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The Tetraploids of Newts Produced by a Shock of Ultrasonic Waves and the Histological Observation of Them.

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(Figs. 1–2; Tables 1–4 ; Plates 1–7)

INTRODUCTION

Although many methods have been tried for producing tetraploids of the amphibia, up to date their methods were comparatively elaborate or the chromosome numbers of produced individuals were not even the double of the diploid ones. In general their methods were low in the percentage of success and poor of reapearance.

The author could produce at a high rate and certainly autotetraploids, allotetraploids and diploid-tetraploid mosaics of newts, using the two species of newts, *Triturus pyrrhogaster* and *Triturus ensicauda.*

Many axolotl tetraploids have been found in the offsprings of triploid females mated with diploid males, besides a considerable number of them have occured in the offspring of diploid parents (FANKHAUSER and HUMPHREY , '50[1]; HUMPHREY and FANKHAUSER, '56[2]). But axolotle is a neoteny species which grows up without metamorphose.

On frogs, one tetraploid frog, *Rana limnocharis,* has been found among many triploids, which developed from cold-treated fertilized eggs (KAWAMURA and MORIWAKI, '53).[3]

One tetraploid with 5 triploids and 12 mosaics have been found among 66 tadpoles obtained from fertilized eggs of *Rana nigromaculata brevipoda,* injected with diploid blastula nuclei of *R. n.nigromaculata.* In case of the reverse combination, one tetraploid has been obtained, too. One tetraploid has been found among 16 tadpoles obtained from unfertilized eggs of *nigromaculata,* injected with nuclei of allotriploid blastula (KAWAMURA and NISHIOKA , '60).[4] By means of the heat shock method 19 tetraploid and 26 diploid-tetraploid tadpoles have been obtaind from fertilized eggs of *Rana nigromaculata* HALLOWELL (KAWAMURA and NISHIOKA, '63).[5] Fourteen autotetraploids have been produced by two methods in *Rana japonica:* seven by heat shock treatment of fertilized eggs and the remaining seven by nuclear transplantation of diploid blastula neclei into fertilized eggs (KAWAMURA , NISHIOKA and MYOREI , '63).[6] But these are anura. That is, there is no report on urodela tetraploids that completed their metamorphosis as yet, although the present author observed on many individuals of them.

Most of autotetraploids produced from *Triturus pyrrhogaster* (BOIE) or *Triturus ensicauda* (HALLOWELL) grew normally during the larval stage and began their metamorphosis simultaneously with the control diploid, but after landing, contrary to control diploid, they became very inactive, did never take food, their external gills did not get completly absorbed, and in a short time most of them died of edema. On the contrary, tetraploids produced from the hybrid of *Triturus pyrrhogaster* and *Triturus ensicauda* could complete their metamorphosis (KAWAMURA and UTSUNOMIYA, '57).[7]

In the previous paper (KAWAMURA and UTSUNOMIYA, '57),[7] some characeristics of tetraploids and diploid-tetraploid mosaics have been reported. In the present paper, more detailed histological observations on tetraploids of subsequent stage are reported.

Before going further I wish to express my gratitude to Emeritus Professor Dr. Toshijiro Kawamura, Hiroshima University for giving a great deal of instruction and advice.

MATERIAL AND METHODS

Autotetraploids of *Triturus pyrrhogaster* and allotetraploids of *Triturus pyrrhogaster* and *Triturus ensicauda* used in the present researches were mostly produced by the ultrasonic waves methods (KAWAMURA and UTSUNOMIYA, '57)[7] in the breeding seasons of the years, 1956 and '57.

Ovulation was induced after 24–48 hours by implantation of several newts pituitaries under the skin of the throat region of newt in the breeding season. The eggs taken out from the oviduct were immediately fertilized in the air and after about 10 minutes they were immersed in water at room temperature. When the slightest sign of the first cleavage furrow appeared on the upper surface 5–9 hours after the fertilization, eggs were put into a test tube together with 5–10 ml. of water and exposed to ultrasonic waves, discharged in an oil basin. The temperature of the water in the test tube was raised by the action of ultrasonic waves. In most cases, many eggs died when the water was heated about 5 minutes up to 40°C, or about 3 minutes up to 43°C. When the temperature of the water in the test tube was raised at 39°C, which required 3–5 minutes by the action of ultrasonic waves, the latter were effective for inducing tetraploidy (KAWAMURA and UTSUNOMIYA, '57).[7] After the treatment the destroyed eggs were taken away and only the surviving eggs were immediately removed in water at room temperature. According to the observation of the eggs treated by ultrasonic waves, the sign of the first cleavage furrow, which was previously observed on the upper surface of the eggs, usually disappeared and frequently a fine whitish line was left behind on the same place. Some hours later, these eggs divided into two blasmeres by a different cleavage furrow, which was in most cases independent of the fine whitish line or the previous furrow. In one experiment two eggs began the cleave into two blastmeres 5 hours after the treatment, when the control eggs were already at the eight-cell stage. Both of them became tetraploid larvae. However, the eggs showing delayed cleavages did not always become tetraploid.

At the about two weeks after hatching, when the larvae began to take food and their tail began to extend, but the pigment of the tail was not yet increasing, the chromosome number of the larvae was examined by the tail-tip method. Besides counting the chromosomes in all good mitotic figures, the size of resting nuclei was measured also. When about 48 chromosomes were counted in a tail-tip and all the nuclei were obviously larger than those of the control diploid larvae, this larva was identified as tetraploid. When there were more than one diploid mitosis or more or less numerous diploid resting nuclei together with many tetraploid mitosis and larger resting nuclei, such a larva was identified as a diploid-tetraploid mosaic. In this present paper the observation on diploid-tetraploid mosaic is not descrived.

The tetraploids and diploid-tetraploid mosaics produced in this research are summarized in table 1.

Table 1
Main results of experiments

Exp. no.	Dosage of ultrasonic waves				Change of water temperature °C	Treated eggs			Number of larvae			
	Intensity		Duration			Parents		Number	Total	4N	2N–4N	2N
	V	mA	min.	sec.		♀	♂					
62.3	1000	210	4	0	22 → 41	pyrrh.	ensic.	22	7	2	0	5
63.2	1000	220	5	0	20 → 41.5	ensic.	pyrrh.	11	1	1	0	0
63.4	1050	230	3	0	20 → 43.5	”	”	16	4	1	0	3
65.1	1000	220	5	0	21 → 40	pyrrh.	ensic.	21	9	1	1	7
65.2	1000	220	2	0	24 → 41	”	”	19	7	1	0	6
66.1	1050	220	4	0	21.5 → 43	ensic.	pyrrh.	8	2	0	2	0
66.4	900	180	4	20	25 → 43	”	”	9	1	1	0	0
67.1	1050	230	3	20	21 → 44	pyrrh.	ensic.	7	4	1+1*	1	1
67.2	1000	200	3	20	22 → 43	”	”	17	4	1	1	2
67.5	900	170	3	30	22 → 43	pyrrh.	pyrrh.	20	5	3	0	2
70.1	1100	200	5	0	23.5 → 42	pyrrh.	ensic.	53	18	1	6	11
70.2	1100	200	3	30	26.5 → 42	”	”	29	6	1	0	5
70.3	1100	200	5	0	24.5 → 42.5	”	” ”	14	6	4	0	2
70.4	1300	200	4	20	26.5 → 43	”	”	40	3	1	0	2
73.1	1200	200	2	30	23.5 → 42.5	pyrrh.	pyrrh.	8	2	2	0	0
73.2	1200	200	3	0	23.5 → 42.5	pyrrh.	ensic.	17	8	0	3	5
73.3	1150	180	3	30	25 → 42.5	”	”	12	2	0	2	0
73.4	1150	180	3	30	25 → 43	”	”	15	6	0	1	5
75.1	1100	180	4	0	23.5 → 42	”	”	12	6	1*	1	4
75.2	1100	180	3	0	24 → 42	”	”	34	13	1	3	9
75.3	1200	200	3	0	23.5 → 41.5	”	”	31	21	7	3	9
76.1	1200	200	4	0	26.5 → 42	”	”	40	10	0	2	8
76.3	1200	200	3	20	28 → 42	”	”	50	5	1	2	2
79.1	1000	160	6	30	19.5 → 42	pyrrh.	ensic.	13	6	2	0	4
79.2	1000	170	4	0	19.5 → 42.5	”	”	14	2	0	1	1
79.3	1000	170	4	0	20 → 43.5	ensic.	ensic.	18	11	3	0	8
80.1	1000	170	6	0	21 → 42	pyrrh.	ensic.	23	3	3	0	0
80.2	1000	160	4	0	22 → 43	”	”	15	1	1	0	0
82.2	950	160	4	0	21 → 40	”	”	39	9	2	3	4
85.1	950	160	6	0	23 → 39.5	”	”	35	13	2	0	11
86.2	950	140	4	0	22 → 42	ensic.	pyrrh.	20	7	3*	0	4
88.2	950	150	4	0	24 → 40.5	pyrrh.	ensic.	11	7	0	1	6
89.1	950	160	5	0	24 → 40	”	”	40	13	1	0	12
89.2	1000	170	4	0	24 → 42	ensic. pyrrh.		45	3	1	0	2
						Total		778	225	45+5*	35	140

