CHANGES IN CONNECTIVE TISSUE AND INTESTINE CAUSED BY VITAMIN A IN AMPHIBIA, AND THEIR ACCELERATION BY HYDROCORTISONE*

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Cartilage may be profoundly depleted of matrix by the action of an excess of vitamin A, both *in vivo* and *in vitro* (1–3). This effect resembles that of the plant protease, papain, which degrades the protein-polysaccharide complex of cartilage matrix, causing liberation of a protein-poor chondroitin sulfate (3–6). Such similarity led to the suggestion that one mode of action of an excess of vitamin A was to release a papain-like enzyme from cartilage cells or their organelles (1, 3). Subsequent experiments have demonstrated that excess of vitamin A does, in fact, increase the proteolytic activity of embryonic avian limb rudiments (7). Since similar proteolytic activity could only be demonstrated in normal rudiments after their exposure to distilled water, it was postulated that vitamin A acted by releasing protease(s) from lysosomes, the intracellular organelles that elaborate acid hydrolases such as cathepsins, after exposure to hypo-osmolar conditions (8, 9). Using isolated lysosomal fractions, Dingle was able to show that "bound" catheptic activity could be released from lysosomes of rat liver by exposure to vitamin A *in vitro* (10).

It seemed desirable, therefore, to study a living system where considerable catheptic activity had already been correlated with the presence of lysosomes. Weber (11) had found a twentyfold increase of catheptic activity in the resorbing tails of *Xenopus laevis* larvae undergoing metamorphosis, and Novikoff (12) had demonstrated abundant lysosomes in such tails. It was predicted that if vitamin A acted upon lysosomes, then the induction of hypervitaminosis A would result in the resorption of amphibian tails *prior* to metamorphosis, because of the premature release of cathepsins from these organelles.

The experiments to be described below have shown that the tails of vitamin A–treated larvae of *Xenopus laevis* are indeed partially resorbed before metamorphosis. In addition, hypervitaminosis A caused remarkable, if unexpected, alterations in the intestines and connective tissue of the amphibia.

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While these experiments were in progress, Fell and Thomas (13) found that the effect of hypervitaminosis A upon bone and cartilage *in vitro* was retarded by hydrocortisone, in direct contrast to the exaggeration of hypervitaminosis A by cortisone in the rat (14). In the present experiments, the effect of hydrocortisone alone, and in conjunction with vitamin A was studied in the *Xenopus* larvae; each of the effects of vitamin A was accelerated by hydrocortisone when the agents were administered together.

Materials and Methods

Larvae.—*Xenopus laevis* larvae were obtained through the generosity of Dr. R. T. Sims, Department of Anatomy, University of Cambridge. They were kept in shallow tanks containing 800 ml of tap water for each group of 14 larvae, and fed three times a week with nettle powder (*Herba urticae*) as recommended by Nieuwkoop and Faber (15). At the beginning of the experimental period of 12 weeks, all larvae were at stages 49 to 51 (15) while at the end, 2 or 3 of the untreated group were undergoing early metamorphosis (stages 59 to 60). Water was changed before each feeding, and the tanks were kept shielded from direct sunlight.

Vitamin A.—Synthetic vitamin A alcohol, 3,360 IU/mg (Roche Products, Ltd., London), was stored under nitrogen (at −20°C) until used. It was ground with the feeding mixture, and added to tanks containing 14 larvae in 800 ml of water. Preliminary experiments had shown that 3.0 mg of vitamin A alcohol given thrice a week caused the death of all animals in one tank within 10 days. In all subsequent experiments, larvae were fed with 2.0 mg of vitamin twice weekly.

Hydrocortisone was obtained as efcortelan (hydrocortisone hemisuccinate sodium, Glaxo Laboratories, Greenford, England). Immediately upon feeding, 25.0 mg were dissolved in water and added to the tanks.

Histology.—At intervals noted below, larvae were killed with nembutal, fixed *in toto* in acetic alcohol, and embedded in paraffin. Sections cut at 7 μ were stained with hematoxylin and eosin, and with acidified toluidine blue (1:1000 in H_2O, acidified with 0.1 ml of glacial acetic acid per 100 ml of stain).

Experimental Design.—In the first series of experiments, 14 larvae were given vitamin A, 2.0 mg, while a similar number served as controls. After pronounced skeletal changes were visible (6 weeks) in the treated group, 6 of the tadpoles were transferred to a "recovery" tank, where they were fed without added vitamin A.[1] In the second series, the larvae were divided into four groups of 14 each: group A was given vitamin A as before, group HC was given hydrocortisone alone, group A + HC was given hydrocortisone and vitamin A, while group C served as controls. During the 3rd week, 1 of each group was killed, fixed, and embedded for histological examination, while for the remainder, treatment continued for 8 weeks. The larvae of each group were measured on the 1st, 28th, and 44th days of the experiments, by isolating them in Petri dishes which had been placed over a millimeter scale. The animals were measured while at rest, body and tail measurements taken separately, the dividing point being the caudal junction of the abdominal sac with the spine. At the end of the 8th week, all dead larvae had been fixed, and the surviving larvae were killed, fixed, and sectioned.

RESULTS

1. The Effects of Hypervitaminosis A.—

(*a*) *Gross morphology:* In larvae exposed to vitamin A, the first change became visible at the end of the 2nd week. The rostral tentacles, appendages useful in the

[1] A preliminary report of these experiments has been published elsewhere (16).

maintenance of equilibrium, began to droop perceptibly instead of holding their naturally erect form. Normally these are resilient structures, with a delicate curve pointing laterally; the affected tentacles became ragged in appearance, shrivelled at their extremities, and pointed medially. They moved passively with each feeding motion, in contrast to those of normal larvae in which their position was unaffected by buccal movement. During the 4th week, this change became so marked that fractures developed in some of these structures, and most of the larvae had lost their sense of equilibrium, a finding which was duplicated in 2 control animals whose tentacles had been broken unilaterally through accidental trauma.

TABLE I

Measurements of Tail and Body Lengths in Xenopus laevis Larvae Given Excess Vitamin A, Hydrocortisone, or Both Agents Together

Day	Group	No.	Tail length (mean, A. D.)	Body length (mean, A. D.)	Ratio, $\frac{\text{body}}{\text{tail}}$
			cm	*cm*	
1	Control	14	1.73 ± 0.17	0.97 ± 0.10	0.56
	Vitamin A	14	1.78 ± 0.15	0.92 ± 0.12	0.52
	Hydrocortisone + vitamin A	14	1.76 ± 0.14	0.90 ± 0.10	0.51
	Hydrocortisone	14	1.86 ± 0.17	0.89 ± 0.13	0.49
28	Control	13	2.63 ± 0.19	1.48 ± 0.11	0.57
	Vitamin A	13	2.41 ± 0.18	1.36 ± 0.12	0.55
	Hydrocortisone + vitamin A	13	2.38 ± 0.19	1.24 ± 0.18	0.54
	Hydrocortisone	13	2.30 ± 0.15	1.19 ± 0.20	0.52
44	Control	13	3.21 ± 0.16	1.77 ± 0.15	0.55
	Vitamin A	13	2.30 ± 0.25	1.51 ± 0.13	0.65
	Hydrocortisone + vitamin A	13	2.33 ± 0.22	1.20 ± 0.17	0.53
	Hydrocortisone	13	2.21 ± 0.40	1.14 ± 0.11	0.52

Another change became noticeable on the 18th to the 20th day. Normal *Xenopus* larvae excrete a well formed, solid, dark green stool which readily sinks to the bottom of the tank. In the tanks of the hypervitaminotic group, however, light green mucinous stools floated to the surface; this was the result of a persistent diarrhea, as was clearly demonstrated by isolating single control and hypervitaminotic larvae in Petri dishes. Addition of vitamin A alcohol at equivalent concentration to a tank containing normal stools of *Xenopus* larvae did not cause a similar alteration of excreta. Neither animals that had been deliberately crowded to slow development, nor others that had been fasted for 3 weeks to simulate the effects of such diarrhea, showed changes that were seen in the group given excess of vitamin A.

