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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TABLE I. The Formation of Nicotinic Acid and Quinolinic Acid by Rat Liver Slices.

Substrate		— Pyridine derivatives found — Nicotinic acid	Quinolinic acid
	3 hr incubation		
		μg per 150 mg	μg per 150 mg
0		9.7	1.4
1 μM	L-tryptophan	11.8	0
10 μM	"	10.7	1.4
1 μM	DL-kynurenine	10.9	1.4
1 μM	3-OH anthranilic acid	11.8	56
1 μM	3,4 di OH anthranilic acid	11	−.5
17 μM	" *	11.9	1.4
1 μM	quinolinic acid	10.2	121
	24 hr incubation		
		μg per g	μg per g
0		144	12
2 mg	L-tryptophan	166	6
3	DL-kynurenine	138	31
—	3-OH anthranilic acid†	—	1000
1.7	3,4- " "	136	22
1.6	quinolinic acid	98	1700
	24 hr incubation		
		μg per 2 g	μg per 2 g
0		195	50
2 mg	L-tryptophan	218	46
3	DL-kynurenine	200	27
1.5	3-OH anthranilic acid	200	1040
1.7	3,4-di OH anthranilic acid	195	49
1.6	quinolinic acid	215	890‡

* Added as a dry powder.

† Portion of this sample was lost after incubation. Quinolinic acid value is the amount in remaining sample.

‡ In 4 hr incubation exp. with 150 mg of hog liver slices and homogenates and 5 μM of quinolinic acid, all of the substrate was recovered.

general problem using hog and rat liver slices in long term *in vitro* experiments. In addition, 3,4-dihydroxyanthranilate has been studied *in vivo* with regard to its growth promoting activity for rats and *Xanthomonas pruni* and its effect on quinolinic acid excretion by the rat.

Experimental and results. In vitro studies. 3,4-dihydroxyanthranilic acid was prepared from vanillin by the method outlined by Makino and associates(5). DL-kynurenine and 3-hydroxyanthranilic acid were the samples used in previous studies(6,7). For the *in vitro* studies, approximately 150 mg of fresh liver as slices or homogenate was suspended in a total volume of 3 ml of Krebs-Ringer phosphate buffer in 10 ml Erlenmeyer flasks or 18 x 150 mm test tubes. The substrate was added in 0.2 ml of buffer. In some experiments, 3,4-dihydroxyanthranilic acid was added in a finely powdered form from time to time during the incubation to preclude destruction by autooxidation during the shaking required to dissolve this compound. Control flasks containing inactivated enzyme plus substrate, or active enzyme but no substrate, were included. The reaction mixtures were incubated exposed to the atmosphere at 38°C with shaking for 30 minutes, 3 hours or 24 hours. The reaction was stopped by immersing the vessels in a boiling water bath for 3 minutes or by the addition of 0.5 ml of a 50% solution of trichloracetic acid. Nicotinic acid and quinolinic acid were determined in the filtrates by the differential assay with *Lactobacillus arabinosus*(8) and in some cases by the cyanogen bromide-aniline method. For the 24-hour incubation experiments, one

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or 2 g of rat or hog liver slices was suspended in 3 ml of 0.9% sodium chloride and 5 ml of M/15 phosphate buffer, and incubated in 50 ml Erlenmeyer flasks with shaking. In some cases, one or 2 drops of toluene was added to the flasks at periodic intervals and found to have no measurable effect on the results. All substrates were added in a dry state at the beginning of the incubation.

In Table I are presented the results of some typical experiments. For purposes of comparison, all figures were converted to the same weight of tissue. In no case was there any significant production of nicotinic acid from any of the substrates used. Only 3-hydroxyanthranilic acid was transformed to quinolinic acid under the conditions of these experiments. The larger quantity of tissue slices and longer period of incubation did not appear to influence the results, since the conversion of 3-hydroxyanthranilic acid was essentially complete in as little as 30 minutes. As in previous work(1), there was no evidence for the *in vitro* decarboxylation of quinolinic acid to form nicotinic acid. Results with the chemical method of analysis agreed with those obtained with the microbiological assay, *i.e.*, there was no net production of nicotinic acid with any of the substrates used.

Rat growth and excretion studies. Two experiments were conducted to determine whether or not 3,4-dihydroxyanthranilic acid could replace tryptophan in supporting the growth of rats on a low tryptophan-niacin diet. Female rats from the Biochemistry Division Colony approximately 4 weeks of age were used. In the first experiment, the supplements were incorporated into the diet at a level of 0.25 mM per 100 g of diet. In the second, 0.025 mM per day was injected intraperitoneally. Control animals received the same volume of isotonic glucose solution. The basal ration contained 9% casein, 0.2% L-cystine and 0.2% DL-threonine(9).