*Probably tetraploid

In the experiments, there are an other 15 experiments, in which neither tetraploids nor diploid-tetraploid mosaics were produced.

Two Matsuda P-560 were used for generating tube, two Matsuda HX-966 were did for rectifier tube. The X-cutt quartz plate was 35 mm in diameter, 4 mm in thickness and 710 K.C. in foundamental frequency.

The circuit diagram of the ultrasonic generator used in the present researches is shown in Figure 1. The ultrasonic generator used in the present study was made by the author own. As the strength of ultrasonic waves can hardly be measured, it was expressed

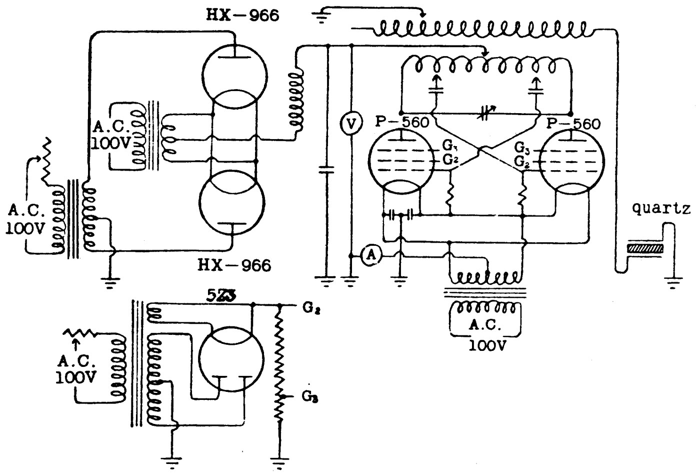

Fig. 1. The circuit diagram of the ultrasonic generator used in the present reserarches.

by both the anode voltage and the anode current of the generating tube for convenience sake. The strength of ultrasonic waves used in this study was 800V-150mA to 1100V-230mA.

The larva identified as tetraploid was continuously raised by giving food (Tubifex). When they died, they were fixed by Navashin's fluid, cut in a paraffin section of 15μ in thickness, dyed by Haidenhain's iron alum haematoxylin solution or Delafield's haematoxylin solution and observed.

OBSERVATIONS

I. Digestive organs

1. Stomach and small and large intestines.

The four kinds of diploid metamorphosed newts, *Triturus pyrrhogaster, T. ensicauda, T. pyrrhogaster* ♀ x *T. ensicauda* ♂ and *T. ensicauda* ♀ x *T. pyrrhogaster* ♂, had stomachs and small and large intestines, which were similar each other in anatomical and histological structures. In the walls of their stomachs innumerable tubular gastric glands, about 127μ long and 38μ in diameter, were closely arranged. In the small intestines, well-developed villi, circular folds and two muscular layers, circular and longitudinal, were distinctly observed. In the epithelium of the large intestines there were numerous goblet cells.

In the four kinds of tetraploid newts, *Triturus pyrrhogaster, T. ensicauda, T. pyrrhogaster* ♀ x *T. ensicauda* ♂ and *T. ensicauda* ♀ x *T. pyrrhogaster* ♂, the gastric glands in the mucous membranes of the stomachs were ill-developed and much fewer in number than those of the diploid newts, although the stomachs themselves were nearly normal in shape and size. The intestines of the tetraploids were remarkably simpler in winding, shorter in whole length, and larger in diameter than those of the diploids. Their villi, circular folds and circular and longitudinal muscular layers were ill-developed, too. In some individuals the muscular layers of the small and large intestines were very thin and the longitudinal and circular layers were not distinguishable from each other.

Every kind of cells which constituted each part of the digestive organs of tetraploid newts were larger and fewer than those of diploid newts. Among the four kinds of newts, *pyrrhogaster, ensicauda, pyrrhogaster* ♀ x *ensicauda* ♂ and *ensicauda* ♀ x *pyrrhogaster* ♂, there were no differences in size and number of the constituent cells (Plate I, Plate II).

2. Liver

No differences were found in size and shape between the livers of the four kinds of diploid newts and those of the four kinds of tetraploid newts. However, there were some differences in histological aspects between diploid and tetraploid livers. The hepatic cells of the tetraploid livers were larger and less numerous than those of the diploid and the sinusoids of the former were distinctly smaller in diameter and far fewer than those of the tetraploid. While melanophores were scarcely found in the livers of diploid *pyrrhogaster,* those of tetraploid *pyrrhogaster* had a considerable number of melanophores. In diploid hybrids, *pyrrhogaster* ♀ x *ensicauda* ♂ *and ensicauda* ♀ x *pyrrhogaster* ♂, the livers had a small number of large melanophores. In contrast with this, the livers of tetraploid hybrids of the same combinations had many large melanophores. Moreover, there were a few tetraploid hybrids, in which such melanophores were so numerous that they covered most parts of the liver (Plate III 1 and 2).

3. Pancreas

The pancreases of the four kinds of tetraploid newts seemed to be similar in size and shape to those of the diploid newts. However, the alveoli of the pancreases of the tetraploid newts were remarkably larger and fewer than those of the diploids. In cross sections the cells of each alveolus of the former were nearly the same in number with those of the latter, while they were distinctly larger. A tetraploid hybrid, *pyrrhogaster* ♀ x *ensicauda* ♂, which was 905-days-old, had an ill-developed pancreas together with an ill-developed alimentary tract (Plate III 3 and 4).

4. Gall bladder

Except the largeness and fewness of constituent cells, the gall bladders of the four kinds of tetraploid newts were the same in size and shape with those of diploid newts. However, a diploid and a tetraploid hybrids, *pyrrhogaster* ♀ x *ensicauda* ♂, had cornified gall bladders, in which the walls looked homogeneous and nuclei were hardly observable.

II. Urogenital organs

1. Kindney

There were no distinct differences in the size and shape of kidneys between diploid and tetraploid newts of the four kinds, *pyrrhogster, ensicauda, pyrrhogaster* ♀ x *ensicauda* ♂ and *ensicauda* ♀ x *pyrrhogaster* ♂. In cross sections of kindneys the mesonephric tubules of the tetraploid newts were generally larger and remarkably fewer than those of the diploid. Accordingly, the kindneys of the tetraploids seemed to be simple in structure. Various kinds of cells in the kidneys of the tetraploid newts were larger and fewer than those of the diploids. In some tetraploids the cavities of the mesonephric tubules were abnormally expanded (Plate IV 1, 2, 3 and 4).

2. Urinary bladder

The urinary bladders of the four kinds of tetraploid newts did not differ in structure from those of diploid newts, except largeness and fewness of constituent cells.

3. Gonads

In diploid *pyrrhogaster,* sex differentiation occurs before completion of the metamorphosis. Diploid *ensicauda* are somewhat delayed in sex differentiation as compared with diploid pyrrhogaster. In general, auto-and allotetraploid newts are more and more delayed in sex differentiation, accordingly sex differentiation is not taken up in present paper. It will be reported when more detailed results will be obtained.