At the end of the 3rd week, another change appeared. Animals in the treated group developed severe kyphoscoliosis; the most obvious deviation of contour was just above the origin of the girdle cartilage (Figs. 1 *A*, *B*). Soon afterwards the tails of the hypervitaminotic animals began to lose their normal, fine flagellar structure. As the larvae swam, the ends of their tails would thrash about aimlessly and soon became

quite shrivelled, while a few developed fractures of the tail. As treatment continued, the treated group showed signs of tail resorption, especially of the fins, whereas in the controls, proportional growth of head and tail continued. Measurements listed in Table I indicate that actual loss of tail substance took place between the 28th and 44th days of treatment.

A change in the normal apposition of upper and lower jaws was noticed during the 4th and 5th weeks. In hypervitaminotic larvae, the lower jaw protruded far beyond the upper; this prognathic appearance caused the lower jaw to resemble that of older animals which had undergone metamorphosis. Concurrently, the angle formed by junction of the cartilagenous skeleton with the spinal column changed from the normal of nearly 90° to a more acute one of 50 to 60°. Surprisingly, there was no inhibition of the protrusion of hind limbs in the treated group, nor any gross structural difference between the limbs formed by the treated and control animals (Figs 1 *A*, *B*).

(*b*) *Microscopic morphology:* The most striking difference between the normal and treated animals was in the gut. In sections of larvae fixed at the onset of diarrhea (3 weeks) there was little difference in the histological appearance of the gut in the two groups, but at 8 weeks there was a great increase in the number of metachromatically staining goblet cells in the intestines of the hypervitaminotic group (Figs. 3, 4). These cells were more prominent in the duodenum and proximal part of the small intestine, and in the rectal mucosa. When such cells were counted at a magnification of × 360 in sections cut at 7μ, they were found to be increased not only in absolute number, but in ratio to the normal cells of the intestinal epithelium. In equivalent sections of proximal intestine of larvae at stages 54 to 55, the ratio of metachromatic to normal cells had risen from 114/1000 in the controls to 753/1000 in group A. The whole character of the gut epithelium had changed. At equivalent stages, the epithelium of treated animals was columnar whereas that of the controls was usually cuboidal. Meanwhile, invagination of submucosa and mucosa, with hypertrophy of the muscle coat, caused the intestine of the treated tadpoles to resemble the more mature organ as it normally appears *after* metamorphosis (Figs. 10 *A*, *B*).

A difference in intestinal contents was also seen. In treated animals, this fecal debris was homogeneous, and was mixed with many thin, metachromatic strands that could be seen to originate in the hypertrophied goblet cells. In contrast, material within the gut of control animals was compact and entirely orthochromatic (Figs. 10 *A*, *B*).

Much extracellular material, which stained metachromatically with toluidine blue, was present in the tails of the control group; this was most prominent in the nonmuscular dorsal and ventral fins (Fig. 5). In most of the hypervitaminotic larvae this material was entirely missing and was considerably reduced in the remainder (Fig. 6). Similar metachromatic strands were visible at the base of the well formed tentacles in the controls; these appendages were collapsed and shrivelled in the treated larvae, and no metachromatic strands could be identified at their base (Figs. 2 *A*, *B*).

"Small cell" cartilage such as that of Meckel's and that of the developing limbs was unaffected in the hypervitaminotic larvae (Figs. 8 *A*, *B*), but there was a moderate loss of metachromasia in the hypertrophic cartilage of the upper chondrocranium (Figs. 11 *A*, *B*). Other organs were similarly unaffected, but the liver showed some cellular granularity, and an increase in sinusoidal spaces which presumably represented loss of parenchymal cytoplasm (Figs. 9 *A*, *B*).

(*c*) *Reversibility of the changes:* 6 animals were placed in tanks without additional vitamin, 42 days after the treatment had begun. Mucinous diarrhea continued for 8 to 10 days, but then gradually ceased. It required another 2 weeks in these "recovery" tanks for the skeletal changes, protrusion of lower jaws (Figs. 7 *A*, *B*), and kyphoscoliosis, to regress. Within 4 weeks, all stigmata of hypervitaminosis A had disappeared, and the tentacles had assumed their normal, resilient character. Histologically, these animals were entirely normal, and their gastrointestinal tract did not show an overgrowth of metachromatic goblet cells.

2. The Influence of Hydrocortisone Alone.—

(*a*) *Gross morphology:* In Table II are listed the results of the second series of experiments. None of the characteristic findings of hypervitaminosis A were duplicated in the group given hydrocortisone alone, but there were two pronounced effects. The melanophores of the steroid-treated group were contracted even when these animals were in the shade so that the larvae looked perpetually blanched. In addition, the animals were much smaller than the controls and their entire development was retarded. Whereas some of the larvae in the control group had progressed to early metamorphosis at the end of the experiment, and some of those in the A group had early forelimb protrusion, none of the hydrocortisone-treated animals had reached this stage. In this developmental retardation the A + HC group resembled the HC group. Although all groups were evenly matched as to larval stage at the outset, the HC animals did not progress beyond stages 54 to 55; there was no loss of tail substance, however, nor did the tentacles become collapsed.

(*b*) *Microscopic morphology:* Little change was found in the ground substance of connective tissue or in the matrix of cartilage. Histologically, this group differed little from the controls except that most structures were smaller. Strands of metachromatic material in the tails, comparable in every way to those of the controls, could be identified. The liver, however, was affected by hydrocortisone treatment. Changes not unlike those seen in the A-treated group were evident, with some increased cytoplasmic granularity and occasional fatty infiltration; an increase in sinusoidal spaces was matched by a corresponding decrease of cytoplasmic mass (Fig. 9 *C*).