The growth rates obtained in both studies are shown in Table II. It is evident that 3,4-dihydroxyanthranilic acid did not promote growth above that observed for the control groups, while tryptophan supported growth in the normal range for rats on low protein diets. After a 4-week growth period, three of the five rats receiving 3,4-dihydroxyanthranilic acid were fed for one week the same diet supplemented with 100 mg of DL-tryptophan per 100 g of diet while the 3,4-dihydroxyanthranilic acid injections were continued. These animals gained an average of 17 g during this 7-day period, while 2 animals continued on the same regimen without added tryptophan gained 0.5 g during the same period. These results indicate strongly that the slow growth of the rats receiving the compound in question could not have been the result of toxicity overcoming a growth response.

Urine was collected for a 3-day period during the 3rd week of the growth experiment. Microbiological analysis for nicotinic acid and quinolinic acid confirmed the growth data. Whereas the tryptophan injections elevated the quinolinic acid and nicotinic acid excretion 3-4 fold, 3,4-dihydroxyanthranilic acid in equimolar quantities did not alter the urinary excretion values significantly. (Table III).

TABLE II. Growth Promoting Effect of Tryptophan and 3,4-Dihydroxyanthranilic Acid for Nicotinic Acid Deficient Rats.

Group	Diet	Growth, g/wk — Exp. I: Supplement in ration,* g	Exp. II: Supplement by I.P. inj. daily,† g
1	Basal ration	3	3
2	Basal ration + DL-tryptophan	19	20
3	Basal ration + 3,4-dihydroxyanthranilic acid	6	2

* .25 mM per 100 g of diet.
† .025 mM per day in isotonic glucose solution.

TABLE III. Excretion of Nicotinic Acid and Quinolinic Acid by Rats Receiving Tryptophan and 3,4-Dihydroxyanthranilic Acid.

Group	Daily supplement (I.P. inj.)	N.A., μg/day	Quinolinic acid, μg/day
1	0	3	25
2	.025 μM DL-tryptophan	16.5	71
3	.025 μM 3,4-dihydroxyanthranilic acid	4.8	17

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Growth tests with Xanthomonas pruni. A strain of the plant pathogen *X. pruni* has been studied and found to respond to all of the postulated nicotinic acid precursors as far back as anthranilic acid(7). It seemed likely that if 3,4-dihydroxyanthranilic acid were an intermediate between 3-hydroxyanthranilic acid and nicotinic acid, it should support growth at levels equal to or less than those at which 3-hydroxyanthranilate is active.

Whereas 3-hydroxyanthranilic acid supported optimum growth at a concentration of 1.2 μM and quinolinic acid at about 200 μM with measurable growth at much lower concentrations, 3,4-dihydroxyanthranilic acid did not show significant activity at levels from 0.12 to 240 μM. Addition of similar concentrations to cultures containing sufficient nicotinic acid (.04 μM) for sub-optimum growth (60-70% transmission) resulted in no growth promoting activity. Some inhibition of growth under these conditions was noted when the 3,4-dihydroxyanthranilic acid concentration was above approximately 100 μM.

To eliminate the possibility that all of the 3,4-dihydroxyanthranilic acid was destroyed during the lag phase of growth, it was added to cultures already actively growing on sub-optimum concentrations of nicotinic acid. Again no growth promoting activity was noted.

Discussion. The explanation of the wide discrepancy between these results and those presented by Makino *et al.*(5) is not known. If 3,4-dihydroxyanthranilic acid were an intermediate as suggested by their data, the intact animal would be expected to utilize it for growth and to excrete nicotinic acid and quinolinic acid as it does when given tryptophan, kynurenine, 3-hydroxyanthranilic acid and quinolinic acid. Likewise, the bacterium tested should respond. The ease with which 3-hydroxyanthranilic acid is converted to quinolinic acid *in vitro* would suggest that any intermediate involved should give rise to large amounts of quinolinic acid when added to such systems. The intermediate reported by Bokman and Schweigert is not 3,4-dihydroxyanthranilic acid‡(10). This intermediate shows an absorption band at approximately 315 mμ in acid solution and 360-375 mμ in buffer at pH 7.4. In trichloracetic acid or ethanol, 3,4-dihydroxyanthranilic acid has two absorption maxima; one at 268 mμ and one at 322 mμ.

Summary. 3,4-dihydroxyanthranilic acid was not converted to quinolinic acid or nicotinic acid by rat or hog liver preparations. Spectrophotometric evidence indicates that this compound is not the transient intermediate formed in the conversion of 3-hydroxyanthranilic to quinolinic acid by liver enzymes. It was not utilized by *Xanthomonas pruni* for growth, or by the rat for growth or formation of urinary nicotinic acid or quinolinic acid.

‡ In a private communication, Dr. Schweigert states that rat liver preparations failed to form quinolinic acid or nicotinic acid when incubated with 3,4-dihydroxyanthranilic acid supplied by Dr. Makino.

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Mechanics of Cell Division. I. The Living Spindle. (19098)

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The destruction of the longitudinal half of a mitotic spindle by X irradiation has been described(1). Chromosomes in the irradiated area of the spindle became pycnotic whereas those in the structurally intact area maintained their separate identities (Fig. 1A).

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