promptly after a single injection of the above specified amounts of meralluride over a one minute period. Previous observations both in cats and dogs have yielded similar results(3,4).

Dogs tolerated large amounts of 1353Ex and 1431Ex without any indication of cardiac toxicity, which might have been anticipated since these two compounds are mercaptides (3). The chlorine substituted mercurial (1347Ex) appeared to have even less cardiac toxicity than meralluride, which is probably the least toxic of the non-mercaptide mercurials(3,4). No electrocardiographic changes occurred in any of the dogs given 1347Ex until the total amount infused was equivalent to 75 mg/kg of mercury and in 4 dogs, the QRS complex was not prolonged until about twice this amount of the compound had been given. These values are many times the amount of meralluride mercury required to produce similar electrocardiographic changes.

Summary. The compounds, 3-chloromercuri-2-methoxypropylurea (1347Ex), 3-carboxymethylmercaptomercuri - 2 - methoxypropylurea (1353Ex), and 3-(α-carboxyethylmercaptomercuri)- 2 -methoxypropylurea (1431-Ex), had 3 or 4 times the diuretic potency of meralluride in the dog. Twenty-four and 48 hour mercury excretion rates were comparable to that of meralluride at the same dosage level. Chronic toxicity studies indicated no essential difference between meralluride and the other compounds, if the greater diuretic potency of the new compounds was considered and the dosage adjusted accordingly. Large amounts of 1353Ex and 1431Ex were infused intravenously without disturbing cardiac function, but the acute cardiac toxicity of 1347Ex, although greater than the other 2 compounds, appeared to be less than meralluride.

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3. Lehman, R. A., PROC. SOC. EXP. BIOL. AND MED., 1947, v64, 428.

4. Chapman, D., and Shaffer, C. F., *Arch. Int. Med.*, 1947, v79, 449.

Distribution of Phosphorus^{32} in the Embryo and Larva of the Frog.* (19096)

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Radioactive substances have been employed in several studies on protein synthesis during development. Recently, Friedberg and Eakin (2) studied the incorporation of C^{14} of radioactive glycine into protein in the embryos and larvae of the Pacific tree-frog, *Hyla regilla*. They reported an increase in the uptake of glycine from the beginning gastrula through the early larval stages. This was interpreted as evidence of acceleration in protein synthesis accompanying differentiation and growth. Dent(1) observed the effects of phosphorus^{32} on regenerating amphibian tissues. He found that tadpoles of *Rana clamitans* immersed for 3 days in solutions of P^{32} (1000 microcuries) before amputation of the tail, regenerated a tail which was half as large as that of the control after 2 weeks. By introducing radioactive phosphorus into the hen's egg and analyzing chemical extracts of the tissues of the embryos, Hevesy, Levi and Rebbe(4) showed that the phosphatide molecule of the yolk was lower in specific activity than that of the embryo and con-

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1. Dent, J. N., *Anat. Rec.*, 1949, v103, 439.

2. Friedberg, F., and Eakin, R. M., *J. Exp. Zool.*, 1949, v110, 33.

3. Hansborough, L. A., and Nicholas, P., *J. Exp. Zool.*, 1949, v112, 195.

4. Hevesy, G., Levi, H. B., and Rebbe, O., *Biochem. J.*, 1938, v32, 2147.

5. Holt, M. W., Cowing, R. P., and Warren, S., *Science*, 1949, v110, 326.

cluded that the more active molecule is synthesized in the embryo. In the embryo itself, a metabolic gradient of phosphorus metabolism has been described as early as the 18th hour of incubation(6). Hansborough and Nicholas(3), using radiophosphorus in the chick embryo found that the uptake of P^{32} increases with the age of the embryo and that the most active regions of the embryo are regions where the population of cells is greatest. They observed no tissue specificity in the process.

Since it is more easily observed and its morphological development is well known, it seemed desirable to extend the latter study to the amphibian embryo.