3. The Influence of Hydrocortisone upon Hypervitaminosis A.—

(*a*) *Gross morphology:* Although the changes produced in the group given both vitamin A and hydrocortisone (A + HC) were of the same type as in the group given vitamin A alone (A) each of the changes began about 7 days earlier in the group receiving both agents (Table II). As in the first experiments, the earliest lesion was a collapse of the tentacles, followed by mucinous diarrhea. Grossly visible kyphoscoliosis and tail resorption developed subsequently, while prognathos appeared later. It may be seen from the table that the lethal effect of either vitamin A or hydrocortisone alone seemed to be accelerated when the two agents were combined. Similarly, at a time when no change in the contour of the spine was visible in the A-treated group, those animals given steroid as well had already developed kyphoscoliosis. From measurements listed in Table I it is seen that total development was arrested in the A + HC group, paralleling the growth arrest of group HC. The A + HC larvae showed tail

TABLE II

Effects of an Excess of Vitamin A, Hydrocortisone and Vitamin A, and Hydrocortisone Alone upon Larvae of Xenopus laevis

Days	Group	No. living	No. dead	No. with collapsed tentacles	No. with mucinous diarrhea	No. with resorption of tail	No. with kypho-scoliosis	No. with prognathos
1 to 7	C*	14	0	0	0	0	0	0
	A‡	14	0	0	0	0	0	0
	A + HC§	14	0	0	0	0	0	0
	HC‖	14	0	0	0	0	0	0
8 to 14	C	14	0	0	0	0	0	0
	A	14	0	2	0	0	0	0
	A + HC	14	0	6	11	0	0	0
	HC	14	0	0	0	0	0	0
15 to 21	C	14	0	0	0	0	0	0
	A	14	0	14	14	3	0	0
	A + HC	14	0	14	14	11	10	0
	HC	14	0	0	0	0	0	0
22 to 28	C	13	0	0	0	0	0	0
	A	13	0	13	13	12	13	1
	A + HC	13	0	13	13	13	13	6
	HC	13	0	0	0	0	0	0
29 to 35	C	13	0	0	0	0	0	0
	A	13	0	13	13	13	13	4
	A + HC	13	0	13	13	13	13	13
	HC	13	0	0	0	0	0	0
36 to 42	C	13	0	0	0	0	0	0
	A	13	0	13	13	13	13	13
	A + HC	13	0	13	13	13	13	13
	HC	13	0	0	0	0	0	0
43 to 49	C	13	0	0	0	0	0	0
	A	12	1	12	12	12	12	12
	A + HC	8	5	8	8	8	8	8
	HC	13	0	0	0	0	0	0
50 to 56	C	13	0	0	0	0	0	0
	A	9	4	9	9	9	9	9
	A + HC	7	6	7	7	7	7	7
	HC	7	6	0	0	0	0	0

* C, controls.

‡ A, vitamin A alone.

§ A + HC, vitamin and hydrocortisone concurrently.

‖ HC, hydrocortisone alone.

resorption and a relative decrease in total body size, in contrast to the A-treated group, which showed tail resorption alone, with no failure of body growth. (Fig. 1 *C*).

(*b*) *Microscopic morphology:* There was no difference in the characteristic overgrowth of metachromatic goblet cells between the group A + HC and group A, and it was impossible to tell from sections of the gut whether or not hydrocortisone had been given. Nor indeed was there any difference between the two groups in loss of metachromasia from the ground substance of the tails and tentacles. One constant feature in the A + HC group was the grossly distorted parenchymal architecture of the liver. The sinusoidal spaces were large and empty, while the cytoplasm of the liver cells was severely diminished: there was evidence of cellular necrosis in some areas. In contrast to the controls and to those treated with either vitamin A or hydrocortisone alone, there was little evidence of lipid or glycogen storage, and the entire liver was reduced in mass (Fig. 9 *D*).

DISCUSSION

The results presented confirm the prediction made at the outset: one effect of an excess of vitamin A upon a structure containing abundant lysosomes, is to cause resorption of that structure, presumably through the liberation of lytic enzymes such as the acid cathepsins. Loss of metachromasia in the ground substance of larval appendages probably represents the same phenomenon that has been observed in bone rudiments exposed to excess vitamin A *in vitro* (2, 3), and in the cartilage of rabbits made hypervitaminotic A *in vivo* (1). The curling of the distal third of the ears of such rabbits is very similar to the collapse of tentacles that has been described above. The suggestion that excess vitamin A might act by causing the release of proteases (1, 3, 9) was originally prompted by the observation that the ears of rabbits can be collapsed by the injection of the exogenous protease, papain. This enzyme, acting upon a protein-polysaccharide complex of high molecular weight (mol wt 4×10^6) liberates protein-poor chondroitin sulfate from the cartilage matrix into the surrounding media *in vitro*, or into the circulation *in vivo* (6, 17). The histologic result of this loss of a readily diffusible polyanion, is the marked decrease in basophilia of the affected cartilage when stained with hematoxylin and eosin, and loss of metachromasia with toluidine blue (4).

In organ culture, the various limb rudiments differ slightly in their degree of response to excess of vitamin A (3). In intact rabbits, there was a striking difference in the susceptibility of various cartilages, articular and epiphyseal cartilage being more regularly and severely affected than that of the trachea or ear (1). In the *Xenopus* larvae, extracellular material which is destined to survive metamorphosis, *e.g.*, small cell cartilage of developing limbs, spinal column, and Meckel's rod, was unaltered by the induction of hypervitaminosis. In direct contrast, two transient structures, tails and tentacles, were drastically affected as shown by the loss of metachromatic ground substance. While the exact chemical nature of this material has not yet been identified, by analogy

with cartilage matrix such loss of metachromasia presumably represents degradation of mucopolysaccharide-protein complexes by proteolytic activity. The only cartilage affected, in these amphibian larvae, was the hypertrophic cartilage of the upper chondrocranium in which calcification progresses during metamorphosis and which eventually is resorbed. This agrees with observations made during hypervitaminosis A in rabbits (1), chicks (18), and rats and guinea pigs (19), which suggested that the hypertrophic chondroblasts of the epiphyseal plate were highly susceptible to the action of excess of vitamin A. If indeed the action of excessive vitamin is to release a protease from the lysosomes, then the "built-in" mechanism of tissue resorption is most readily activated in those structures that are destined *not* to survive metamorphosis in their larval form. At the time of metamorphosis, more permanent structures presumably do not contain susceptible lysosomes. In this regard, Novikoff (12) has demonstrated a high concentration of one lysosomal enzyme, acid phosphatase, in precisely that area of hypertrophic cartilage in the chick embryo (the calcifying edge) where Wolbach and Hegsted (18) had demonstrated susceptibility to excess vitamin A in the newly hatched chick.

Another mechanism that may protect the cartilage of amphibians during metamorphosis, is the presence of a serum protein which would bind the cathepsins which have been released from lysosomes. Such protease binding by the $alpha_2$ globulin fraction of normal rabbit and human sera has already been shown (5, 20, 21); the sudden appearance of a protein with similar electrophoretic characteristics during metamorphosis (22) might represent a mechanism for the protection of permanent structures against the harmful effects of endogenous or exogenous proteases entering the circulation.