4. Sex

The sex ratios of diploid and tetraploid newts of the four kinds, *pyrrhogaster, ensicauda, pyrrhogaster* ♀ x *ensicauda* ♂ and *ensicauda* ♀ x *pyrrhogaster* ♂, are presented in Table 2. There were 20 females and 13 males in the tetraploid newts, in contrast with 13 females and 9 males in diploid.

Table 2. The sex ratio of tetraploid

Parents		Diploids		Tetraploids	
♀	♂	♀	♂	♀	♂
pyrrh.	pyrrh.	5	3	6	4
ensic.	ensic.	1	1	1	0
pyrrh.	ensic.	5	4	10	7
ensic.	pyrrh.	2	1	3	2

III. Skin, muscular tissue and bone

1. Skin

The skin of autotetraploid newts, *pyrrhogaster* and *ensicauda* was soft, smooth and wettish for a long time after landing, differing from those of the diploid ones. They were very similar to those of larvae in appearance. The skin of allotetraploid newts, *pyrrhogaster* ♀ x *ensicauda* ♂ and *ensicauda* ♀ x *pyrrhogaster* ♂, was quite similar in appearance to those of the diploid newts and became somewhat granulous.

In cross sections of the skins of diploid newts the epidermis consisted of 4 or 5 cell layers and there were numerous dermal glands. The cavities of those dermal glands were filled with secretory granules. In contrast with this, the epidermises of autotetraploids consisted of 2 or 3 layers of flattened cells, which were very similar in appearance to the two layers of the epidermis at the larval stage. However, there were so many dermal glands as found in the diploid newts, although no distinct cavity was found in each gland. The skin of allotetraploids was nearly the same in structure to that of the autotetraploids even in a 876-days-old *ensicauda* ♀ x *pyrrhogaster* ♂ and a 905-days-old *pyrrhogaster* ♀ x *ensicauda* ♂ tetraploid newts. They consisted of 2 or 3 layers of flattened cells and contained numerous dermal glands, which were very similar to those of the autotetraploids (Plate V 1, 2, 3 and 4).

2. Muscular tissue

Muscular tissues of tetraploid newts of the four kinds, *pyrrhogaster, ensicauda, pyrrhogaster* ♀ x *ensicauda* ♂ and *ensicauda* ♀ x *pyrrhogaster* ♂, were distinctly different in texture from those of diploid newts. The bundles of muscle fibers in the tetraploid newts were larger in diameter and coarser in arrangement than those of the diploid newts. Accordingly, the muscular tissues of the tetraploid newts were somewhat similar in texture to those of larvae.

3. Bone

Differentiation of bone tissue was observed by ossification of a part of the vertebra. Among the diploid newts of four mating, *Triturus pyrrhogaster* ♀ x *Triturus pyrrhogaster* ♂, *Triturus ensicauda* ♀ x *Triturus ensicauda* ♂, *Triturus pyrrhogaster* ♀ x *Triturus ensicauda* ♂, *Triturus ensicauda* ♀ x *Triturus pyrrhogaster* ♂, the vertebra of *T.ensicauda* and hybrid diploids produced from *T.ensicauda* were observed as stronger and their ossification were observed as more progressive.

On tetraploids, the same facts as the above can be stated, too, but the ossification of tetraploids generally seemed to be delayed a little as compared with controls. (Plate VII)

IV. Heart, blood vessels, spleen, lung and blood corpuscles

There were no distinct differences in anatomical and histological aspects of the heart, blood vessels, spleen and lungs between the diploid and the tetraploid newts of the four kinds, *pyrrhogaster, ensicauda, pyrrhogaster* ♀ x *ensicauda* ♂ and *ensicauda* ♀ x *pyrrhogaster* ♂, except differences in size and number of constituent cells. As a matter of course, the red blood corpuscles and their nuclei of the tetraploid newts were distinctly larger than those of the diploid ones (Plate VI 1 and 2).

V. Inner secretory organs

1. Pituitary body

The pituitary bodies of autotetraploid newts, *pyrrhogaster* and *ensicauda, were smaller* than those of diploids. Their glandular tissues were ill-developed. The pituitary bodies of all allotetraploid newts,*pyrrhogaster* ♀ x *ensicauda* ♂ and *ensicauda* ♀ x *pyrrhogaster* ♂, were similarly small and ill-developed, except one of the tetraploid hybrids, *pyrrhogaster* ♀ x *ensicauda* ♂. This exceptional newt, 905-days-old, landed 105-days after insemination. The pituitary body of this newt was nearly normal in size and structure.

2. Thyroid gland

The thyroid glands of nearly all auto- and allotetraploid newts were smaller than those of diploid newts or consisted of fewer follicles distributed dispersedly, even if they were of nearly normal size. However, there was an exceptional tetraploid hybrid, *pyrrhogaster* ♀ x *ensicauda* ♂, which was 251-days-old. This newt had a pair of well-developed thyroid glands, although the epidermis of the skin consisted of 2 or 3 layers of cells.

VI. Eyes

The eyes of the autotetraploid newts, *pyrrhogester* and *ensicauda,* were not so clear as those of diploid newts. Among allotetraploid newts, *pyrrhogaster* ♀ x *ensicauda* ♂ and *ensicauda* ♀ x *pyrrhogaster* ♂, there were also some individuals with such abnormal eyes. There were no distinct differences in size of the eye balls and the lenses and in thickness of the retinae between diploid and tetraploid newts.

In histological aspect the eyes of the tetraploid newts of the four kinds, *pyrrhogaster, ensicauda, pyrrhogaster* ♀ x *ensicauda* ♂ and *ensicauda* ♀ x *pyrrhogaster* ♂ showed some differences from those of diploid newts.

The epithelium of the cornea in the diploid newts consisted of about 3 layers of cells. In contrast with this, that in the tetraploid newts usually consisted of one layer of larger cells. The stromae of the corneas of the tetraploid newts distinctly varied in thickness, differing from those of the diploids, which were nearly uniform in thickness.

The autotetraploid newts, *pyrrhogaster* and *ensicauda,* had no or only rudimental eyelids and lacrymal glands. The allotetraploid newts, *pyrrhogaster* ♀ x *ensicauda* ♂ and *ensicauda* ♀ x *phrrhogaster* ♂ had ill-developed eyelids and lacrymal glands, although they were somewhat better developped than those of the autotetraploid newts (Plate VI 3 and 4).

VII. Volume of nuclei

As the chromosome number of the tetraploid is twice to one of the diploid, it is supposed that nuclear substance too, should be twice, and therfore the volume of resting nucleus of tetraploid should be twice to one of the diploid. So in fact the resting nuclei of some organs were measured. That is, the major and minor diameter of 50 nuclei in some organs were measured, and assuming an ellipsoid shape of the nucleus, its volume was calculated. That is, if a is the minor and b is the major diameter, its volume V is expressed the following equation:

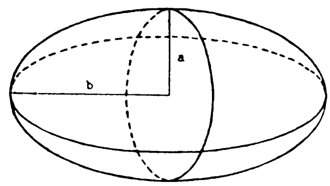

Fig. 2. The assuming ellipsoid shape of cell nucleus.

$$V = \frac{4}{3} \pi a^2 b$$

Volumes of kidney and small intestines cell nuclei are showed in table 3. As clearly from it, the volume of kidney and small intestines cell nuclei of the most parts of the tetraploid are far larger than the double of those of the diploid and there is no smaller than twofold.

In liver, some tetraploid were measured. The volume of tetraploid cell nuclei was about $2116.2\mu^3$, while that of diploid was about $980.9\mu^3$, showing the same tendency as above.