Methods. Male and female frogs *(Rana pipiens)* in amplexus were collected at the Kenilworth Aquatic Gardens, Washington, D. C. Fertilized eggs were obtained from these, divided into 3 groups of 225 each and placed in finger bowls containing 750 ml of radioactive NaH_2PO_4. The solution in the first container had an activity of 550 microcuries; the second, 300 microcuries and the third, 65 microcuries.† The developing eggs were allowed to remain in these solutions until the desired stages were reached. Similar groups were prepared and exposed periodically for 2 hours every 24 hours and then returned to tapwater. The stages were determined according to the stages of development outlined by Rugh(7) after Shumway. The temperature and water level in each container were kept constant. Embryos and larvae were prepared for autoradiographs according to the method of Holt, Cowing and Warren(5). Whole embryos used for autoradiographic study were embedded in paraffin blocks. Those prepared for sectioning were cut at 10 microns and mounted on microscope slides. Triple-X panchromatic sheet film was used in preparing autoradiographs. The film was placed in close contact with whole mounts and sections, wrapped tightly in black paper, bound with cellophane tape and rubber bands and exposed for 5 days. They were then developed in Kodak Selectol (D-52) and fixed according to the usual procedure. For microscopic study, sections were stained in Delafield's hematoxylin and eosin and mounted in permount. All photographs were made with a Leica Microcamera. The magnifications of microphotographs and autoradiographs are indicated under explanation of figures.

Results and conclusions. Embryos exposed to different concentrations of P^{32} show the highest uptake of phosphorus in the solutions of greatest concentration of phosphorus (550 microcuries), which is to be expected. Those in the lowest concentrations showed correspondingly fainter autoradiographs. This is true of all stages of development. Those exposed periodically for 2 hours in every 24 hours and returned to tapwater gave autoradiographs similar to those exposed continuously, except that the blackening on the film was less intense. Hence, variations in the time of exposure did not affect the distribution pattern of the phosphorus.

Since the embryos in the highest concentration of phosphorus gave clear yet typical results, they will be described in detail according to the developmental stages observed.

1. Mid-cleavage (Fig. 1). Autoradiographs show that the peripheral cells are mainly involved in the uptake of P^{32}. This is to be expected since the surface cells are in direct contact with the medium. Both the micromeres at the animal pole and the yolk-laden cells at the antipole show an equal distribution of P^{32}. Cells toward the center of the embryo, however, show only slight activity.

2. Mid-gastrula (Fig. 2). Autoradiographs of the whole embryo show blackening of the same intensity as that of the preceding stage. Those of the sectioned embryo show a concentration of P^{32} not only at the periphery but in the yolk-laden cells of the gut as well. Sections also show a concentration of P^{32} at the blastopore where population of cells is high and cell division is rapid.

3. Neural Tube (Fig. 3). The whole embryo shows less activity than the midgastrula.

6. Hunt, E. L., and Wolken, J. J., *J. Exp. Zool.*, 1948, v109, 109.

† We are indebted to Dr. Herman Branson of the Departmment of Physics for the preparation of these solutions.