Before commenting on the remarkable overgrowth of goblet cells in the intestines of hypervitaminotic larvae, we must consider whether induction of diarrhea *per se* with subsequent malnutrition caused the skeletal changes. Since collapse of tentacles occurred before, and resorption of tail substance began immediately after the onset of diarrhea, this explanation seems unlikely. Furthermore, neither larvae that had been crowded, nor others which had been fasted, showed any of the changes seen in the animals given excess vitamin A.

Embryonic avian skin undergoes mucous metaplasia in response to vitamin A *in vitro* (23), and Lawrence and Bern (24) have described mucous gland formation in keratinised epithelium of the cheek pouch of the adult golden hamster, as a result of local exposure to high concentrations of vitamin A. The findings described in this report are the first to show histological changes in gastrointestinal epithelium caused by excess of vitamin A. Recent work by Wolf and Varandani has indicated that vitamin A plays a role in the synthesis of sulfated mucopolysaccharides of the intestine (25). In the intestines of vitamin A–*deficient* rats, they found mucopolysaccharide formation to be diminished, and were able to stimulate the incorporation of S^{35} sulfate into

mucopolysaccharide by the addition of vitamin A but not of other fat-soluble vitamins. Wolf and Varandani (25) then framed the hypothesis that "vitamin A, acting like a hormone, regulates the formation of mucopolysaccharides; excess vitamin A leading to an increase in the mucus type, a deficiency to an increase in the connective tissue type." The overgrowth of metachromatic mucus-secreting cells, in the *hyper*vitaminotic A larvae supports this hypothesis. Whether this hyperplasia of goblet cells is associated with activation of lysosomes in the epithelium, remains to be investigated.

Acceleration of the effects of hypervitaminosis A by cortisone was first described, in the rat, by Selye (14), who found that the steroid greatly increased the bone resorption caused by an excess of the vitamin. Fell and Thomas (13), however, found that the effect of vitamin A on cartilage (mouse and chick) and bone (mouse) growing in organ culture, was much retarded by hydrocortisone added simultaneously. We have found in preliminary experiments (26) that pretreatment with hydrocortisone retarded the release of catheptic activity from isolated lysosomes by excess vitamin A. Thus, *in vitro* studies and organ culture techniques offer opposing data.

The discrepancy between the experiments on isolated preparations and those on intact animals may be due to systemic factors that modify the distribution of the vitamin in the animal. Clark and Colburn (27) found that cortisone reduced the amount of vitamin A stored in the liver of rats deficient in vitamin A, while Wang *et al.* (28) were able to elevate serum levels of vitamin A by administering corticotropin to patients with rheumatic fever. It is thus not unlikely that one effect of hydrocortisone is to elevate serum levels of the vitamin, by decreasing the liver stores. This view is supported by the appearance of degeneration and diminution of cytoplasm in the livers of *Xenopus* larvae receiving both steroid and vitamin A.

SUMMARY

In view of the theory that an excess of vitamin A causes release of cathepsins from intracellular lysosomes, hypervitaminosis A was induced orally in the larvae of *Xenopus laevis*. It was predicted that the tails of these amphibia would undergo resorption prior to metamorphosis, since the presence of abundant lysosomes, associated with measurable increases of catheptic activity, had previously been demonstrated in the resorbing tails of amphibia during metamorphosis. This prediction was confirmed; after 3 to 4 weeks of hypervitaminosis A, the tails of treated animals underwent partial resorption.

Other transitory appendages, the rostral tentacles, collapsed after 2 weeks of treatment with an excess of vitamin A, an effect analogous to the collapse of rabbits' ears after intravenous papain. These effects were related to the loss of metachromatic extracellular material in these appendages. Excess of vitamin A caused kyphoscoliosis and prognathos in the larvae.

The hypervitaminotic larvae always developed a mucinous diarrhea, which was associated with a remarkable overgrowth of metachromatic goblet cells of the intestine. The entire intestine of the treated animals was more advanced in development than that of control larvae at equivalent stages.

All the effects of hypervitaminosis A were accelerated by the simultaneous administration of hydrocortisone. This was held to be due to liberation of vitamin A from hepatic stores by the steroid, and is in contrast to the retardation of hypervitaminosis A by hydrocortisone *in vitro*.

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EXPLANATION OF PLATES

PLATE 51

FIG. 1 *A*. Normal larva of *Xenopus laevis*, with hind limb development. Fixed, cleared specimen. × 2.

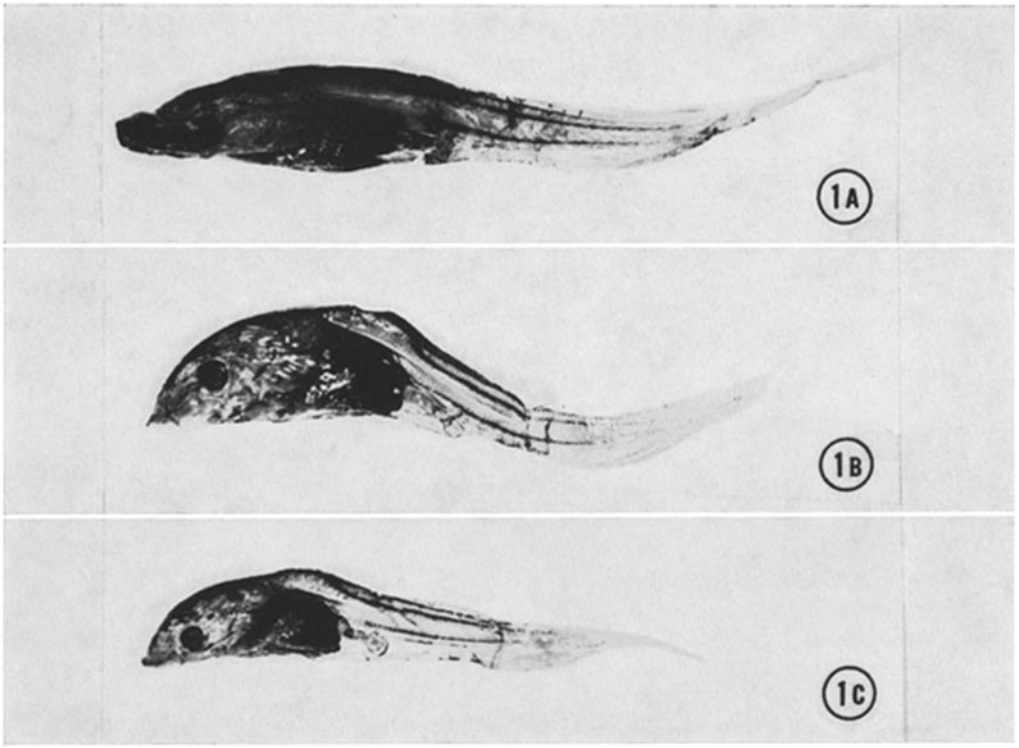
FIG. 1 *B*. Larva of *Xenopus laevis* after 44 days of hypervitaminosis A. Note the marked kyphoscoliosis, evidence of tail resorption, and protrusion of lower jaw. Fixed, cleared specimen. × 2.