Table 3. Volume of kidney and small intestines cell nucleus

Tetraploids

Individual no.	Parents ♀	Parents ♂	Age (days)	Total length (mm)	Volume of cell nucleus (μ^3) Kindney	Volume of cell nucleus (μ^3) Small intestin	Sex
19.2	pyrrh.	pyrrh.	83	23.0	2789.0	2228.0	♀
14.4.1	"	"	336	32.5	4116.4	4675.7	♀
14.4.2	"	"	340	34.0	3003.2	4843.9	♀
14.4.3	"	"	103	28.6	2893.0	2552.0	♀
14.4.5	"	"	159	35.0	2700.1	4048.7	♂
14.4.9	"	"	320	–	3249.1	3906.6	?
15.2.7	"	"	131	31.6	2396.4	4747.2	♀
15.3.4	"	"	157	34.5	2934.3	3304.8	♀
26.1.3	"	"	159	32.5	3261.7	4763.0	♂
26.1.5	"	"	97	22.3	2401.7	5062.7	♂
37.4.1	pyrrh.	ensic.	110	34.2	5355.0	5995.4	♀
37.5.2	"	"	112	34.0	4702.8	4105.2	♀
47.1.7	"	"	31?	39.0	2465.7	2531.1	♀
Controls (diploid)*							
35.1.8	pyrrh.	ensic.		35.2	1161.2	1149.1	♀
37.B.1	"	"		23.0	1209.6	1291.5	♀
37.C.2	"	"		34.0	992.0	1078.6	♂
37.4.3	"	"		35.5	1300.6	1068.0	♂
H	pyrrh.	pyrrh.	152	32.8	935.3	1159.2	♂
I	"	"	152	32.0	896.8	1068.5	♀
J	"	"	152	26.6	949.5	1086.3	♀
K	"	"	152	25.1	759.8	1048.9	♂
Controls (diploid, wild)							
a	pyrrh.	pyrrh	106	33.3	1258.0	640.8	♂
b	"	"	106	34.3	1140.6	524.7	♀
c	"	"	106	34.0	1073.5	702.0	♀

* Diploid which was treated by ultrasonic waves, but was neither tetraploid nor diploid-tetraploid mosaics.

Table 4. Volume of blood corpuscles nucleus

Individual No.	Parents ♀	Parents ♂	2N or 4N	Diameter $b(\mu)$**	Diameter $a(\mu)$**	Volume (μ^3)
45.5	pyrrh.	pyrrh.	2N	13.32	6.04	242.95
'56 cont.	ensic.	ensic.	"	12.15	6.58	263.05
85. 82	pyrrh.	ensic.	"	12.94	6.23	251.10
74. cont.	ensic.	pyrrh.	"	12.91	6.17	245.74
67. 5.3	pyrrh.	pyrrh.	4N	17.27	7.76	519.99
79. 3.6	ensic.	ensic.	"	18.51	9.73	876.17
70. 2.4	pyrrh.	ensic.	"	16.18	9.28	696.71
63.2.1	ensic,	pyrrh.	"	15.64	9.41	692.46
*91. cont.	pyrrh.	ensic.	2N	15.27	6.96	359.84
*91.2.3	pyrrh.	ensic.	4N	21.97	10.14	1129.48

* smeare ** a, b in Fig. 2

DISCUSSION

What mechanism of ultrasonic action are produced tetraploids by? As mentioned above, when the temperature of the water in the test tube was raised above 39°C, which required about 5 minutes by the action of ultrasonic waves, tetraploids were effectively produced. By heat shock of hot water, too, tetraploids were produced, but the percentage of success was lower than the former.(KAWAMURA and UTSUNOMIYA '57) For these two reasons, it is no doubt that tetraploids are produced by both the stirring and the heat action of ultrasonic waves. That is as eggs are exposed to ultrasonic waves in metaphase or when chromosoms increase double at the equatorial plate and do not begin yet to move toward the two poles, it is supposed that the traction fibers in the eggs are cut off and chromosomes are apart from each other by stirring action of ultrasonic waves and therefore chromosomes can not move toward two poles and soon become resting state. That the traction fibers may be cut off by the action of ultrasonic waves is supposed from the fact that the first cleavage furrow of eggs after the treatment by ultrasonic waves raises up soon. The heating action of ultrasonic waves, by its heating shock, is supposed to force the eggs to enter in a resting state, after a few hours, the eggs begin to creave again, each of all the 48 chromosomes divide in two, and so become two tetraploid nuclei.

If the mechanism which tetraploids are produced is supposed as above, the time to treate by ultrasonic waves must be in metaphase. By the author's ovservation, the metaphase of newt eggs, is out of common sense and very late, it is when the slightest sign of the first cleavage furrow appears on the upper surface of the egg.

These were ascertained by fixing, staining, cutting in praffing section, observating by microscope the eggs when the slightest sign of the first cleavage furrow appeared on the upper surface, that is, immediately before the first cleavage.

If newt eggs are treated by ultrasonic waves except in these periods, tetraploids or diploid-tetraploid mosaics of newts are not produced at all.

In tetraploids, cell nuclei are larger, accordingly the cell size is larger too, but the numbers of cells are less. These are supposed nearly from the fact that many tetraploid mitotic figures are seen than diploid ones when mitotic figures are oboerved by microscope. That is, it is supposed that mitosis of tetraploid cells require much more time than the ones of diploid.

SAMMARY

1) Many auto- and allotetraploids and diploid-tetraploid mosaics of newts were produced by treating the eggs of two species of newts, *Triturus pyrrhogaster* and *Triturus ensicauda,* by ultrasonic waves. When the temperature of the water around the eggs in the test tube was raised at 39°C, which required 3–5 minutes by the action of ultrasonic waves, the latter were effective in inducing tetraploid and diploid-tetraploid mosaics.

2) All the autotetraploids produced from *Triturus pyrrhogaster* or *Triturus ensicauda*

had a same vital power as the control (diploid) until their metamorphosis, on the contrary after the landing, they could not complete their metamorphosis and died within half a year wholly.

The allotetraploids of *Triturus pyrrhogaster* and *Triturus ensicauda* could complete their metamorphosis and have a same vital power as the controls approximately. Especially the allotetraploids of *Truturus ensicauda* ♀ x *Triturus pyrrhogaster* ♂ were stronger in vital power.

3) Among most of the organs of diploid newts of the four mating, *Triturus pyrrhogaster* ♀ x *Triturus pyrrhogaster* ♂, *Triturus ensicauda* ♀ x *Triturus ensicauda* ♂, *Triturus pyrrhogaster* ♀ x *Triturus ensicauda* ♂, *Triturus ensicuada* ♀ x *Triturus pyrrhogaster* ♂, especially conspicuous differences were not seen, but the vertabra of *Triturus ensicauda* and hybrid diploids produced from *Triturus ensicauda* were observed as stronger and their ossification were observed as more progressive.

On tetraploids, the same facts as the above can be stated, too, but the ossification of tetraploids was generally seemed to be delayed a little as compared with that of the controls.

4) All the cells and cell nuclei consisted of every organs of tetraploids were larger than twice of diploid one in volume and less in number. Then the constitution of tetraploid organs was simple in general. Especially, all the gland tissues (gastric glands, lacrimal glands, salivary glands, thyroid gland, hypophysis etc.) of tetraploids were very simple in structure and very few in number.

5) The sex ratio made clear in tetraploid newts up to date, was 6 females and 4 males in 14 *Triturus pyrrhogaster* ♀ x *Triturus pyrrhogaster* ♂, 1 female in 1 Triturus ensicauda ♀ x *Triturus ensicauda* ♂, 10 females and 7 males in 18 Triturus pyrrhogaster ♀ x *Triturus ensicauda* ♂, 3 females and 2 males in 5 *Triturus ensicauda* ♀ x *Triturus pyrrhogaster* ♂.

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EXPLANATION OF PLATES

Plate 1.

The external appearance of the intestines of two diploids (4 and 5) and three tetraploids (1, 2 and 3). Less winding of tetraploid intestine in comparison with diploid one are showed. x 5

1. Individual no. 67.2.3,4N, T.pyrrh. ♀ x T.ensic. ♂, 545 days in age.
2. Individual no. 65.1.3,4N, T.pyrrh. ♀ x T.ensic. ♂, 630 days in age.
3. Individual no. 79.3.6,4N, T.ensic. ♀ x T.ensic. ♂, 318 days in age.

4 and 5. Individual no. 85.1.a, 2N, T.pyrrh. ♀ x T.ensic. ♂ , 243 days in age.

Plate 2

Cross section of diploid (1) and tetraploid (2) stomach, and diploid (3) and tetraploid (4) small intestine. In comparisson whith diploid, the intestine of tetraploid is ill-developed.