7. Rugh, Roberts, *Experimental Embryology*, 1948, Rev. Ed., Burgess Publishing Co.

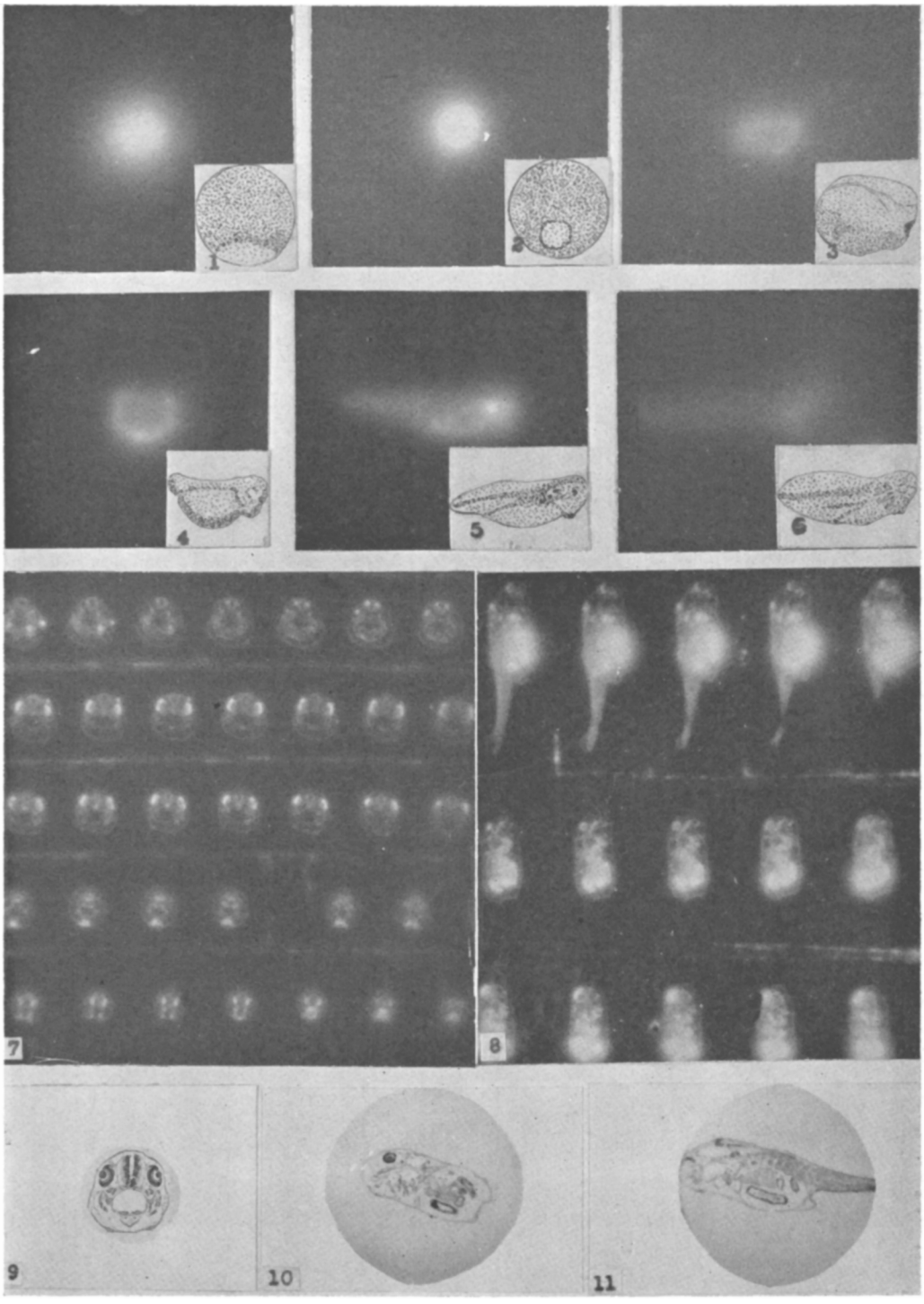

FIG. 1-6. Autoradiographs and drawings of embryos in the (1) mid-cleavage stage, (2) mid-gastrula stage, (3) neural tube stage, (4) muscular response stage, (5) gill circulation stage and (6) tail-fin circulation stage. × 4.

FIG. 7. Autoradiograph of sections of an embryo in the operculum complete stage at the level of the eyes and otocyst. × 4.

FIG. 8. Autoradiograph of longitudinal sections of an embryo in the same stage of development as that shown in Fig. 7. × 4.

FIG. 9. Microphotograph of one of the sections (through the eye) shown in Fig. 7. × 8.

FIG. 10. Microphotograph of one of the sections (middle row) shown in Fig. 8. × 4.8.

FIG. 11. Microphotograph of one of the sections (top row) shown in Fig. 8. × 4.8.

Sections, however, show that the phosphorus is no longer concentrated in the surface epidermis but also in the oral suckers, neural tube, eye, fore-gut and the liver. Mesodermal areas and the ears show less activity.

4. Muscular Response (Fig. 4). The whole embryo shows high activity on the outer surface, particularly in the anterior, posterior and ventral regions. Sections, however, show that there are several internal regions which give a more intense blackening on the photographic film. These regions of high activity include oral suckers and the muscles. Next in activity are surface ectoderm, gut, neural tube, eyes, liver, gills and notochord.

5. Gill Circulation (Fig. 5). Less activity is shown by the whole embryo in this stage than that in stage 18 (see chart), except in the eyes and gut. Sections show that in addition to the eyes and gut, the oral suckers and gills are also regions of high activity. Muscles and notochord show less activity than in the preceding stage, while mesenchyme, lateral mesoderm, heart, notochord and tail are the least active structures.

6. Tail-fin Circulation (Fig. 6). The embryo of this stage appears to be the least active of the series. Sections show that the gut, particularly the fore-gut, is most active. This is probably due in part to the entrance of water containing the phosphorus during respiratory movement. Next in activity are the eyes, ears, kidney tubules, liver, muscles, tail and teeth. The heart, lateral mesoderm and mesenchyme show only slight activity.

7. Operculum Complete (Fig. 7). The greatest activity is observed at this stage. The whole embryo produces intense blackening on the photographic film (not shown). In sections, the following structures show the highest activity: gut, eyes, gills, teeth and cartilage of the head. Other structures of the embryo are only slightly less active than these regions.

A study of the table will show that regions of high activity may show slight shifts in different stages of development. It is difficult to account for such shifts unless we assume that the shift of high activity from a specific region of an embryo in an early stage of development to a different region in a later stage

TABLE I. Distribution of Phosphorus^{32} in Embryos of Different Development Stages.