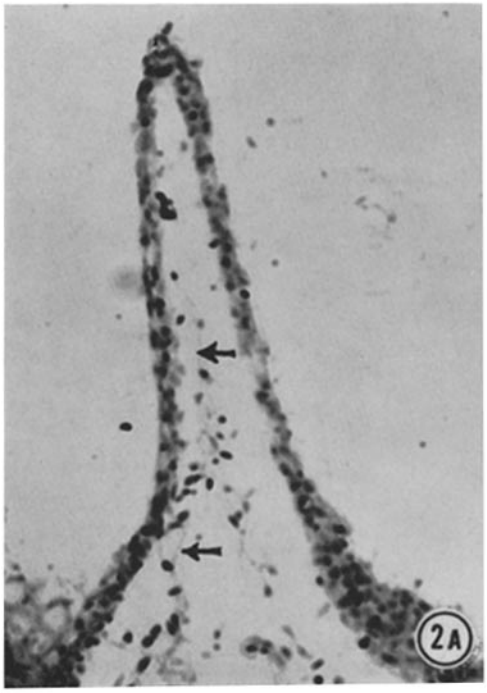
FIG. 1 *C*. Larva of *Xenopus laevis* after 44 days of hypervitaminosis A with added hydrocortisone. In addition to the changes seen in the larvae given the vitamin alone, there is a general reduction in size of the whole animal. × 2.

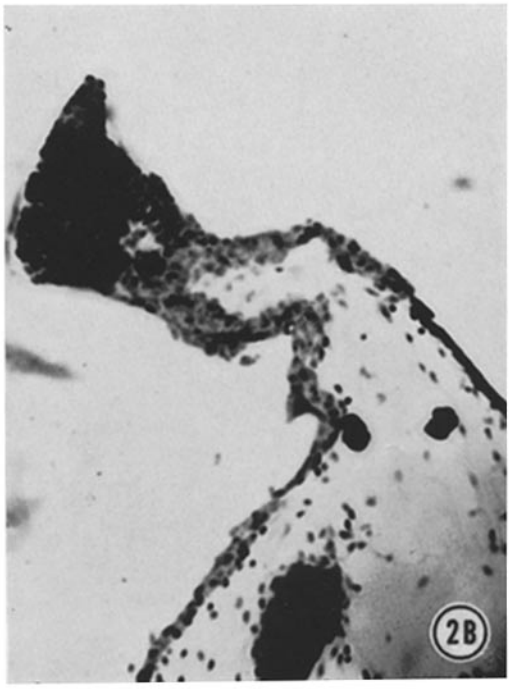
FIG. 2. Rostral tentacles of larvae of *Xenopus laevis*.

FIG. 2 *A*. Normal tentacle. There are delicate metachromatic strands at the base (indicated by arrows).

FIG. 2 *B*. Collapsed, shrivelled tentacle of animal after 44 days of hypervitaminosis A. No metachromatic strands are visible. Both, toluidine blue, × 180.





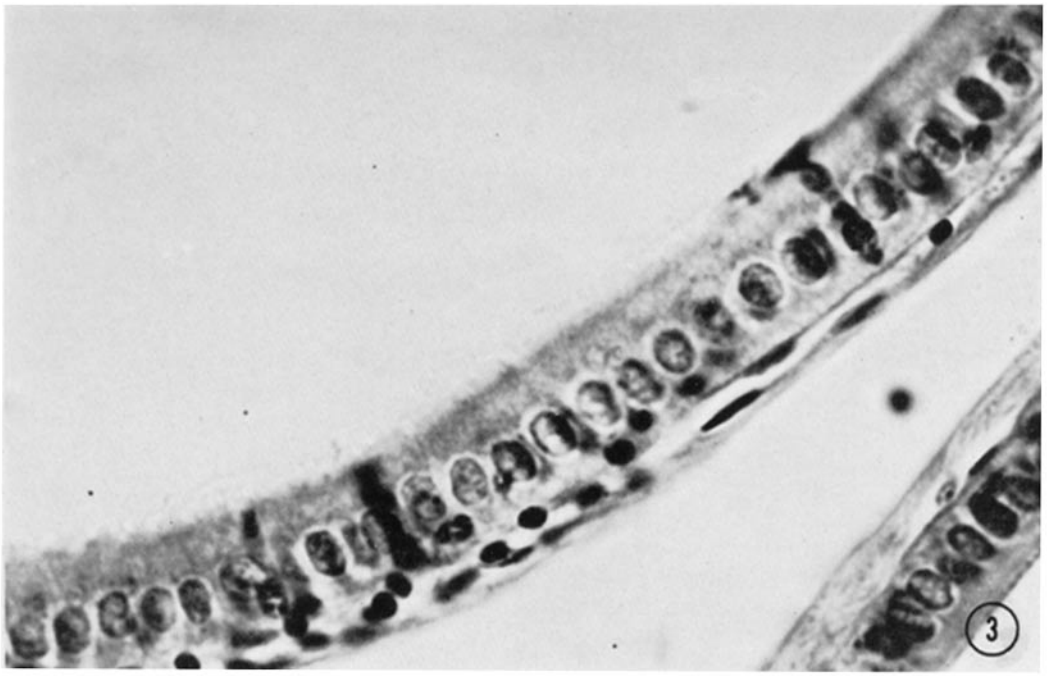


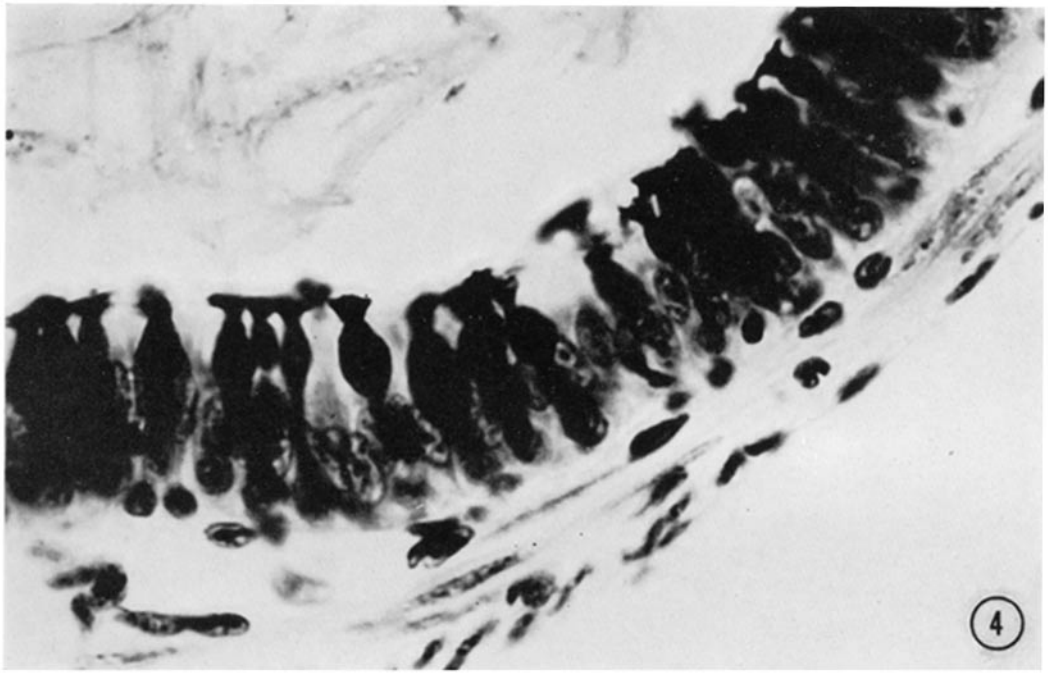
(Weissmann: Vitamin A in amphibia)

PLATE 52

FIG. 3. Normal proximal small intestinal loop of larva of *Xenopus laevis* (stage 55 to 56); three metachromatic goblet cells are present, and the epithelium is still cuboidal. Toluidine blue, × 760.

