A METHOD OF COUNTING THE ACTUAL NUMBER OF PURKINJE CELLS PRESENT IN A GIVEN AREA OF CEREBELLUM, AND ITS APPLICATION IN TEN CLINICAL CASES.*[1]

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In making extensive studies of nerve cell changes, it was found that while a qualitative method was of certain value, a quantitative method would be essential in order to obtain the greatest possible accuracy. This led to the formation of an arbitrary classification of nerve cell changes (Crile) and to the method of making differential Purkinje cell counts by means of which cell activity could be reduced to percentages of active, fatigued, and exhausted cells. The application of these differential counts has been of wide range, and the more counts that have been made under many carefully checked and counterchecked conditions, the more confidence has been felt in their relative accuracy.

The problem of the exhausted cell as presented by the differential counts is of considerable interest. If we assume that a destroyed nerve cell is never replaced, the problem becomes one of great practical importance. The body can regenerate many of its component cellular parts, but if it cannot regenerate nerve cells, if it has but one supply for the use of the individual during his life, every step taken to conserve this supply would be a step toward maintaining efficiency and probably toward prolonging life itself.

The arbitrary definition of an exhausted cell is that it is one in which, after loss of practically all the intracellular and intranuclear chromatin granules, the nucleolus itself begins to disintegrate. It

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[1] This method was elaborated in the course of the routine work done in the laboratory of Dr. G. W. Crile. Thanks are due to Dr. Crile, to Dr. J. B. Austin for the differential Purkinje cell count figures, and to Dr. H. C. King, for assistance in making the actual Purkinje cell counts.

is not known absolutely that a cell in such a condition cannot recuperate, but there is little doubt but that this is so. Beginning nucleolar disintegration is the first stage of exhaustion. With increasing disintegration or disappearance of the nucleolus the major structural changes occur, from rupture and disappearance of the nucleus down to rupture and disappearance of the entire cell.

The assumption is that an exhausted cell in any stage of exhaustion is a dying cell which cannot be recuperated. What becomes of the exhausted cells? Many observers have found evidence of the removal of nerve cells through phagocytic action, and the consensus of opinion is that the phagocytes are not only derived from the white blood corpuscles but also from the neuroglia cells themselves.

The object of this research is to endeavor to show, by a method of making actual Purkinje cell counts, that the observed facts of cell disappearance are supported by demonstrable numerical losses of the cells themselves. With certain reservations it would be expected that in cerebellar tissue showing very high percentages of exhausted cells there would be a corresponding actual loss of cells. If extreme nerve cell activation were to cause extreme nerve cell exhaustion and death in a short time, it would not be expected that many of the exhausted cells would be removed, but in a severe chronic activation the processes of exhaustion and removal would have time to go on and evidence of removal would be found.

THE METHOD FOR MAKING ACTUAL PURKINJE CELL COUNTS.

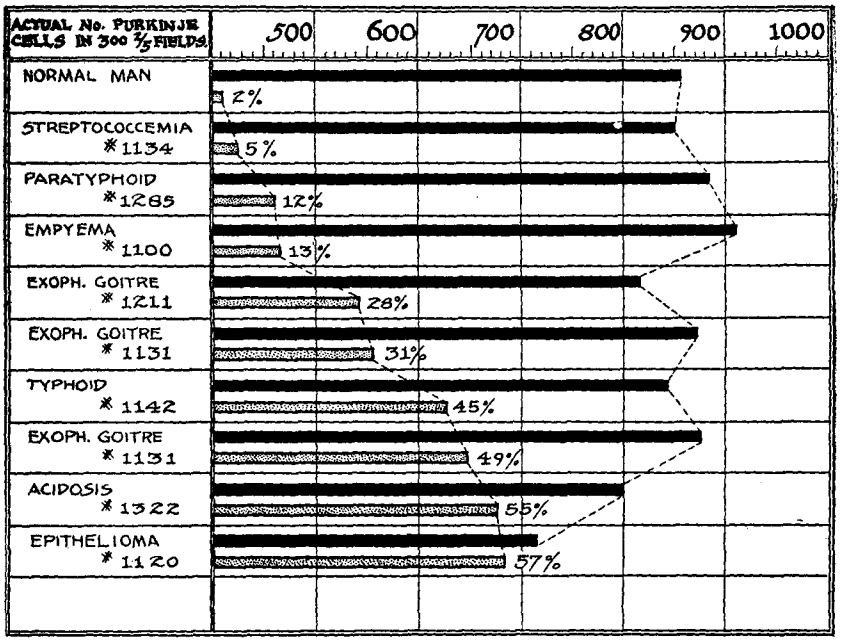
The method consists in counting, with the help of a mechanical stage, the number of Purkinje cells found in 300 fields of the size made by using a No. 2 ocular and No. 5 objective (Zeiss). This can readily be done owing to the linear arrangement of the cells, it being easy to estimate the distance equivalent to the diameter of a field when counting the cells in the angles made by the cerebellar folds. When the cell row is straight it is made to lie in a diameter of the field so as to include as many cells as possible. By following the margins of the cerebellar folds a count begun in one corner of a section can be made to cover the entire section without covering the same area twice. The sections should be of uniform thickness, preferably five microns, and of such a size that an entire count can

be made from one section, or else sections not subjacent should be taken if more than one has to be used.

After counting the cells in the row in a given field the slide is moved exactly one field away, and so on until three hundred fields have been counted, thus covering what is arbitrarily considered to be a large enough area to give a fair idea of the number of cells present, and giving a definite basis for comparison of material from different individuals.

APPLICATION OF THE METHOD.

In applying this method to animals it was found that there was too much variation among normal animals to establish a standard without more labor than could be given at the time (over 100,000 cells were counted). From human brains ten counts were made



TEXT-FIG. 1. The relation between the number of Purkinje cells of all kinds, as shown by actual cell counts, and the number of exhausted Purkinje cells, as shown by differential Purkinje cell counts.

The black lines represent actual counts, while the stippled lines represent the percentages of exhausted cells. Marked actual cell loss is shown only in the cases in which large percentages of exhausted cells were present.

as shown in text-figure 1. The actual Purkinje cell counts are indicated by the solid horizontal lines while the stippled horizontal lines show the percentage of exhausted cells found in the respective differential Purkinje cell counts. The exhausted cell counts are arranged in sequence.

It will be seen that the largest percentage of exhausted cells was in the case of epithelioma and that in this same case the smallest actual number of cells was found (16.6 per cent. less than in the count from the normal man). On the whole, while the curve of actual cell loss does not diminish so evenly nor so markedly as the curve of exhausted cell increase, there is enough convergence to suggest that the underlying principle is correct.

CONCLUSIONS.

1. This method of determining the actual number of Purkinje cells present in a given area of cerebellum is practicable and of sufficient accuracy to make it another useful means of studying nerve cell activity.

2. In its application to clinical cases it is found that increasing nerve cell exhaustion is accompanied by increasing nerve cell disappearance, although it is recognized that theoretically complete nerve cell exhaustion could be present without nerve cell disappearance on account of the individual dying before phagocytic action could take place.

3. This disappearance of nerve cells corroborates the theories and observations made on phagocytosis of nerve cells, inasmuch as it shows that nerve cells disappear from the brain.

4. While there are too few cases to establish a normal actual Purkinje cell count, it is of interest to note that there were 16.6 per cent. fewer cells in the case with the maximum cell exhaustion (57 per cent.) than in the case of the normal man (2 per cent.).