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The Influence of Growth Rate Upon Brain development in *Rana Pipiens* Tadpoles.

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In the course of studies on the development of the optic tectum in the frog (Kollros, '53), considerable variation in the cell populations of the outermost tectal layers was noted, with some involvement of the deeper layers as well. The greatest differences appeared toward the end of metamorphosis, with cell counts in some animals being 30 to 40 per cent less than those in others. The animals with low counts were generally smaller than those with the high counts, and frequently they grew more slowly. In order to study the variations in tectal development which might be associated with variations in growth rate, the growth of tadpoles was inhibited through partial starvation and by crowding (see Rugh, '34), and the cell counts of the tecta of these animals were compared with those of well fed, uncrowded control animals.

Material and Methods

The animals of this study were collected as eggs from ponds near Iowa City, Iowa. They were kept in fingerbowls in groups of 25 or fewer until the start of feeding. Control animals were then placed in individual fingerbowls. Boiled lettuce was available to them at all times. Animals which were to be starved were kept in individual fingerbowls, but they were given less than one-half as much lettuce as the control animals of the same size consumed. Animals to be crowded were kept in groups of 10 in fingerbowls; lettuce was available to them at all times. Since all fingerbowls contained 125-150 ml. of water throughout the experiment, the degree of crowding increased progressively as the tadpoles grew, except as one or two animals (the largest in each case) were removed from the bowl after they had developed to stage V. All animals were fixed at stage V (V to VI-), following the staging plan of Taylor and Kollros ('46), in order to have a group relatively uniform as to both stage and size. Had the crowded or starved animals been kept to later stages, they would have been substantially shorter than control animals of the same stages.

The head was trimmed, and embedded in paraffin. It was cut in serial sections 10 microns thick, and stained with Ehrlich's acid hematoxylin. Counts were made of the cells in layers 7-8-9

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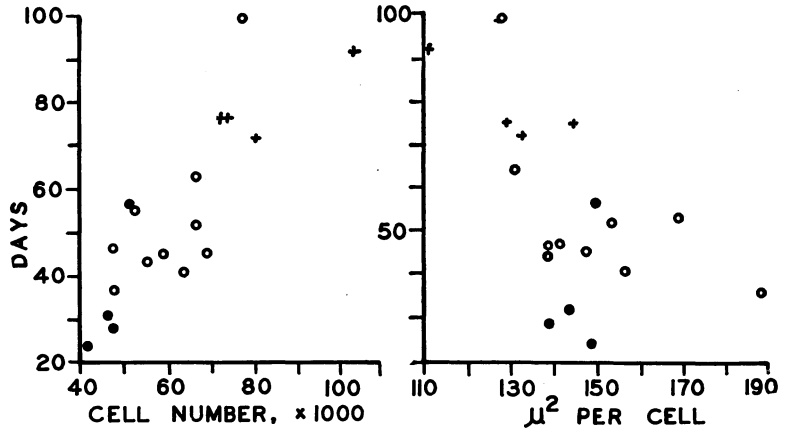

Figure 1. Scatter diagrams to indicate correlation between age and cell number (left), and between age and space available to the cells (right). The solid circles represent the control tadpoles, open circles the crowded ones, and the plus marks the starved tadpoles.

(Larsell, '29) as described in Kollros ('53). At least 10 equally spaced sections were counted on both sides in each animal, and commonly up to 14 were counted. Brain lengths varied from 78 to 105 sections. Subsequently the area of each counted section was drawn with the aid of a microprojector, and measured with the aid of a polar planimeter. The cell counts were then multiplied by the section interval to yield an estimate of the total cell population. The figures for cross-sectional area of layers 7-8-9 were utilized in determining the area on a cellular basis (i.e., μ^2 per cell).

Results and Discussion

The cell counts and the measurements of cross-sectional areas are presented in Table 1. It can be seen that the cell counts of 8 of the 10 crowded animals exceed those of all of the control animals. The 4 starved animals uniformly show higher counts than do the control tadpoles, in one case being 256 per cent of the lowest control count. A strong correlation between age and cell number exists (Figure 1), whereas one between length and cell number is unlikely. It is also seen that there is some variation in the amount of space available to the cells in each case. In general, the older the animal the less the space available, i.e., cells tend more to migrate from the deeper to the more peripheral layers, and to be somewhat crowded there. In metamorphic stages and the adult, with the accretion of white matter, there is progressively more space available to the cells in layers 7-8-9 (Kollros, '53).

It is obvious from these results that animals of the same length, of the same stage, and therefore of essentially identical external appearance may show very different levels of development intern-

Table 1

Record of cell numbers, and of space available to the cells, in layers 7-8-9 of the optic tectum on both sides, of starved, crowded and control tadpoles.

Group	Age in days	Length in mm.	Total cell count	Total cross section area, μ^2 per cell
Control	23	36	40710	148
	27	45	48120	139
	31	42	45920	143
	56	46	51660	150
Ave.	35	42	46602	145
Starved	72	42	80364	132
	76	41	73094	145
	76[1]	41	72338	129
	92[2]	40	104454	110
Ave.	79	41	82563	129
Crowded	36	37	46944	188
	41	36	65358	155
	43	40	54793	138
	45	43	68908	138
	45	37	59562	147
	46	38	46256	141
	52	41	65850	153
	53	43	52780	168
	63	39	66430	131
	99	43	76734	126
Ave.	52	40	59758	149

[1]Tadpole fixed at stage V+.
[2]Tadpole fixed at stage VI–. All others fixed at stage V.

ally, particularly in the midbrain. It will be important therefore in future studies on quantitative aspects of amphibian development, either to use animals paired as to developmental history except for the specific features of the experiment, or to supply sufficient details of the developmental history of each animal.

Summary

1. Tadpoles of Rana pipiens were raised under conditions designed to influence growth rate. They were fixed at stages V to VI-, at an average length of 41 mm.

2. In general, the older the animal, the greater was the number of cells in the peripheral layers of the optic tectum, and the greater the degree of crowding of these cells.

3. Implications of this study for future quantitative work on brain development were noted.

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