Developmental stage from Shumway's table	Stage No.	Ecto-derm	Ent. (gut)	Mesen-chyme	Neur. tube	Eye	Ear	Lateral mesod.	Heart	Kidney tubule	Liver	Muscles	Oral sucker	Gill	Notch	Tail fin	Teeth
Mid-cleavage	9	2+	1+														
Mid-gastrula	12	2+	2+														
		Blastopore 3+															
Neural tube	16	2+	2+		2+	2+	1+	1+		1+	2+		2+		2+		
Muscular response	18	2+	2+	1+	2+	2+	1+	1+	1+	1+	2+	3+	3+	2+	2+		
Gill circulation	20	2+	3+	1+	2+	3+	1+	1+	1+	1+	2+	2+	3+	3+	1+	1+	
Tail-fin circulation	22	2+	4+	1+	2+	3+	3+	1+	1+	3+	3+	3+	2+	2+	2+	3+	3+
Operculum complete				Cartilage													
	25	3+	4+	4+	3+	4+	3+	2+	2+	3+	3+	3+	3+	4+	3+	3+	4+

Degree of blackening: 1+, slight; 2+, low; 3+, intermediate; 4+, deepest.

is an indication of a shift in the rate of the utilization of P^{32} in these regions.

It is concluded from a study of the autoradiographs of whole and sectioned embryos that P^{32} is rapidly utilized in those regions where cell duplication is rapid and hence, cell population is high. This is in agreement with the observations on the distribution of P^{32} in the chick embryo (Hansborough and Nicholas)(3). The process is a cumulative one, increasing with the age of the embryo.

Summary. (1) Fertilized eggs of the leopard frog, *Rana pipiens*, were exposed to solutions of radiophosphorus of different activities (65, 300 and 550 microcuries). (2) Autoradiographs and microphotographs were prepared from whole and sectioned embryos in 7 different stages of development. (3) Autoradiographs show that the uptake of phosphorus^{32} is greatest in embryos exposed to the most active solutions and also that the process is a cumulative one, increasing with the age of the embryo. (4) The most active areas of the embryo in each of the stages studied are those areas where cell division is high and the population of cells is largest. (5) With the appearance and differentiation of organ primordia, the rate of phosphorus turnover increases, thus indicating that phosphorus is probably being utilized more rapidly in those areas in the synthesis of nucleoproteins.

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Proposed Intermediary Role of 3,4-Dihydroxyanthranilic Acid in the Biosynthesis of Nicotinic Acid.* (19097)

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The conversion by rat liver slices and homogenates of 3-hydroxyanthranilic acid to quinolinic acid, but not to nicotinic acid has been reported(1,2). Neither tryptophan nor kynurenine gives rise to either of these pyridine derivatives under similar conditions(1). It has been assumed, that in the conversion of 3-hydroxyanthranilate to quinolinate there are at least two enzyme catalyzed steps, since the reaction involves a four electron oxidation and the conversion of a benzene to a pyridine nucleus. The work of Stanier *et al.*(3), and other evidence bearing on the mechanism of metabolic breakdown of aromatic compounds led to the suggestion(4) that 3,4-dihydroxyanthranilic acid might be an intermediate in the postulated 3,4 fission of 3-hydroxyanthranilate. Mitchell *et al.*(4) found that this compound was not active for *Neurospora crassa*. Likewise, a sample kindly supplied by Dr. Mitchell, failed to give rise to quinolinic acid when incubated with rat liver slices. More recently Makino *et al.* (5) have reported that slices of liver from a number of species form nicotinic acid as estimated by the cyanogen-bromide-aniline color reaction, when incubated with tryptophan, 3-hydroxyanthranilate, 3,4-dihydroxyanthranilate, and quinolinate. They used 24-hour incubation periods with beef, hog and horse liver. Since these findings are at variance in several respects with our results with rat liver preparations, we have reinvestigated the

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1. Henderson, L. M., and Ramasarma, G. B., *J. Biol. Chem.*, 1949, v181, 687.

2. Schweigert, B. S., and Marquette, M. M., *J. Biol. Chem.*, 1949, v181, 199.

3. Stanier, R. Y., and Hayaishi, O., *Science*, 1951, v114, 326.

4. Mitchell, H. K., *Vitamins and Hormones*, 1950, v8, 144.

5. Makino, K., Itoh, F., and Nishi, K., *Nature*, 1951, v167, 115.