Reports

Effect of Tetraploidy on Learning and Retention in the Salamander

The effect of polyploidy on learning in the salamander has been studied in the larvae of the newt *Triturus viridescens* (*1*). In that study it was found that the maze performance of a triploid (3n) animal was significantly worse than that of a normal diploid (2n) animal. The triploid condition in the salamander can be produced by subjecting the fertilized egg to a temperature of about 36°C for about 10 minutes during the meiotic division period. The temperature treatment brings about a suppression of the second meiotic division so that the normal complement of chromosomes is not reduced to the haploid number but instead effects the retention of both sets of chromosomes. Thus, when fertilization occurs there is an addition of an extra set of chromosomes. Offspring possessing three sets of chromosomes are known as triploid organisms (*2*).

The addition of the third set of chromosomes causes an increase by about 50 percent in the size of the cell nucleus, and since the cell size is roughly proportional to the nucleus there is also an increase in the cell size by about 50 percent. Despite the fact that the cells are larger, there is no change in over-all body size, a result which is brought about by a compensatory reduction in the number of cells (*3*). In the case of the triploid animals, the total number of cells is reduced by about 33 percent.

Tetraploid animals possess four sets of chromosomes, a condition which can be produced in the following manner (*4*). Triploid salamanders are usually sterile; however, the species *Amblystoma mexicanum* is something of an exception to this rule. The triploid *A. mexicanum* can be produced by the temperature method indicated in the first paragraph. These triploid animals mature after about 2 years instead of the usual 1 year, at which time about 50 percent of the females are fertile although they lay a greatly reduced number of eggs. When these eggs are fertilized by diploid males, about 3 percent of them will develop into tetraploid animals. Such a tetraploid animal has four sets (4n) of chromosomes; thus the cell nucleus is twice as large as normal and hence the cell size is also double the usual size. Once again, however, there is no change in the body size, which means a compensatory reduction in the cell number that must be by a factor of about 50 percent.

The purpose of the present study was to investigate the learning ability of organisms possessing very large brain cells. The animals were trained to perform a position habit on a water T-maze. They were motivated to traverse the maze in order to escape punishment which was applied in the form of tactual prodding at the tail region. A correct turn at the choice point rewarded the animal with 1½ minutes of rest, while an incorrect turn brought on a period of compound punishment, consisting of continued prodding plus the addition of a 200-watt light. Since the intensity of the prodding was only roughly constant, being administered manually by *E*, the animals were coded, and *E* was not informed of the nature of the code. The criterion of learning was ten consecutive errorless trials. The experimental group was composed of three mature tetraploid salamanders, and the control group was composed of four mature diploid salamanders, all of which were *Amblystoma mexicanum*. The water in the maze was maintained at 68°F.

From Table 1 it can be seen that the tetraploid salamander is a much slower learner than the normal salamander; the differences in their trials to criterion is significant at slightly better than the 5 percent level of confidence, and the difference in their errors is significant at the 1 percent level of confidence. Thus it may be concluded that tetraploidy in the *Amblystoma mexicanum* reduces maze learning ability. It may not be concluded that the poor learning performance of the tetraploid animal was due to its being less active or less responsive to the tactual stimulus for the following reason. The relative activity and reactivity of the animals was tested by a technique devised by Detwiler (*5*). The animals were placed in a circular maze, the bottom of which was marked off into equal sectors. The animals were prodded on the tail, and the number of sectors traversed per prod was recorded. This procedure was repeated 20 times for each animal in each group where *E* was again not informed of the nature of the coding of the animals. The Detwiler test revealed that the diploids traveled, on the average, 5.9 sectors per stimulus, while the tetraploids averaged 7.3 sectors per stimulus. The difference between these two means is *not* statistically significant, and if there is a trend at all it would appear to favor greater activity on the part of the tetraploids.

Seven days after each animal had reached criterion on the original learning task, it was retrained on the same maze for the same task to the same criterion. Table 1, under the heading "Relearning," presents the data on relearning. Note that both groups show some evidence of retention. This retention, when measured by the savings technique, is about 33 percent in both groups for trials to criterion. In the case of error scores, the diploids save about 32 percent, while the tetraploids save 50 percent. Thus, relative to their original learning, the tetraploids retain as well as the diploids.

The data of the present study reveal that the tetraploid condition has a detrimental effect on maze learning in the salamander. They complement earlier findings for the triploid condition in salamanders. Since these two studies are being compared, it should be pointed out that the mature *Amblystoma mexicanum* seems to learn a simple maze task much more readily than the larva of *Tri-*

Table 1. Trials and errors to criterion.

Positive maze stem	Trials	Errors	Relearning	
			Trials	Errors
		Diploid control group		
R	40	6	33	5
R	47	14	11	1
L	34	11	36	13
L	51	13	36	11
Mean	43	11	29	7.5
		Tetraploid experimental group		
L	125	42	85	24
L	67	31	56	19
R	78	36	39	12
Mean	90	36	60	18

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turus viridescens. The present study also reveals that retention over a period of 7 days, when measured by the savings technique, was equal for diploid and tetraploid salamanders, a somewhat surprising result in view of the tetraploid's poor learning ability.