1. Individual no. 91 cont., 2N, T.pyrrh. ♀ x T.ensic ♂, 250 days in age.
2. Individual no. 79.1.2, 4N, T.pyrrh. ♀x T.ensic.♂, 211 days in age.
3. Individual no. 82.1.A, 2N, T.pyrrh.♀ x T. ensic.♂, 449 days in age.
4. Individual no. 65.1.3, 4N, T.pyrrh. ♀x T. ensic.♂, 630 days in age.

Plate 3.

Cross section of the liver of diploids (1) and tetraploids (2). The latter is seemed that the function is insufficient, and there are many pigment, 3 (diploid) and 4 (tetraploid) are pancreas.

1. Individual no. 82.85.1.a, 2N, T.pyrrh.♀x T.ensic.♂ , 434 days in age.
2. Individual no. 65.1.3, 4N, T.pyrrh.♀ x T.ensic.♂, 630 days in age.
3. Individual no. 85.88, 2N, T.pyrrh.♀x T.ensic.♂, 485 days in age.
4. Individual no. 70.2.4, 4N, T.pyrrh.♀x T.ensic.♂, 905 days in age.

Plate 4.

Cross section of the kidney of diploids (1 and 2), and tetraploids (3 and 4). The latter is seemed to have insufficient function.

1. Individual no. 85.1.Aa, 2N, T.pyrrh. ♀x T.ensic. ♂ , 243 days in age.
2. Individual no. 82.85.1.a, 2N, T.pyrrh. ♀ x T.ensic. ♂, 449 days in age.
3. Individual no. 75.3.14, 4N, T.pyrrh. ♀ x T.ensic. ♂, 207 days in age.
4. Individual no. 70.4.3, 4N, T.pyrrh. ♀ x T.ensic. ♂, 600 days in age.

Plate 5.

Cross section of diploid (1 and 3) and tetraploid (2 and 4) skin.

1. Individual no. 85.1.Aa, 2N, T.pyrrh. ♀ x T.ensic. ♂, 243 days in age.
2. Individual no. 75.3.14, 4N, T.pyrrh. ♀ x T.ensic. ♂, 207 days in age.
3. Individual no. Cont. H, 2N, T.pyrrh. ♀x T.pyrrh.♂ , 152 days in age.
4. Individual no. 15.2.6, 4N, T.pyrrh. ♀ x T.pyrrh.♂, 118 days in age.

Plate 6 .

Red blood corpuscles of diploid (1) and tetraploid (2) by smear, and cross section of diploid (3) and tetraploid (4) eye balls.

1. Individual no. 91 cont., 2N, T.pyrrh. ♀ x T.ensic. ♂, 250 days in age. smear.
2. Individual no. 91.2.3, 4N, T.pyrrh. ♀ x T.ensic. ♂, 250 days in age. smear.
3. Individual no. 74.cont., 2N, T.ensic. ♀ x T.pyrrh. ♂, 836 days in age.
4. Individual no. 63.2.1, 4N, T.ensic. ♀ x T. pyrrh. ♂, 376 days in age.

Plate 7.

Cross section of vertebra. Ossification of tetraploids are seemed to be delayed.

1. Individual no. 82.85, 2N, *T.Pyrrh.* ♀ x *T.ensic.*♂ , 434 days in age.
2. Indivicual no. 67.5.3 4N, *T. pyrrh.*♀ x *T. pyrrh.* ♂ , 117 days in age.
3. Individual no. 85.88, 2N. *T.pyrrh.* ♀ x *T.ensic.* ♂ , 485 days in age.
4. Individual no. 70.2.4, 4N, T.pyrrh. ♀ x T. ensic. ♂ , 905 days in age.

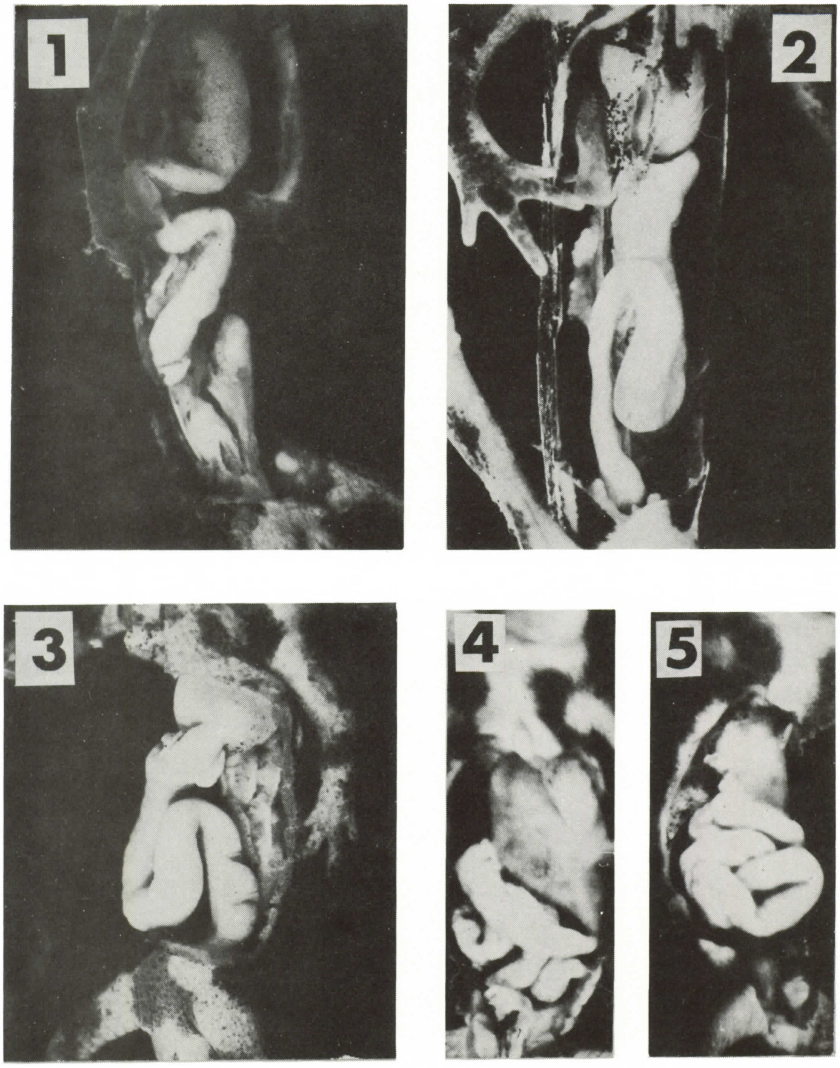

Plate 1. The anatomical aspect of the intestines of two diploid (4 and 5) and three tetraploids (1, 2 and 3). x 5

