

## INFLUENCE OF ACUTE ALCOHOL POISONING ON NERVE CELLS.

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(From the *Physiological Laboratory of Clark University, Worcester.*)

### PLATE XXVI.

IN 1895 Dehio \* demonstrated changes in the ganglion cells of the cerebellum produced by acute alcohol poisoning. In his research eight rabbits, of which two were controls and one, being diseased, was useless, formed the first series for experiment. Alcohol was administered by the stomach and by subcutaneous injection, the latter method producing the greatest result in the shortest time. The usual dose was 7 to 10 cubic centimetres of ninety-six per cent alcohol, reduced with water to about forty per cent, followed, as consciousness returned, by a further dose of 5 cubic centimetres, until in all 20 or 25 cubic centimetres had been given. Death occurred in from one to thirty-four hours, according to the amount given, though the rabbit living thirty-four hours was allowed to recover during the night.

Slides were prepared by Nissl's methylene-blue method. The alcohol effect was not observed with any certainty in those animals which died in the earlier stages of intoxication, while the others showed the following definite changes in the appearance of Purkinje's cells: The cells showed, instead of the normal, fine-meshed, stained network, fine irregularly arranged granules of approximately uniform size, while the normally unstained substance had taken on a pale blue tone. This change affected sometimes the whole of the cell, sometimes a part only, while nucleus, nucleolus, and the granules of

\* Heinrich Dehio, *Experimentelle Untersuchungen über die Veränderungen der Ganglienzellen bei der acuten Alkoholvergiftung. Centralbl. für Nervenheilkunde und Psychiatrie*, 1895, N. F., vi, 113.

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the cell processes remained unchanged. By no means all the ganglion cells were affected. In most cases whole rows of normal cells were observed, with pathologically altered cells scattered singly or in groups. Other parts of the central nervous system were studied in the same way, but nothing was observed to warrant any conclusions as to experimentally induced changes.

A second series included three dogs, one living five hours, one thirty hours, and one a normal control. The one living five hours showed nothing, but the one intoxicated for thirty hours gave more pronounced results than the rabbits.

With the hope of confirming and, if possible, extending Dehio's results, three adult male cats were chosen, and absolute alcohol was given to two of them, according to the following scheme:

	Cat I.	Cat II.	Cat III.
May 27th.	.....	.....	2.45 P. M.: 12 c. c. absolute alcohol, diluted to 40 per cent with a 0.6 per cent salt solution, injected into the abdominal cavity.
May 28th.	.....	.....	3 P. M.: 12 c. c. more, in the same way. Under the influence of alcohol all day; partially recovered during the following night.
May 29th.	7.30 P. M.: Killed by decapitation.	6 P. M.: 22.5 c. c. of absolute alcohol, 50 per cent solution in 0.6 per cent salt solution, injected into the abdominal cavity. Stupor commenced before the alcohol was all injected. 6.50 P. M.: Cat dead.	8.45 A. M.: 8 c. c., given as before. 2 P. M.: 8 c. c., given as before, neither dose quite sufficing to produce complete unconsciousness. 8.45 P. M.: 20 c. c. absolute alcohol, 50 per cent solution in 0.6 per cent salt solution, injected as before. 9 P. M.: 5 c. c. of the same injected subcutaneously, repeated after 5 minutes. 9.15 P. M.: Cat dead.

Blocks of tissue of equal size from corresponding portions of the right cerebral hemisphere and vermis, and from the spinal cord opposite the sixth pair of cervical spinal roots, were excised and placed immediately in one common vessel of absolute alcohol. Next morning, about twelve hours later, this was replaced by fresh alcohol, and on the following day, after about thirty-six hours, sections of a uniform thickness of 10 micra were cut under absolute alcohol and placed in separate dishes of fresh alcohol.