FIG. 4. Proximal intestine of larva of *Xenopus laevis* (stage 55 to 56) after 44 days of hypervitaminosis A. There is a remarkable overgrowth of metachromatic goblet cells. The epithelium is more columnar in appearance, and there is hypertrophy of the muscle coats, when compared to Fig. 3. Toluidine blue, × 760.



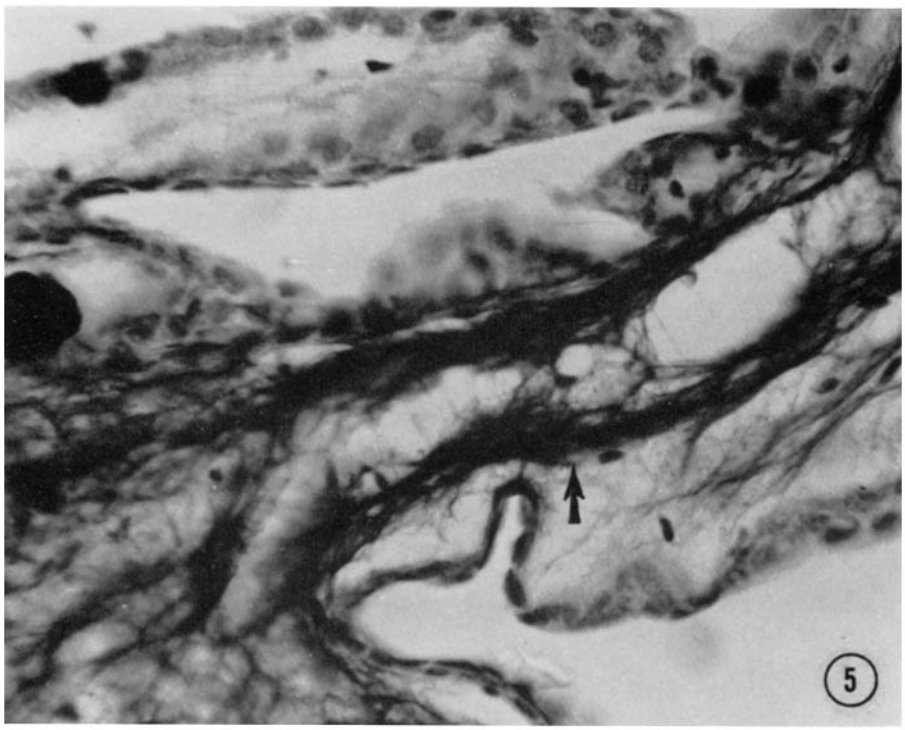


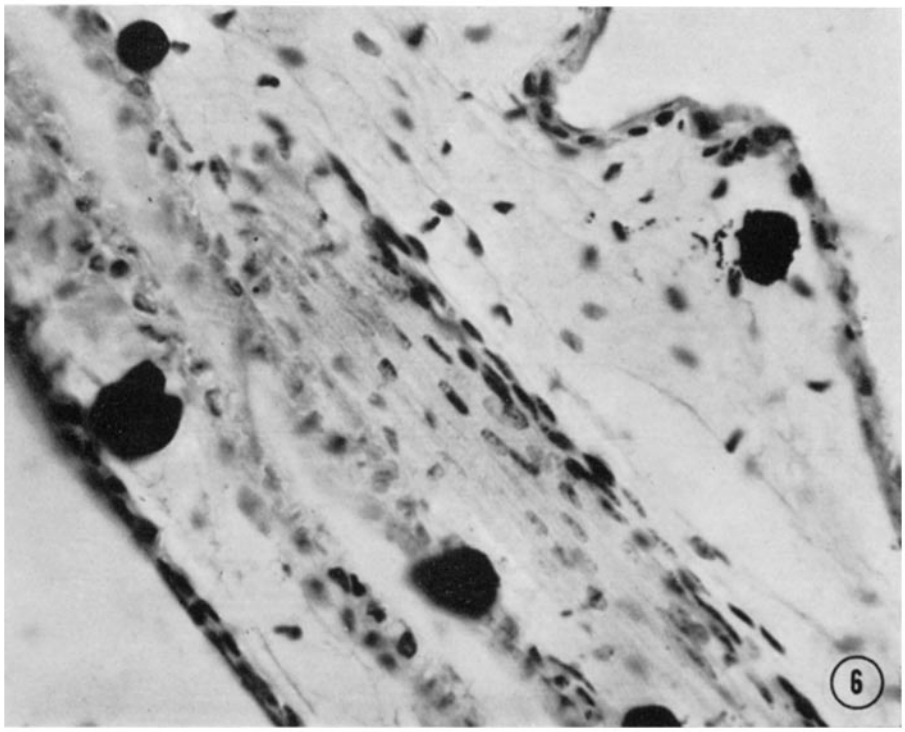
(Weissmann: Vitamin A in amphibia)

PLATE 53

FIG. 5. Section of tail of *Xenopus laevis* larva. Densely packed metachromatic fibers are seen in the ground substance, as indicated by the arrow. Toluidine blue, × 520.

FIG. 6. Section of tail of *Xenopus laevis* larva, after 44 days of hypervitaminosis A. There is practically no metachromatic material left in the tail at this point. The dark spots are melanophores. Toluidine blue, × 520.





(Weissmann: Vitamin A in amphibia)

PLATE 54

FIG. 7. Upper and lower jaws of larvae of *Xenopus laevis*.

