinje cells is rarefied or absent. In other words, both the afferent and efferent cerebellar systems are severely impaired.

In addition, distinct though minor changes are present in the nerve cells of the dentate
and roof nuclei. Here the nerve cells show one large vacuole added to many small ones. There are a few remnants of eccentrically located chromatin (fig. 3, left), the nucleus is enlarged, bright, with a small nucleolus. The cell prolongations are curved and stain at greater distances from the cell body than normally.

Comment. The pathological process in the central nervous system of dogs kept on a diet containing NCl₃ treated flour, is characterized by a patchy liquefaction necrosis in the deep layers of the cerebral cortex and of the "U" fibres extending slightly into the white matter. These changes are wide-spread in the cerebral cortex but most pronounced in the hippocampus. Severe changes of a different character are found also in the cerebellum, specifically in the Purkinje cells, their afferent and efferent fibres, and in the inter-connecting system of the basket cells and their axiscylinders. Milder alterations are found in the nerve cells of the dentate and the roof nuclei.

The pathological changes seem to affect pre-eminently the nerve cells, to a lesser degree the glia, and are not accompanied by any marked degree of reaction of either glia or mesodermal tissue.

No pathological changes are found in the peripheral nerves or in any of the body organs of these dogs, other than the brain.

In contrast with the described findings in dogs fed with NCl₃ treated flour, no changes whatsoever could be recognized in the nervous system of dogs which had been on a diet containing ClO₂ treated flour even in doses 100 times the normal content.

DISCUSSION

It is a well-known fact that infections and intoxications alike may be species bound. Good examples of the first group are Syphilis and Poliomyelitis. Both fail to occur spontaneously in any animal, and can be transferred artificially only to a few species with great difficulty. Examples of the second group are numerous.

The predilection of the cortical disturbances for the hippocampus have been reported in a number of conditions connected with insufficient blood supply. Cell loss in certain areas of the hippocampus—called Sommer's sector—were once believed to be characteristic of epilepsy. Spielmeyer (7) has shown that the hippocampus is most vulnerable to decreased circulation as in arteriosclerosis. Thorner and Lewey (8) have demonstrated a similar sensitivity of the cells of the hippocampus to true anoxia. This experience raises the question whether the toxicity of the reaction product of NCl₃ with a protein constituent may be due to its effect upon one of the respiratory enzymes.

Another similarity between the pathological findings in poisoning with NCl₃ treated flour and pure anoxia consists in the metabolic changes in the nerve cells which prevent them from taking up basic aniline dyestuffs while they can still be visualized by means of hematoxylin staining and silver impregnation. It is understandable that this first stage of cell damage is not necessarily accompanied by glia or mesodermal reactions. It is self-explanatory that repeated intoxications of comparatively short duration will not increase the initial effect—as stressed in so-called chronic CO poisoning. If, on the other hand, the NCl₃ action is powerful from the beginning, the glia becomes destroyed along with the nerve cells and only the mesenchyme is left to produce histiocytes and eventually fibroblasts. This phase did not play any rôle in our NCl₃ dogs because these dogs (as a rule) developed seizures after being exposed to the agenized
flour for not more than two or three days, and if the causative agent was not withdrawn, they invariably died. The experiment can be repeated several times but each single exposure has to remain relatively light and of short duration. The situation reminds one of the pharmacologic action and the pathologic results in the case of streptomycin [Winston et al (9)], and of the findings of Haymaker et al. (10) in the cerebellum of dogs poisoned with DDT. They also have described nerve cell changes without glial reaction.

The loss of the “U” fibers connecting adjacent cerebral convolutions is another feature of the predominantly cortical nerve cell loss in contrast to the diseases of the white matter which, independent of the extent of the demyelination, leave the “U” fibers intact.

The cerebellum was of particular interest to the physiologists before the histopathological findings became known. Some of the physiologists were of the opinion that the seizures in these animals were—in contrast to all those observed in man—of cerebellar origin. This statement was based on the occurrence of bursts of fast spikes in the cerebellar electrocorticograms of animals under a diet containing NCl$_3$ treated flour or poisoned with DDT, obtained before and during their epileptoid attacks (11, 12). This seizure-like cerebellar ECG, if verified, would have been of singular interest in view of the pathological findings in the cerebellum. Unfortunately, this seems not to have been the case. Adrian had shown long ago that the normal EEG led off the cerebellum shows spikes of high frequency. In fact, the ink writer of the EEG apparatus used in connection with these NCl$_3$ and DDT experiments is much too slow an instrument to permit true records even of the normal electrical output of the cerebellum. Be this as it may, the focal cerebral changes are certainly sufficient to explain the excitatory phenomena which find their expression in convulsions. Again, one is reminded of the action of streptomycin or penicillin which, on intracisternal injection or local cortical application, may produce epileptoid seizures [Walker (13)].

The high degree of ataxia, astasia and abasia of the NCl$_3$ dogs is readily explained by the cerebellar changes. Thus, the clinical and pathological picture of dogs during a diet rich in NCl$_3$ treated flour is that of a combined cerebral cortical and cerebellar syndrome.

It remains to be seen whether “running fits” and “dog hysteria” will disappear in the future with the elimination of NCl$_3$ from flour and bread, whether they are only facilitated and triggered off by the chemical in predisposed animals or whether they are independent of it. Whatever the outcome of these observations will be, it is gratifying to find that both the U. S. Government and Industry have taken note of the noxious effects of NCl$_3$ as a bleaching agent of flour by replacing it promptly by ClO$_2$.

**SUMMARY**

Nitrogen trichloride, commercially called agene, is used in most wheat consuming countries to bleach and soften the hard-to-bake yellow wheat flour. This substance has been found to be toxic to dogs, rabbits and ferrets but not
to monkey, cat, guinea pig and man. Dogs when on a diet rich in NCl3 treated flour become atactic within a few days and develop running fits. EEG records of these dogs suggest that these fits are epileptoid in character.

The pathological changes in the central nervous system are characterized by a patchy liquefaction necrosis in the deep layers of the cerebral cortex and of the "U" fibers extending slightly into the white matter. These changes are widespread in the cerebral cortex but most pronounced in the hippocampus. Severe changes of a different character are found in the cerebellum, specifically in the Purkinje cells, their afferent and efferent fibers, and in the interconnecting system of the basket cells and their prolongations. Milder alterations are found in the nerve cells of the dentate and the roof nuclei.

This chemical is no longer employed and was replaced by ClO2, which is harmless even for dogs.

REFERENCES