In staining, every endeavour was made to have specimens to be compared subjected to exactly the same conditions. Three corresponding sections, one from each animal, were lifted to a glass slide, drained, stained in the same large drop of methylene-blue solution, drained again and placed together in the same dish of anilin alcohol, washed, dehydrated and cleared together, and mounted under the same cover slip. So that with the single exception of placing the cut specimens in exactly similar dishes in the same alcohol between the time of cutting and the time of staining, *any three comparable sections were treated as one specimen.*

The sections were stained for from forty seconds to five minutes in the following solution:

Methylene-blue, Grübler, B. Pat.....	3.75
Sapo. venet.....	1.75
Distilled water.....	1,000.

Slightly better results were obtained if the slide carrying the pool of stain in which the sections lay was warmed until steam was given off. After staining, the sections were bleached in

Anilin oil.....	10
Absolute alcohol.....	90

for five or ten minutes, according to the depth of the stain. The anilin oil was then washed out in two or three washes of absolute alcohol, after which the sections were cleared in xylol and mounted in xylol balsam.

A variation in the intensity of the stain of any three sections of a comparable set was apparent from the first to the naked eye. The normal stained always bluer than the second of the series, and the second always bluer than the third or most heavily poisoned. A microscopical examination of the mounted specimens showed that while this variation in depth of tone was due in the cerebrum to variations in the tone of the cortical cells themselves, and of the Purkinje cells and cells of the granular layer in the cerebellum, yet in the gray matter of the cord it was produced in great measure by changes in the nuclei of the smaller cells. The large multipolar cells in the cord in both the pathological specimens, however, gave evidence of a great

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decrease in the number of their chromophile granules (see Plate XXVI, Figs. 10, 11, 12), and the cells from the cord of the animal under the influence of alcohol for the longest period of time (fifty-four hours and a half) show fewer granules than those from the animal dying in fifty minutes.

There are, of course, as was true of Dehio's \* specimens, some cells unaffected and some not so much so as others. To give an approximate expression of the proportion of pathological cells a scale was chosen corresponding to the scale of the three multipolar cells drawn for the accompanying plate, and the cells of the slide from which the drawings were made were counted and classified according to that scale. The following table will show the result of that classification:

	Class 1. (See Fig. 10.)	Class 2. (See Fig. 11.)	Class 3. (See Fig. 12.)
	Per cent.	Per cent.	Per cent.
Cat I, normal, 136 cells . . . . .	30·9	44·9	25·2
Cat II, 50 minutes, 107 cells . . . . .	24·3	31·8	43·9
Cat III, 54½ hours, 115 cells . . . . .	5·2	29·6	65·2

In the cerebellum (see Figs. 7, 8, and 9 of Plate XXVI) a great decrease in the number of granules in the pathological cells is also to be observed. All the Purkinje cells from the three specimens on the slide from which the drawings have been made were counted and classified according to the scale which the drawings show. The following is a tabulation of the result:

	Class 1. (See Fig. 7.)	Class 2. (See Fig. 8)	Class 3. (See Fig. 9)
	Per cent.	Per cent.	Per cent.
Cat I, normal, 356 cells . . . . .	81·2	18·8	· . . .
Cat II, 50 minutes, 286 cells . . . . .	1·1	72·4	26·5
Cat III, 54½ hours, 309 cells . . . . .	0·7	54·7	44·6

The cerebellum gives much more definite results than the cord, for, whereas in the section from the normal cat we have only 18.8

\* Dehio, *loc. cit.*

per cent of the cells showing even a moderate deficiency in granules, we have in the two pathological sections only 1.1 per cent and 0.7 per cent respectively which can be compared with the normal cells as to granulation; and, too, the section from the animal receiving alcohol for the longer time shows in its cells a greater decrease in granules than the other.

To estimate, if possible, the extent of other changes in the ganglion cells of the cerebellum, measurements were made for twenty nucleated cells in each section, those cells being chosen which were nearest to the cell drawn for the plate from each section. These measurements will be given with the reservation that they may be an expression of variations due to original differences in the animals used in the experiment.