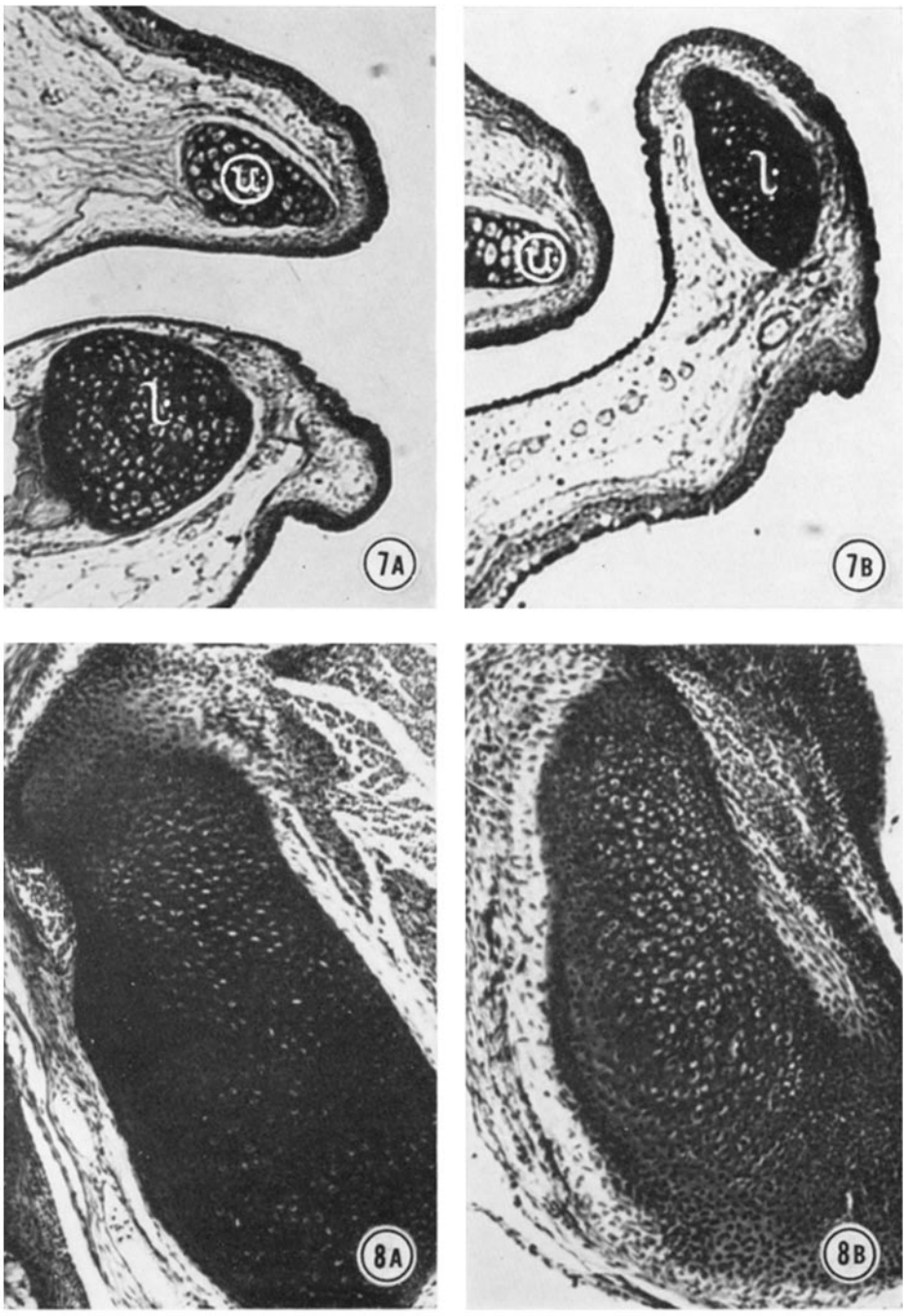
FIG. 7 *A*. There is good apposition of the upper (*u*) and lower (*l*) jaw cartilages in the normal animal.

FIG. 7 *B*. Larva after 44 days of hypervitaminosis A. Reduction in size of upper chondrocranium, with little effect upon Meckel's rod, has resulted in prognathic appearance; lower jaw protrudes before upper. Both, hematoxylin and eosin, × 75.

FIG. 8. Developing hind limb rudiments of larvae of *Xenopus laevis*.

FIG. 8 *A*. Normal, densely metachromatic cartilage.

FIG. 8 *B*. Little difference in this small-cell cartilage in larva after 44 days of hypervitaminosis A. Compare with Figs. 11 *A*, *B*. Both, toluidine blue, × 84.



(Weissmann: Vitamin A in amphibia)

PLATE 55

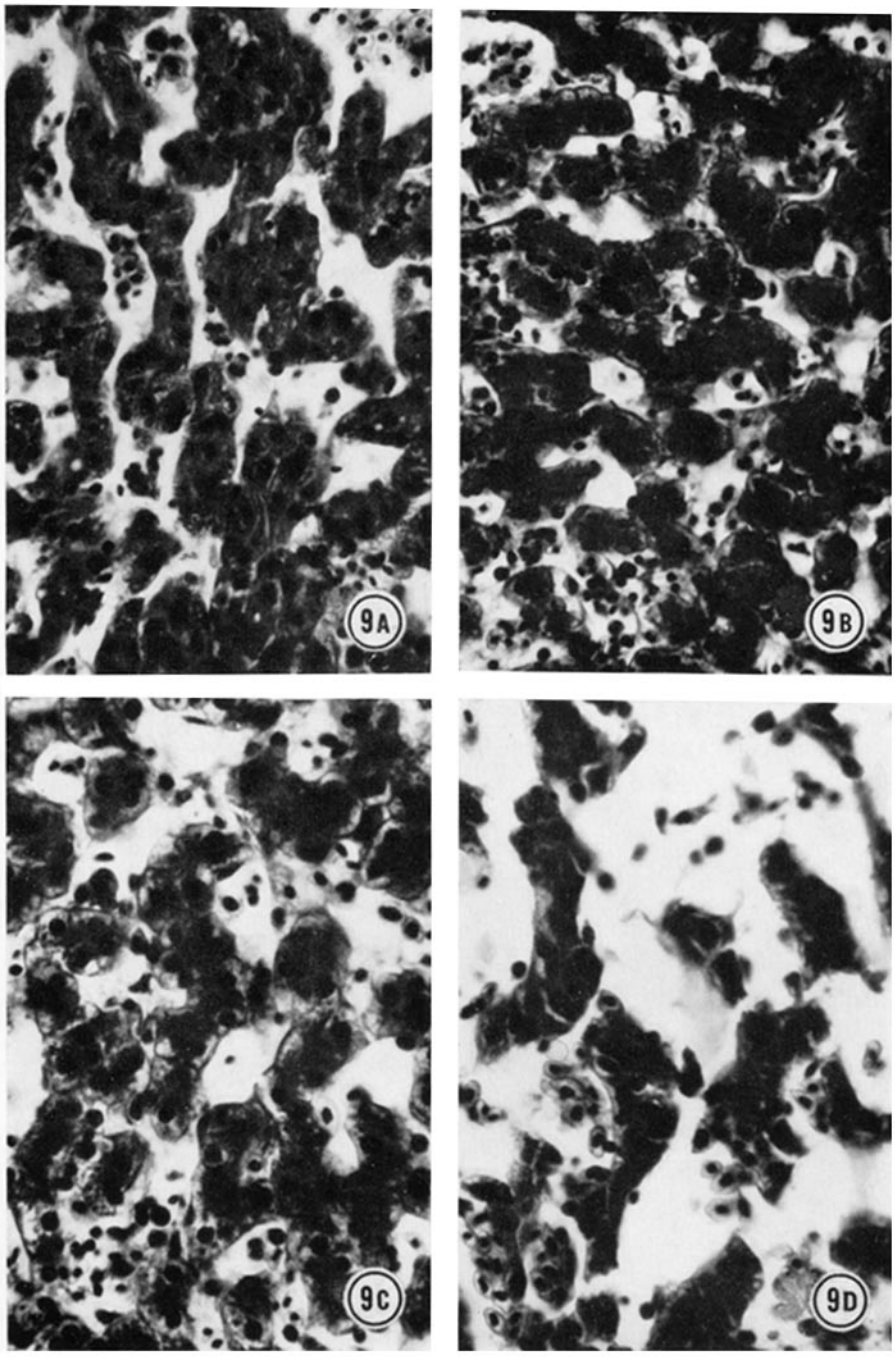
FIG. 9. Livers of larvae of *Xenopus laevis*.