At this point, it appears safe to conclude that polyploidy, whether diploid or tetraploid, brings about a decrease in maze learning ability. It is not possible, however, to state whether such an effect is the result of the increase in cell size, the reduction in number of cells, or the reduction in number of neural connections that probably results from the reduced number of cells.

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References and Notes

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2. G. Fankhauser and R. C. Watson, *Proc. Natl. Acad. Sci. U.S.* 28, 436 (1942).
3. G. Fankhauser, *J. Morphol.* 68, 161 (1941); *Quart. Rev. Biol.* 20, 20 (1945); G. Fankhauser and B. W. Scott, *J. Exptl. Zool.* 121, 105 (1952).
4. Appreciation is expressed to R. R. Humphrey, professor of histology at the University of Buffalo Medical School, for making available the animals used in this study.
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Observations on Local Spread of Pox Viruses in Tissue

In the course of experimental work on the local spread of pox viruses in the infected chorioallantoic membrane of the embryo, we frequently encountered (*1, 2*) peculiar ring zones consisting of concentric rings around a central focus of infection (Fig. 1). In plants, ring-zone formation has been known for a long time. The ringspot viruses, which cause concentric necrotic and non-necrotic areas on infected leaves, are typical examples. As far as we know, similar findings with animal viruses have not been reported. The ring zones appear to be a suitable model for the study of the mode of local spread of virus in infected tissues. This report is a brief summary of our observations on this problem.

Our experiments were carried out with two strains of dermovaccinia, one of neurovaccinia, one of cow pox, one of variola, and two of fowl pox virus. All strains had been passaged in eggs more than ten times. Embryonated 10-day eggs were inoculated on the chorioallantoic membrane according to a previously described modification of Burnet's method (*1, 3*). The amount of seed virus varied between 10 and 1000 minimal infective doses, and the membranes were harvested at various intervals before and after the death of the embryos. Our experimental material comprised nearly 1000 eggs. For macroscopic examination, the membranes were placed in Kaiserling solution and imbedded in glycerine gelatine. For histological study, they were fixed in phosphate-buffered formalin (4.37 percent by weight of formaldehydus solutus, *p*H 7.4) and imbedded in paraffin. The sections were stained with hematoxylin and eosin.

Macroscopically typical ring zones were found around both the primary and the secondary foci, the latter appearing after the generalization of the infection (Fig. 1).

The number and the intensity of the zones depended on the time of survival of the host: the longer the embryo survived, the more marked the ring zones. The zones were always more distinct and more constant with fowl pox than with the other pox viruses examined. This may be owing to the different character of the fowl-pox infection. The chorioallantoic picture, the much longer incubation period, the lower virus content of the affected tissues, the relatively high survival rate of the infected embryos, and the slower course of the infection differentiate fowl pox from the more active vaccinia, cow pox, and variola viruses in incubated eggs. For this reason, histological examinations were carried out only with fowl pox-infected chorioallantoic membrane.

The results obtained show that the ring zones in fowl pox-infected chorioallantoic membrane are caused by stepwise centrifugal spreading of the virus in the tissue. The phenomenon is the result of a complex process in which both the proliferating ectoderm with its concentric exfoliations and the entoderm with its concentric rampartlike proliferations play a part. The latter are particularly prominent at the periphery of the foci (Fig. 2).

There can be little doubt that the ectodermal exfoliations are primarily caused by the virus, while the viral cause of the entodermal proliferations is proved by the characteristic vacuolizing degeneration of the cells (Fig. 2). There is no fundamental difference between the primary and the secondary foci.

Fig. 1. Ring-zone formation around an older fowl-pox focus in the chorioallantoic membrane. Two dim rings show around the pocks. (× 6)

Fig. 2. Peripheral ring zone of a secondary focus of fowl pox. The rampartlike entodermal proliferation (Ent.) extends far beyond the altered zone of the ectoderm (Ect.). (× 73)

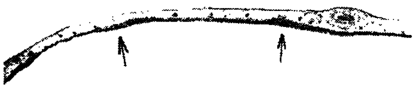

Fig. 3. Cross-section through two rings at the periphery of a fowl-pox focus. Note that such rings are caused by entodermal proliferation exclusively (arrows point to the thickened entodermal areas). (× 27½)

The appearance of rampartlike, almost undulated entodermal proliferations around some foci strengthens the concept of a local centrifugal spread of the virus from cell to cell connected with rhythmical differences in viral activity. In the course of spreading, the pathogenicity of the virus appears to be exhausted and restored periodically. Zones of high virus activity and marked tissue damage alternate with zones of low viral activity and little or no pathogenic effect.

This hypothesis of rhythmic virus spread is strengthened by the presence of shaggy ectodermal proliferations that appear at almost regular intervals. The extent of these proliferations is greatly reduced toward the periphery of a focus.

Of special interest in this connection is the fact that, in the course of generalization, the secondary virus implantation in the chorioallantoic membrane begins in the entoderm. In the ectoderm, there is extensive viral spread in the early stages of focus formation (Fig. 3). Later, especially in secondary foci, the peripheral proliferating ramparts in the entoderm extend far beyond the last ectodermal alteration (Fig. 2).

A striking feature of this intermittent local spread of virus is the fact that the intensity decreases toward the periphery and finally stops. In other words, the local spread of virus does not proceed unlimitedly, but is checked at a certain point. The reason for this phenomenon