	Average long diameter of cell.	Average transverse diameter of cell.	Average mean diameter of nucleus.	Average diameter of nucleolus.
Cat I, normal . . . . .	30·1 $\mu$	20·95 $\mu$	.....	2·94 $\mu$
Cat II, 50 minutes . . . . .	28·3 $\mu$	23·45 $\mu$	13·15 $\mu$	2·85 $\mu$
Cat III, 54½ hours . . . . .	29·1 $\mu$	17·4 $\mu$	11·25 $\mu$	2·76 $\mu$

It was found impossible to measure the nuclei of the cells in the section from the normal cat with an oil-immersion lens, on account of the heavy granulation always present. Very little importance, too, can be attached to the measurements of the cell diameter, for a small difference in the plane of the section would make an enormous difference, more especially in the apparent transverse diameter of the cell. But measurements of the nucleoli are absolute, and were taken accurately, so that the serial decrease in diameter must be due either to a constant original difference in the research animals or to an alcohol effect, equally constant, which must be decided by further experiment.

In the cerebrum almost all of the cells of a section are of the type shown in the corresponding figures of Plate XXVI. Figs. 1 and 2 are from the normal cat; Figs. 3 and 4 are from Cat II; Figs. 5 and 6 are from Cat III. So few exceptions were found that a count was considered unnecessary. No attempt was made to measure nuclei or

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nucleoli, on account of the difficulty of selecting cells that could be accurately compared; but if further experiment should show the importance of such measurements, the specimens will be gone over again for that purpose.

No alteration of the nucleus or disintegration of the nucleolus, such as Berkley \* describes for chronic alcoholism in rabbits, has been observed. Fig. 11 shows what appeared to be vacuolation of the nucleolus—the only such case found.

Preparations by the quick Golgi method, and by the phosphomolybdic-acid method used by Berkley, showed nothing conclusive as to the presence of moniliform swellings of the cell dendrites and degeneration of contact granules, such as are described by Andriezen † and Berkley ‡ for chronic alcoholism.

With regard to the clearness of results obtained in this experiment, it may be noted that Dr. Hodge, # in his work on fatigue of nerve cells, obtained always much clearer demonstrations of the fatigue effect in cats than in rabbits or frogs.

Since the results obtained in this experiment are a confirmation and extension of Dehio's work, already referred to fully, it has been thought better to publish. With regard to the significance of the changes described, much more can possibly be said when the methylene-blue staining method has been applied for the demonstration of normal and abnormal fatigue effects in the nerve cell in a series of experiments already contemplated for the laboratory of Clark University.

This experiment is one of a series on the physiology of alcohol now being carried on for the Committee of Fifty in the Physiological Laboratory of Clark University, under the direction of Dr. C. F.