FIG. 9 *A*. Normal liver. The cytoplasm is dense and the architecture is regular.

FIG. 9 *B*. Liver from larva after 44 days of hypervitaminosis A. There is some granularity in the peripheral cytoplasm.

FIG. 9 *C*. Liver from larva given hydrocortisone for 44 days. There is some increase in sinusoidal space, together with peripheral granularity.

FIG. 9 *D*. Liver from larva given vitamin A and hydrocortisone for 44 days. The parenchymal architecture is completely distorted with a marked increase of the sinusoidal spaces. All, hematoxylin and eosin, × 270.



(Weissmann: Vitamin A in amphibia)

PLATE 56

FIG. 10. Sections of intestinal loops, larvae of *Xenopus laevis* at stage 56 to 57.

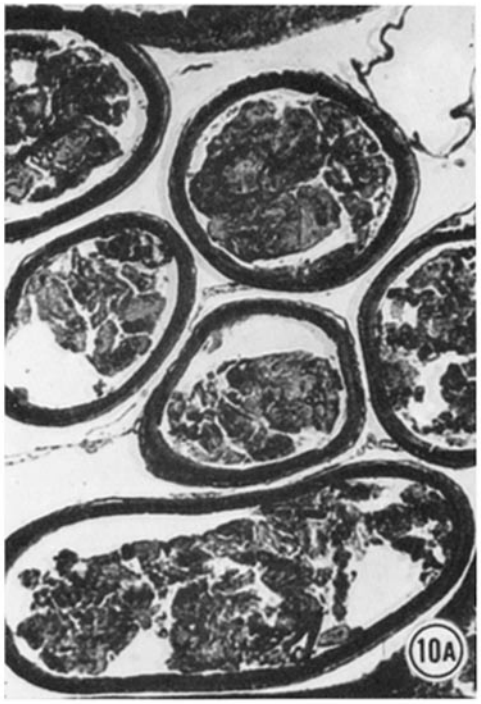
FIG. 10 *A*. Normal larva. The loops are flat with little invagination. Intestinal contents are particulate.

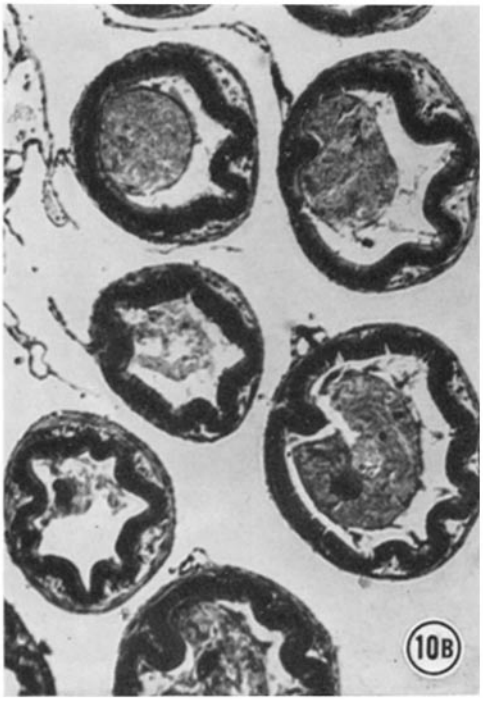
FIG. 10 *B*. Larva after 44 days of hypervitaminosis A. The intestinal loops are far more developed with invaginations, development of a submucosa, and hypertrophy of muscle. These intestines resemble the structures seen in more advanced larvae. The intestinal contents are more homogeneous and are surrounded by many secretory strands. Both, hematoxylin and eosin. × 60.

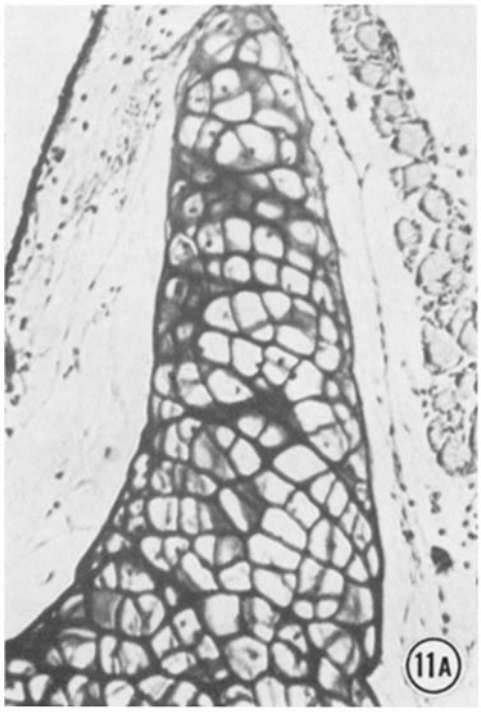
FIG. 11. Chondrocranial cartilage of larvae of *Xenopus laevis*.

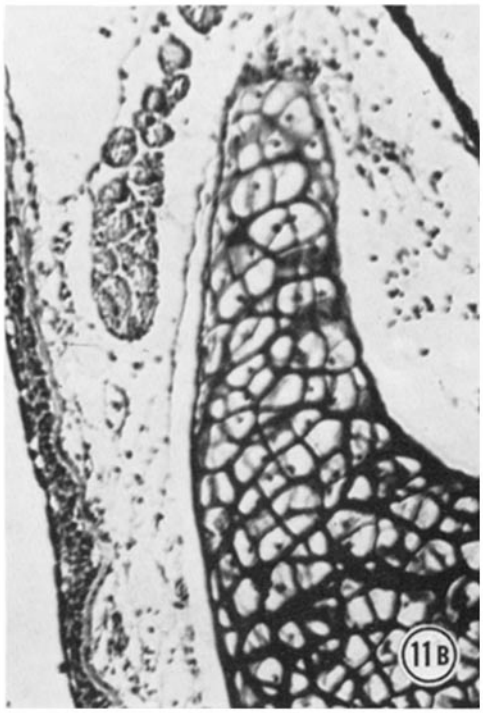
FIG. 11 *A*. Normal large cell cartilage. Good metachromasia of cartilage matrix, while the tip is well formed.

FIG. 11 *B*. Cartilage of larva after 44 days of hypervitaminosis A. The whole cartilage is reduced in size, while at the tip there is evidence of resorption, together with loss of metachromatic matrix. Both, toluidine blue, × 80.









(Weissmann: Vitamin A in amphibia)