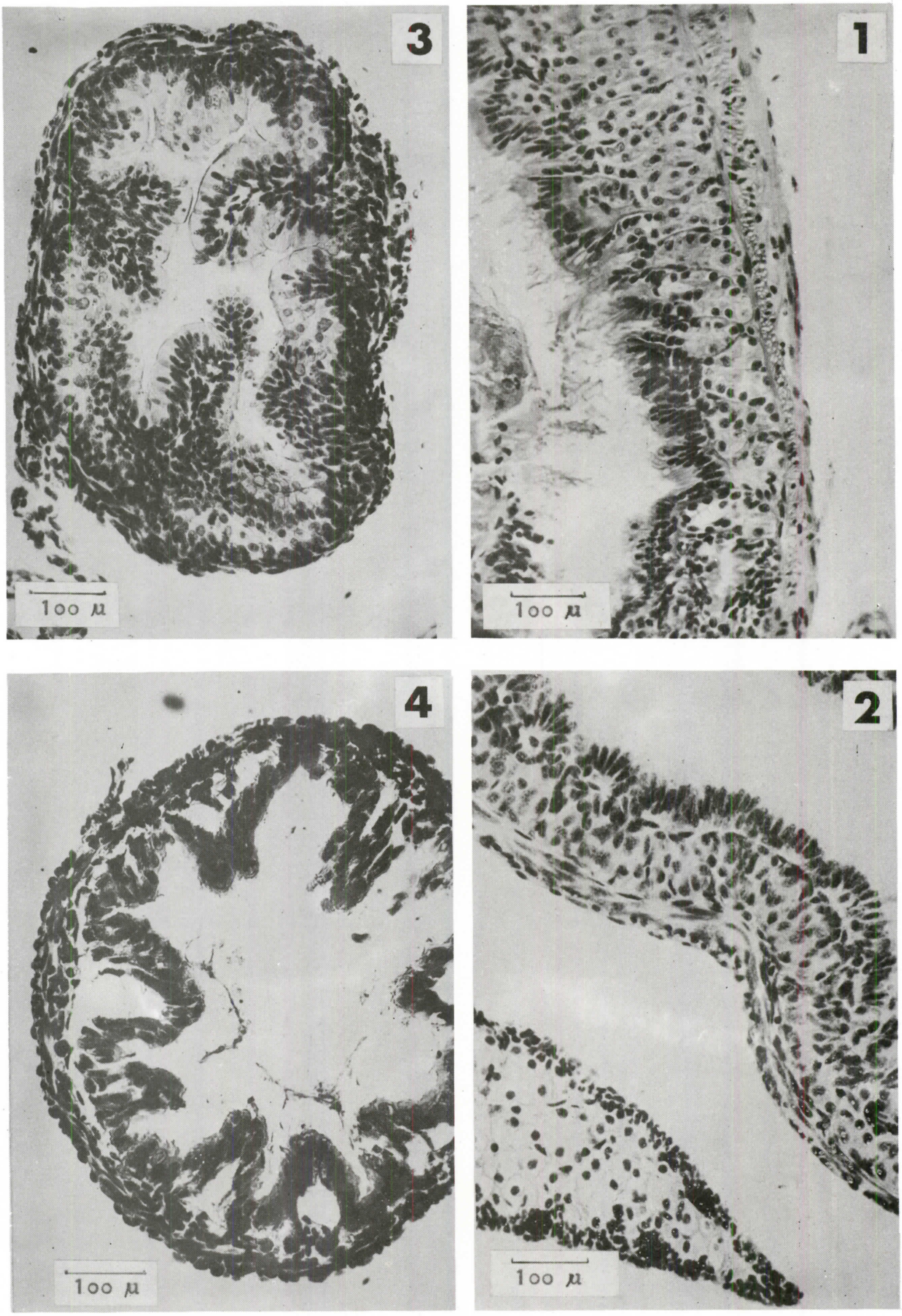

Plate 2. Cross section of diploid (1) and tetraploid (2) stomach and diploid (3) and tetraploid (4) small intestine.

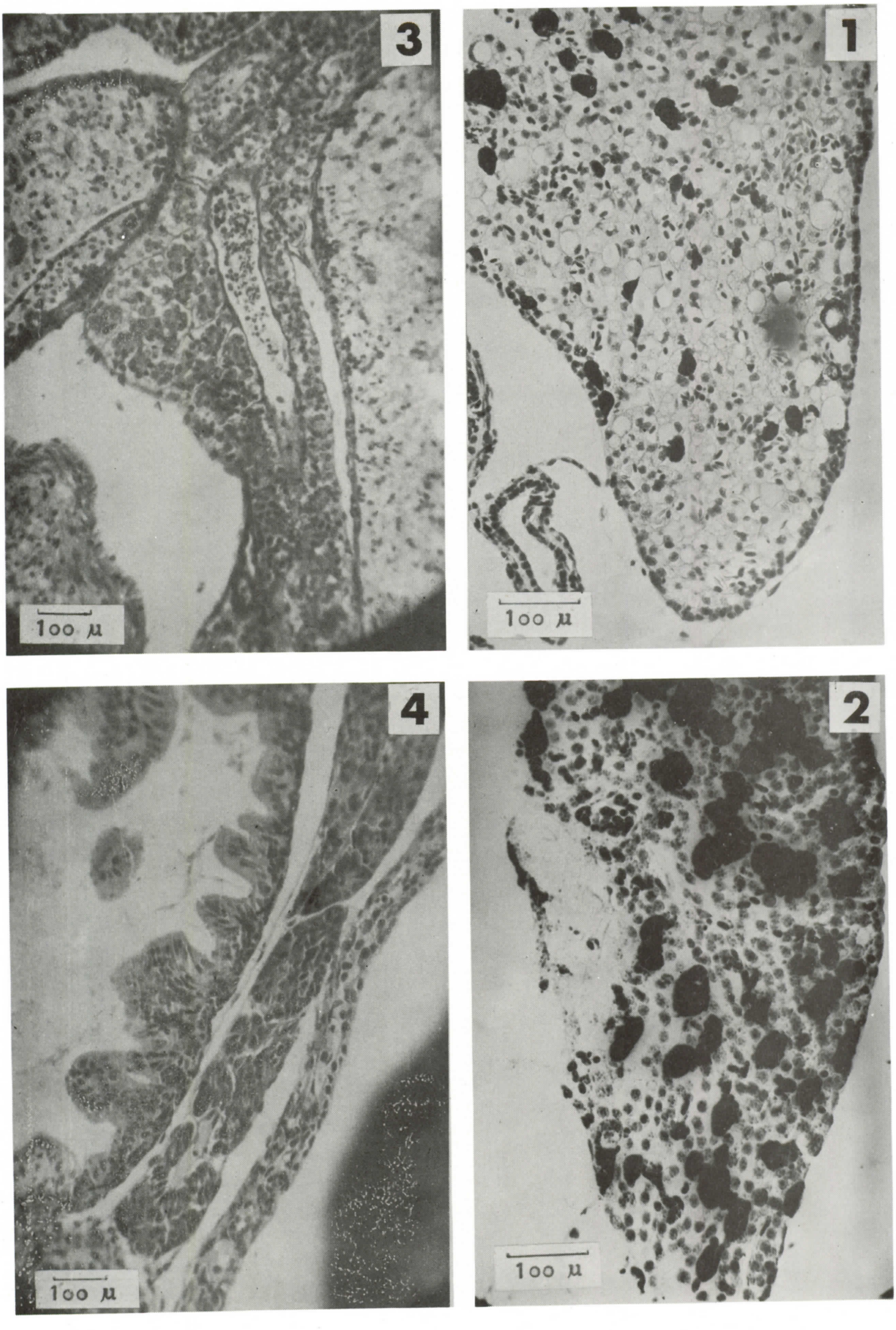

Plate 3. Cross section of diploid (1) and tetraploid (2) liver and diploid (3) and tetraploid (4) pancreas.