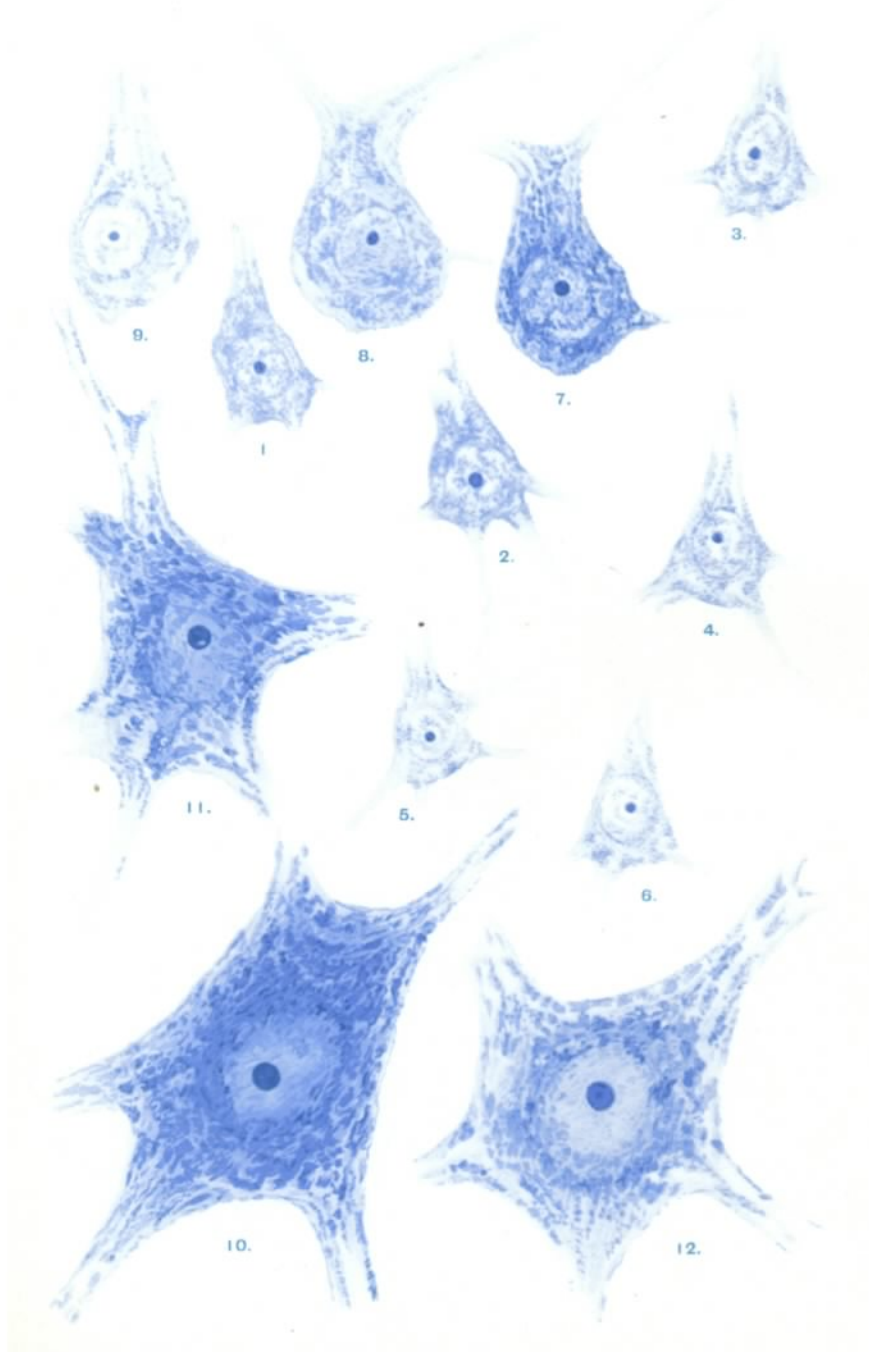
\* Henry J. Berkley, Studies of the Lesions Produced by the Action of Certain Poisons on the Cortical Nerve Cell. I. Alcohol. *Brain*, Part LXXII, Winter Number, 1895, p. 473.

† W. Lloyd Andriezen, On Some of the Newer Aspects of the Pathology of Insanity. *Brain*, Part LXVIII, Winter Number, 1894, p. 668.

‡ Henry J. Berkley, *loc. cit.*

# C. F. Hodge, Ph. D., A Microscopical Study of Changes Due to Functional Activity in Nerve Cells. *Journal of Morphology*, vol. vii, No. 2.

NOTE.—Dr. Hodge kindly permits me to say that rabbits were used in his experiments, though results were not reported.—C. C. S.



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#### EXPLANATION OF PLATE XXVI.

Figs. 1, 2, 3, 4, 5, and 6.—Large pyramidal cells from the central cortex. Figs. 1 and 2 are from Cat I, normal; 3 and 4 are from Cat II, alcohol for fifty minutes; 5 and 6 are from Cat III, alcohol for fifty-four hours and a half.

Figs. 7, 8, and 9.—Purkinje cells. Fig. 7 is from Cat I, normal; 8 is from Cat II, alcohol for fifty minutes; 9 is from Cat III, alcohol for fifty-four hours and a half.

Figs. 10, 11, and 12.—Multipolar cells from the spinal cord. Fig. 10 is from Cat I, normal; 11 is from Cat II, alcohol for fifty minutes; 12 is from Cat III, alcohol for fifty-four hours and a half.

Drawings are made with Zeiss Oc. 6, and homog. imm. lens, 2 millimetres, aperture 1.30. Outlines were drawn with Zeiss camera lucida after Abbe.