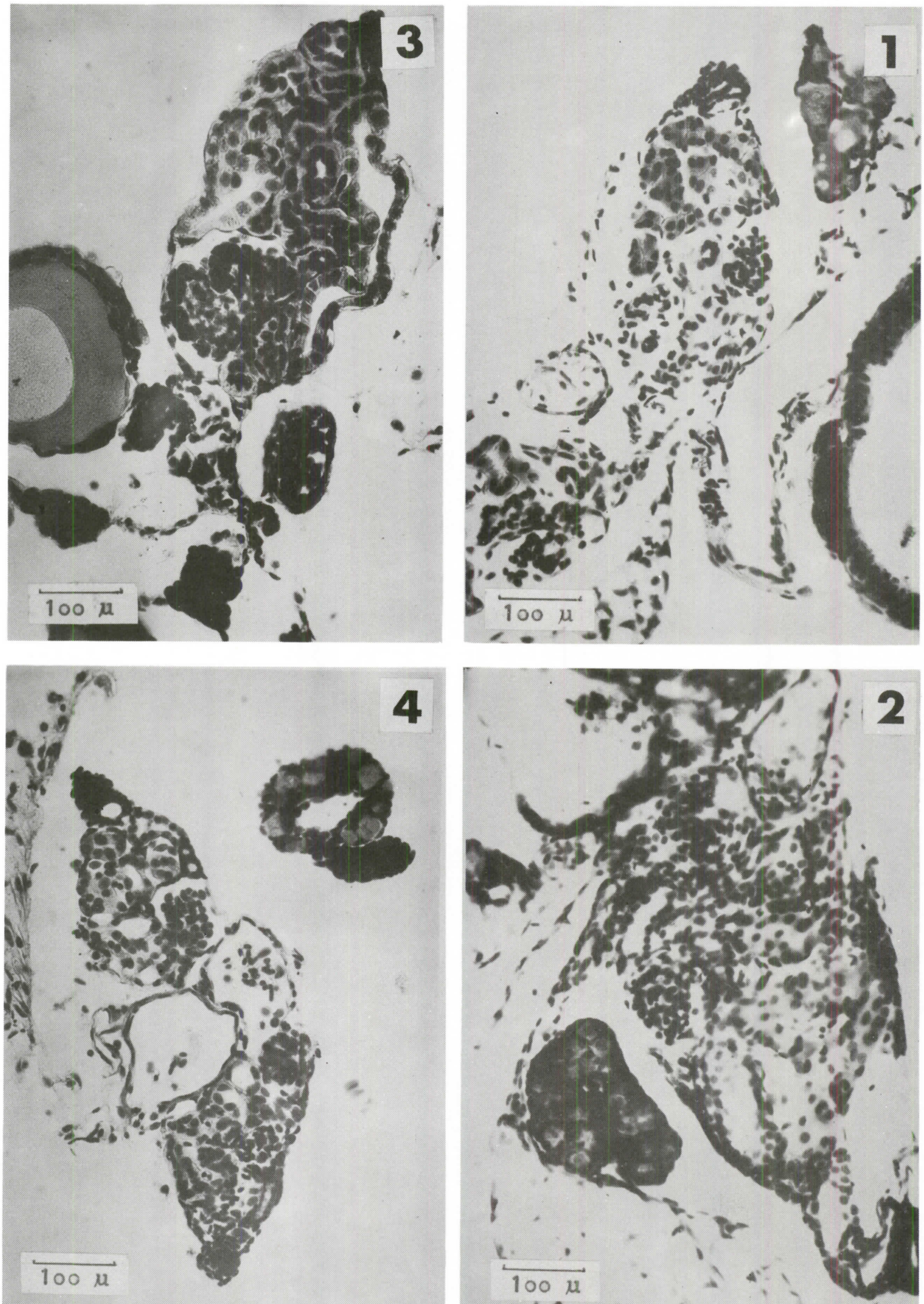

Plate 4. Cross section of diploid (1 and 2) and tetraploid (3 and 4) kidney.

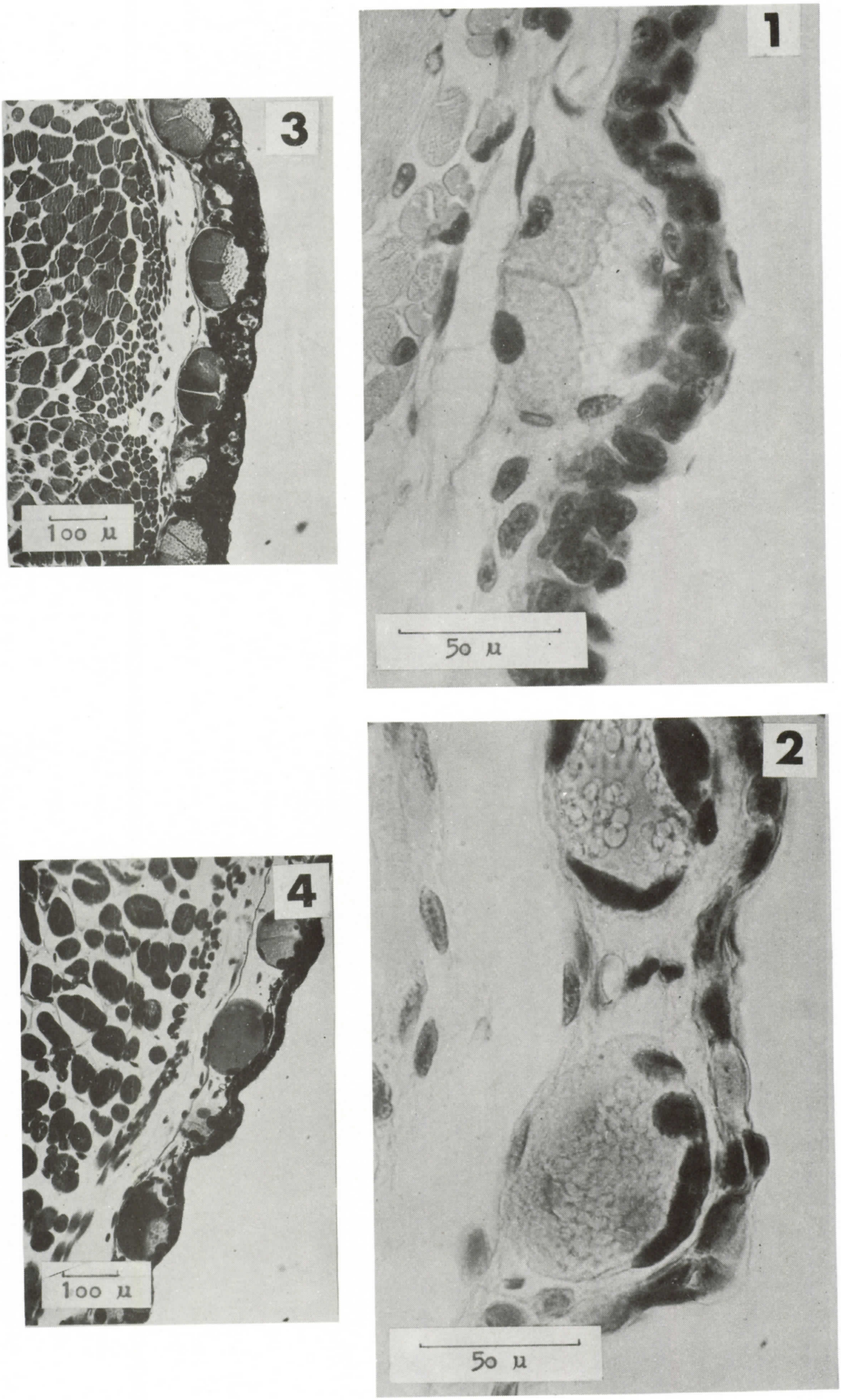

Plate 5. Cross section of diploid (1 and 3) and tetraploid (2 and 4) skin.

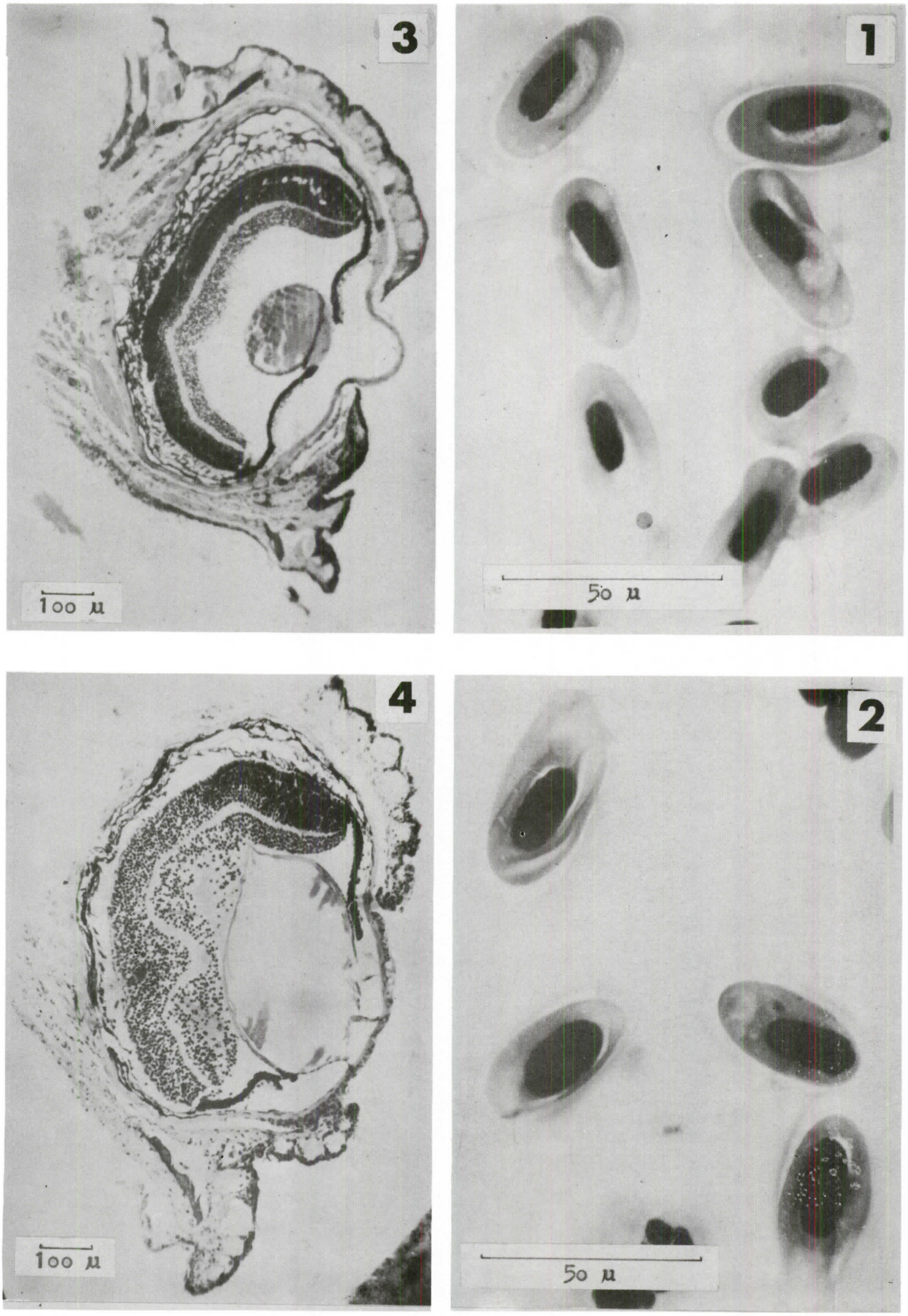

Plate 6. Red blood corpuscles of diploid (1) and tetraploid (2) smear. and cross section of diploid (3) and tetraploid (4) eye balls.

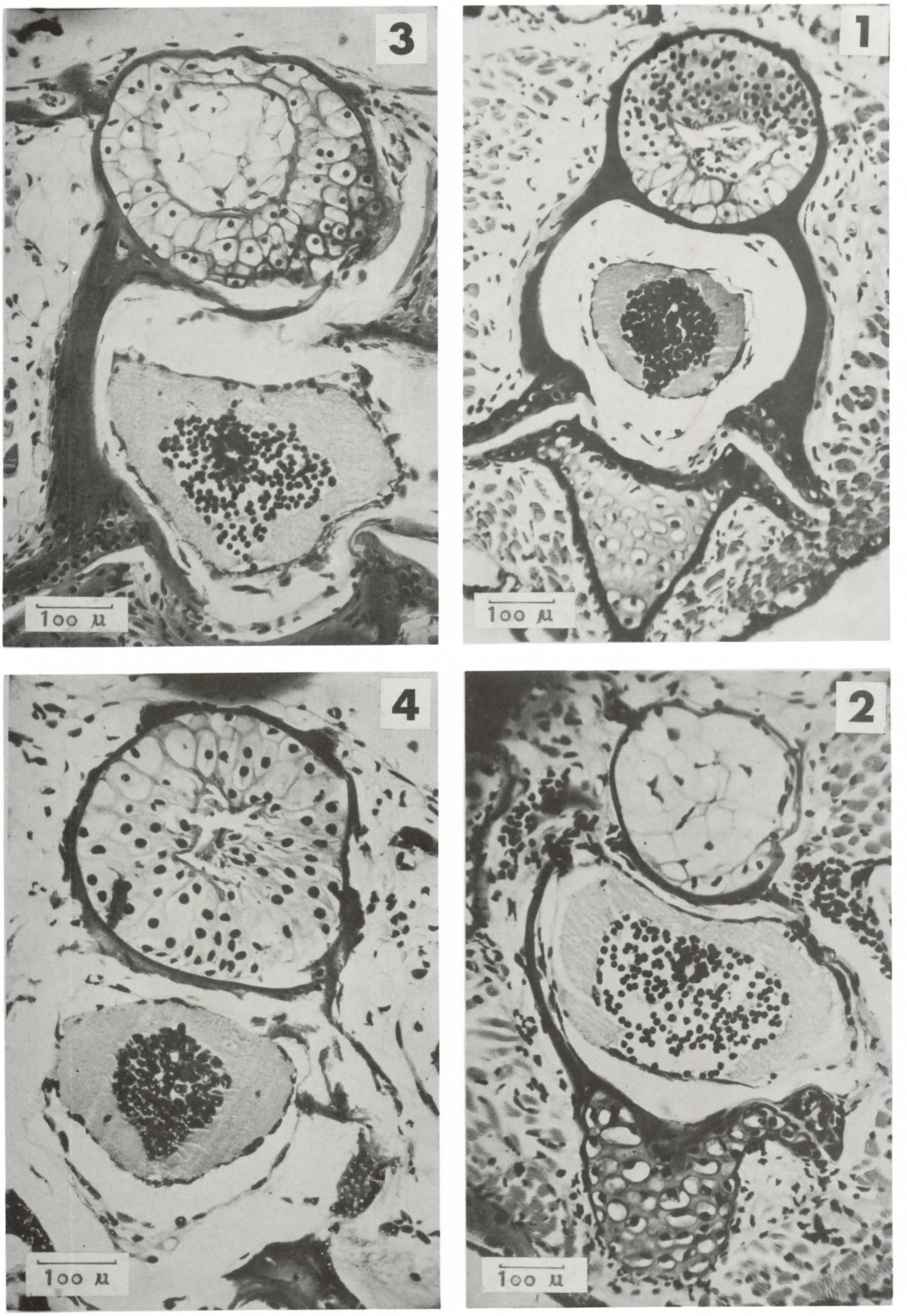

Plate 7. Cross section of diploid (1 and 3) and tetraploid (2 and 4) vertebrae.

超音波刺激によるイモリの四倍体の作成とそれらの組織学的観察

宇 都 宮 泰 明

イモリ (*Triturus pyrrhogaster*) とシリケンイモリ (*Triturus ensicauda*) の受精卵を超音波で刺激することにより多数の四倍体を二倍一四倍性モザイクと共に作成することができた。受精卵の二分割のかすかな条溝が現われた時超音波を照射するのがよく，その量は超音波で撹拌しながらその熱作用により５分間で39℃ないし３分間で42℃に昇温するときが効果的であった。同質四倍体はいずれも幼生の時は二倍体とほぼ同様に成長したが，上陸は遅れた。上陸後も外鰓は残り，皮膚は幼生のようで変態を完了できず，ほとんどの個体は半年以内に死滅した。これに対し異質四倍体は，二倍体とほぼ同様に変態を完了した。両者のうちシリケンイモリの雌にイモリの雄をカケ合わせた雑種から作った四倍体のほうが生活力旺盛であった。イモリとシリケンイモリの二種からの同種，異種四種の組み合わせの二倍体の内臓諸器官の間には特に著しい差はなかった。而しシリケンイモリおよびシリケンイモリ雌×イモリ雄の雑種はその脊椎骨は丈夫に見え，また骨化が他より進んでいるようだった。四倍体についても同様なことが云える。また四倍体の骨化が二倍体に比較して少し遅れるということが云える。

四倍体の内臓諸器官を構成する細胞並びに細胞核は全て二倍体に比較してその体積において二倍以上，数において少なかった。従って四倍体の器官の構造は一般に簡単である。特に四倍体の多くの線（胃線，涙線，唾液線，甲状線，脳下垂体等）は構造が非常に簡単で，数が少なかった。

四倍体の性比は，イモリ雌×イモリ雄からの14個体中雌６雄４，シリケンイモリ雌×シリケンイモリ雄からの１個体中雌１，イモリ雌×シリケンイモリ雄からの18個体中雌10雄７，シリケンイモリ雌×イモリ雄からの５個体中雌３雄２であった。